23 Gastric Cancer: Epigenetic Mechanisms: Aberrant DNA Methylation and Dysregulation of MicroRNA

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Abstract

Like many human cancers, global DNA hypomethylation and promoter CpG island hypermethylation in tumor suppressor or tumor-related genes are frequently observed in gastric cancer, and aberrant DNA methylation occurs in a gene-specific manner during the multistep gastric carcinogenesis. Chronic *Helicobacter pylori* (*H. pylori*) infection induces pro-inflammatory cytokines and reactive oxygen and nitrogen species in the gastric mucosa, which is known to be associated with the accumulation of aberrant DNA methylation. Aberrant DNA methylation caused by *H. pylori*-associated gastritis persists even after the disappearance of *H. pylori*, and epigenetic alterations induced by *H. pylori* correlate with the risk for gastric cancer. Numerous microRNAs (miRNAs) are dysregulated during the gastric carcinogenesis, and some of these miRNAs are known to be also dysregulated by *H. pylori* infection. miRNAs dysregulated by *H. pylori* infection play an important role in gastric carcinogenesis by modulating inflammation and immune response of the host, cell cycle progression, apoptosis and proliferation, and tumor invasion and metastasis.

Keywords

Helicobacter pylori • Epigenetics • Gastric cancer • Methylation • MicroRNA

23.1 Introduction

Aberrant DNA methylation is a major epigenetic mechanism associated with gene silencing; it is observed in many human cancers [1]. Global DNA hypomethylation and promoter hypermethylation in the specific genes have been associated with genomic instability and inactivation of tumor sup-

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pressor genes, respectively. Global hypomethylation mostly occurs in the intergenic region, and it precipitates chromosomal instability which leads to chromosomal mutations such as recombination, translocation, deletion, and rearrangement [2, 3]. On the other hand, promoter CpG island hypermethylation induces carcinogenesis by gene silencing of mostly tumor suppressor genes. These epigenetic changes are known to be replicated with a high fidelity in mammalian cells, mediated by DNA methyltransferases (DNMTs) which add methyl groups to cytosines and in result serve as a long-term memory of cells.

In gastric cancer, tumor suppressor or tumor-related genes are more frequently inactivated by CpG island hypermethylation than by mutations [4]. To date, promoter hypermethylation of such genes as *p16*, *CDH1*, *MGMT*, *MLH1*, *APC*, and *RUNX3* has been reported in gastric cancers. Global DNA hypomethylation is frequently observed in gastric cancer cells [5]. Interestingly, promoter CpG island hypermethylation has also been found both in the adjacent noncancerous tissues of patients with gastric cancer and in nonneoplastic gastric mucosae of subjects without gastric cancer. From the results of previous studies, aberrant DNA methylation occurs in a gene-specific manner along the multistep gastric carcinogenesis [6].

MicroRNA (miRNA) is a small noncoding RNA molecule (containing approximately 21–23 nucleotides) that functions as RNA silencing and posttranscriptional regulation of gene expression. miRNAs can inhibit translation of the target messenger RNAs (mRNAs) by binding to complementary sequences in the 30 untranslated regions (UTRs) of the mRNAs. Approximately more than one-third of human genes are known to be regulated by ~1,000 miRNAs, and a single miRNA can regulate hundreds of unique mRNAs [7, 8]. miRNAs play important roles in the regulation of almost all biological processes, including proliferation, apoptosis, cell differentiation, metabolism, and epithelial-to-mesenchymal transition [9]. Therefore, miRNA dysregulation has been determined to correlate with cancer development and progression. The first report on miRNA dysregulation in cancer was in chronic lymphocytic leukemia [10]. To date, specific miRNAs have subsequently been found to have links with various malignancies including lung, colorectal, and breast cancers.

The role of miRNAs has also been addressed in gastric carcinogenesis. According to the results of miRNA chip studies, numerous miRNAs were demonstrated to be associated with gastric cancer. For example, miR-21, miR-17-92 cluster, miR-106b-25 cluster, and miR-150 were overexpressed in gastric cancer. On the other hand, miR-451, miR-141, miR-31, miR-218, and miR-9 were reported to be downregulated in gastric cancer tissue [11]. Alterations of miRNAs in gastric mucosa or blood or gastric juice may provide important data on early diagnosis and prognostication of gastric cancer [12]. More studies are warranted in the future.

23.2 *H. pylori*-Induced Gastric Carcinogenesis and Aberrant DNA Methylation

Helicobacter pylori (*H. pylori*) infection is an established risk factor for gastric cancer and is known to be associated with the accumulation of epigenetic alterations such as aberrant DNA methylation in gastric mucosa. A recent study on genome-wide methylation profiling shows that a number of genes were differentially methylated by *H. pylori* infection [13] (Fig. 23.1). Previous studies have shown that *H. pylori* infection can induce promoter hypermethylation of *CDKN2A* (*p16*), *CDH1*, and *MLH1* [4]. Since the aberrant DNA methylations of these genes are closely related to gastric carcinogenesis, *H. pylori* infection seems to be associated with promoter hypermethylation with gene type-specific methylation profiles in the multistep process of carcinogenesis [14, 15].

23.2.1 Underlying Mechanisms of Induction of Aberrant DNA Methylation by *H. pylori* Infection

Animal studies may address the underlying mechanisms regarding how *H. pylori*-induced chronic

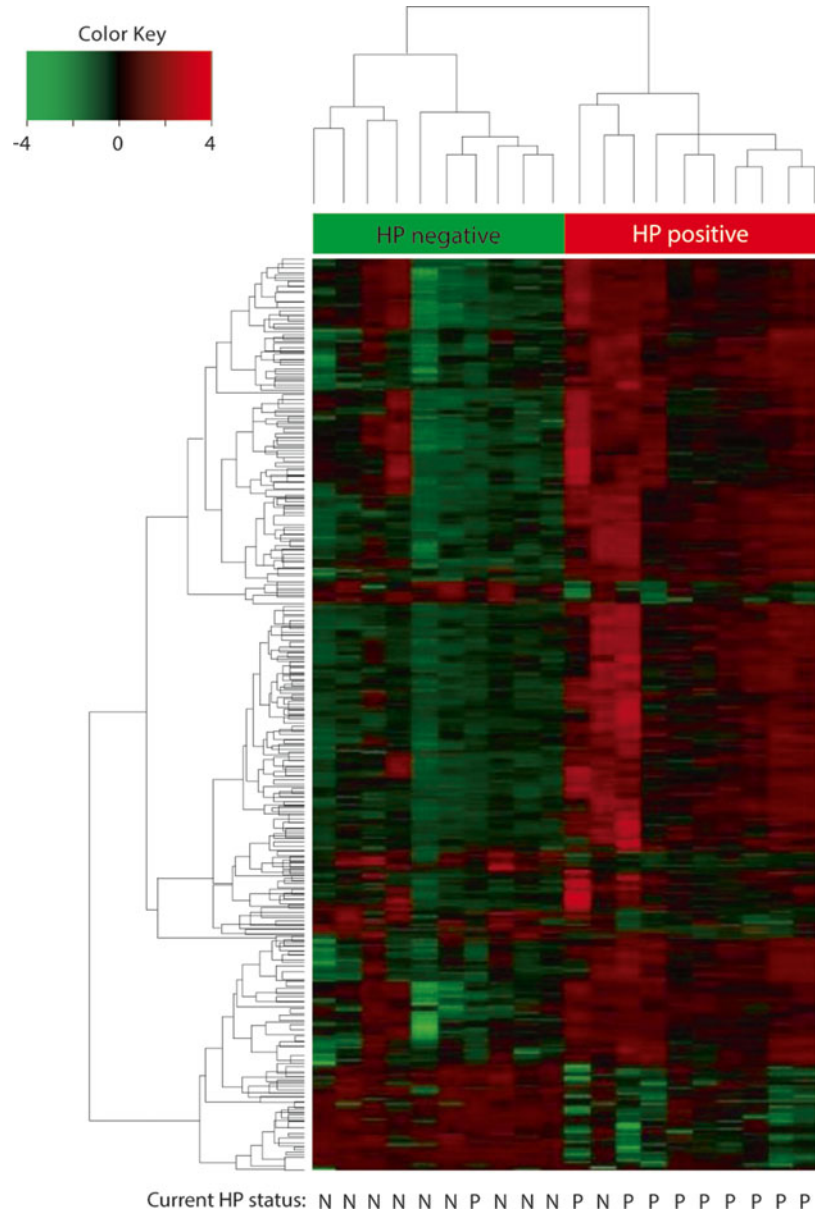

Fig. 23.1 Genome-wide DNA methylation profiles obtained from 10 *H. pylori*-positive patients and 10 *H. pylori*-positive controls. *Hp Helicobacter pylori*, *N H. pylori* negative, *P* current *H. pylori* infection positive (Adapted from Shin et al. [13])

inflammation induces aberrant DNA methylation. If chronic active inflammation induced by *H. pylori* infection was suppressed by cyclosporin A in Mongolian gerbils, aberrant DNA methylation was significantly reduced, while the number of *H. pylori* was unaffected in gastric mucosae [16]. This study indicates that not *H. pylori* itself but inflammation is important in the induction of aberrant DNA methylation [17]. However, repeated induction of acute inflammation by ethanol or high salt diet could not induce aberrant DNA methylation [18]. *Il-1β*, *Nos2*, and *Tnf-α* were specifically upregulated by the *H. pylori*-induced inflammation. Probably, chronic active *H. pylori* infection activates macrophage, and its secretion of interleukin (IL)-1β and tumor necrosis factor (TNF)-α, as well as the production of active oxygen species by nitric oxide synthase (NOS), may induce DMNT1 and in result aberrant DNA methylation in gastric mucosae [17].

23.2.2 Reversibility of Aberrant DNA Methylation Following *H. pylori* Eradication

To date, changes of DNA methylation levels in gastric mucosae after anti-*H. pylori* treatment have not been clarified yet. Previous studies using nonquantitative methods have reported that hypermethylation in several tumor suppressor genes such as *CDH1* in gastric mucosae decreases following *H. pylori* eradication [19, 20]. On the other hand, more recent studies using quantitative methods have shown that aberrant DNA methylation induced by *H. pylori* seems to be partially reversible by *H. pylori* eradication [21, 22].

23.2.3 Epigenetic Fingerprint of *H. pylori* Infection and Epigenetic Field for Cancerization

In viewpoint of the prediction for gastric cancer using methylation biomarkers, DNA methylation profiles obtained from noncancerous gastric mucosae may be useful in identifying a high-risk group for gastric cancer. As mentioned above, *H. pylori* infection is recognized as one of the most important risk factors for gastric cancer. As mucosal atrophy and intestinal metaplasia progress, however, the bacteria are slowly removed from the gastric mucosa and active inflammation gradually decreases. Thus, it is difficult to demonstrate a causal relationship between *H. pylori* infection and gastric cancer. From the epigenetic point of view, *H. pylori*-associated chronic inflammation is responsible for the promoter CpG island hypermethylation and global DNA hypomethylation. DNA hypermethylation caused by *H. pylori*-associated gastritis persists even after the disappearance of *H. pylori* (epigenetic fingerprint of *H. pylori* infection). The duration of *H. pylori* exposure and the epigenetic alterations induced by *H. pylori*, not *H. pylori* infection *per se*, are known to be correlated with the future risk for gastric cancer (epigenetic field for cancerization) [23] (Fig. 23.2). From this background, it has been suggested that the DNA methylation levels in the specific CpG loci obtained from blood or gastric mucosae or gastric fluid might be used as a marker for gastric cancer.

23.3 *H. pylori*-Induced Gastric Carcinogenesis and miRNA

The role of miRNAs in *H. pylori*-induced chronic inflammation has been evaluated in recent studies. According to a miRNA profiling study of 470 human miRNAs in noncancerous gastric mucosae of *H. pylori*-positive and *H. pylori*-negative subjects, a total of 30 miRNAs were significantly downregulated with *H. pylori* infection, while only one miRNA, miR-223, was upregulated with *H. pylori* infection [24]. Interestingly, eradication of *H. pylori* normalized 14 of 30 miRNAs of which the expressions were downregulated [24]. One recent study has reported that 219 of 3,523 miRNAs showed at least two-fold increased or decreased expressions in *H. pylori*-positive gastric cancer tissues compared with *H. pylori*-negative gastric cancer tissues [25]. According to a review article, miRNAs such as miR-17, miR-20a, miR-21, miR-25, miR-146a, miR-155, miR-196, and miR-223 were upregulated both with *H. pylori* infection and gastric cancer, while miRNAs such as let-7a, miR-31, miR-34b, miR-34c, miR-101, miR-141, miR-203, miR-210, miR-218, miR-375, and miR-449 were downregulated both with *H. pylori*-infected gastric mucosae and gastric cancer tissues [16] (Tables 23.1 and 23.2). However, miRNAs that are dysregulated in response to *H. pylori* infection may not be the same miRNAs that are dysregulated in later stages of gastric carcinogenesis. For example, miR-106b is known as an oncogenic miRNA and is upregulated in gastric cancer, but its expression was reported to be suppressed in *H. pylori*-infected gastric mucosa [68, 69]. Likewise, miRNAs such as miR-34b, miR-34c, miR-103, miR-200a, miR-214, and miR-372 have been

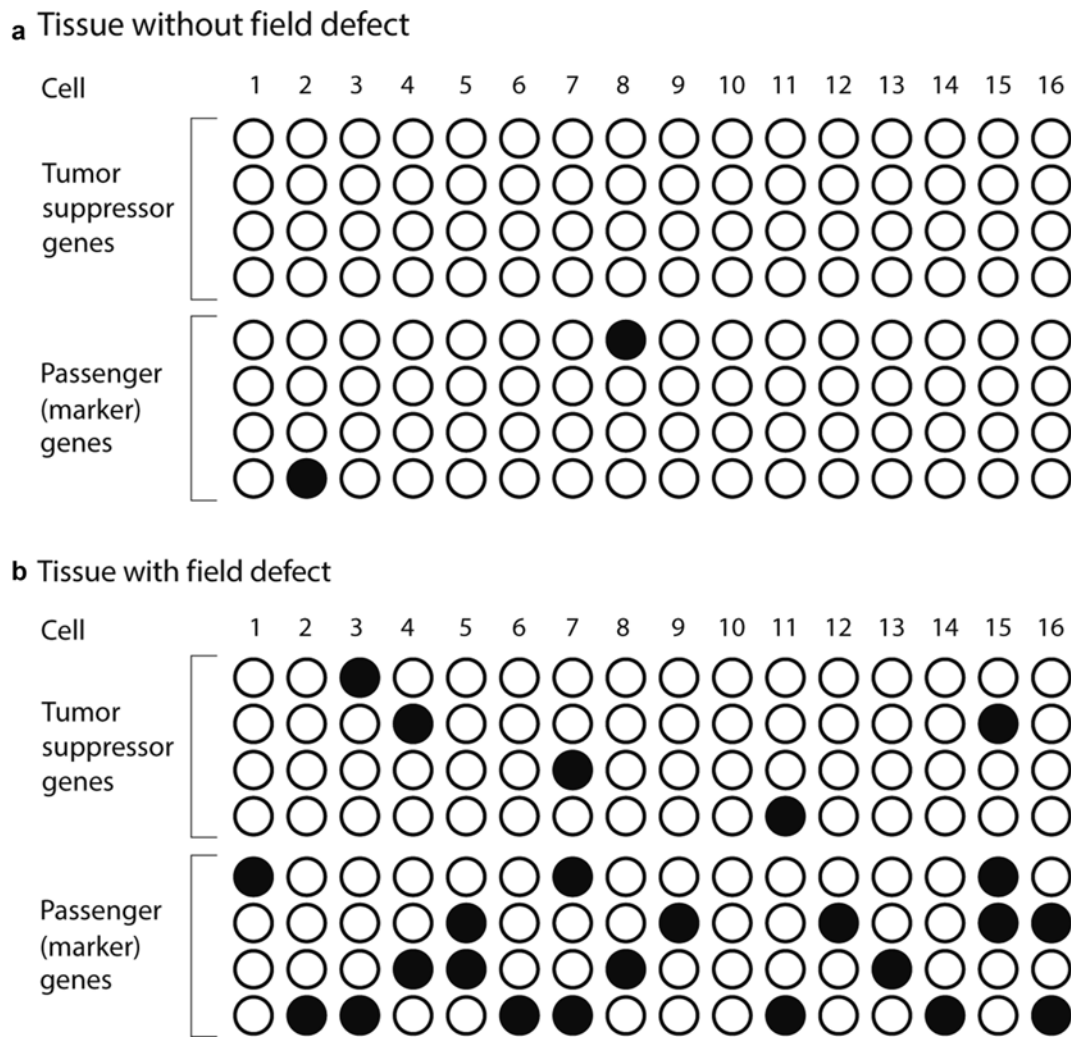

Fig. 23.2 Methylation of tumor suppressor and passenger (marker) genes. (**a**) Gastric mucosae without epigenetic field defect. (**b**) Gastric mucosae with field defect. In gastric mucosa with field defect, tumor suppressor genes may have low methylation levels, but passenger genes showed high levels of DNA methylation. Although methylation of passenger genes is not directly involved in carcinogenesis, their methylation levels correlate with those of tumor suppressor genes and reflect the future risk for gastric cancer. *Open circles* unmethylated genes, *closed circles* methylated (Modified from Ushijima [4])

reported to be overexpressed in gastric cancer, but these are downregulated in *H. pylori*-infected gastric mucosa [16]. MiR-146a was reported to be upregulated in *H. pylori*-infected gastric mucosae [18, 58, 70]. However, its expression has been reported to be upregulated with gastric cancer in one study but downregulated in the other studies [71–74].

23.3.1 *H. pylori* and miRNA: Underlying Mechanisms

Underlying mechanisms are still unclear regarding how miRNAs dysregulated by *H. pylori* infection can be involved in the gastric carcinogenesis. Nevertheless, it appears to be related to (1) modulating host inflammatory immune

Table 23.1 MicroRNAs downregulated in response to *Helicobacter pylori*

MicroRNAs	Downregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
let-7a	Yes	RAB40C	Cell cycle progression, proliferation	[24, 26, 27]
		HMGA2	Invasion	
let-7b		HMGA2	Invasion	[24, 26, 28, 29]
		TLR4, NF-kB, COX-2, cyclin D1, IL1B	Immune response	
let-7d		HMGA2	Invasion	[24, 26]
let-7e		HMGA2	Invasion	[24, 26]
let-7f		HMGA2	Invasion	[24, 26]
miR-1		ND	Proliferation	[30]
miR-31	Yes	ND	ND	[24]
miR-32		ND	ND	[24]
miR-34b	Yes	ND	ND	[31, 32]
miR-34c	Yes	ND	ND	[31, 33]
miR-101	Yes	COX-2, FOS	Proliferation	[24, 34, 35]
		MCL1	Apoptosis	
		EZH2	Invasion, migration	
miR-103		ND	ND	[24]
miR-106b		p21	Cell cycle progression	[24, 36, 37]
			Proliferation	
		BIM	Apoptosis	
miR-125a		ERBB2	Proliferation	[24, 38]
miR-130a		ND	ND	[24]
miR-133		ND	Proliferation	[30]
miR-141	Yes	FGFR2	Proliferation	[24, 39]
miR-200a		ZEB1, ZEB2	EMT	[24, 40, 41]
miR-200b		BCL2, XIAP	Apoptosis	[24, 40–42]
		ZEB1, ZEB2	EMT	
miR-200c		BCL2, XIAP	Apoptosis	[24, 41, 42]
			EMT	
miR-203	Yes	ABL1	Proliferation	[24, 43]
			Invasion	
miR-204		EZR	Proliferation	[24, 44]
miR-210	Yes	ND	ND	[24]
miR-214		ND	ND	[24]
miR-218	Yes	ECOP	Proliferation	[45, 46]
			Apoptosis	
		ROBO1	Invasion, metastasis	
miR-320		ND	ND	[24]
miR-370		FOXM1	Proliferation	[47]
miR-371-5p		LATS2	Cell cycle progression	[48]
miR-372		LATS2	Cell cycle progression	[48]
miR-373		LATS2	Cell cycle progression	[48]

Table 23.1 (continued)

MicroRNAs	Downregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
miR-375	Yes	PDK1, 14-3-3	Apoptosis	[24, 49, 50]
		JAK2	Proliferation	
miR-377		ND	ND	[24]
miR-379		ND	ND	[24]
miR-429		BCL2, XIAP	Apoptosis	[24, 42, 44]
		MYC	Proliferation	
miR-449	Yes	GMNN, CCNE2, MET, SIRT1	Cell cycle progression	[51, 52]
miR-455		ND	ND	[24]
miR-491-5p		ND	ND	[24]
miR-500		ND	ND	[24]
miR-532		ND	ND	[24]
miR-652		ND	ND	[24]

Modified from Noto et al. [16]
EMT epithelial-to-mesenchymal transition, *ND* target mRNA or biological process not determined

Table 23.2 MicroRNAs upregulated in response to *Helicobacter pylori*

MicroRNAs	Upregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
miR-17	Yes	p21	Cell cycle progression	[53]
miR-20a	Yes	p21	Cell cycle progression	[53]
miR-21	Yes	PTEN, PDCD4	Apoptosis	[54, 55]
		RECK	Metastasis, angiogenesis	
miR-25	Yes	ND	ND	[55, 56]
miR-93		ND	ND	[55]
miR-146a	Yes	IRAK1,TRAF6	Immune response	[18, 57, 58]
			Proliferation	
		SMAD4	Apoptosis	
miR-155	Yes	IKK-ε, SMAD2	Immune response	[59–62]
		FADD, PKIα	Apoptosis	
miR-194		ND	ND	[55]
miR-196	Yes	ND	ND	[55, 63, 64]
miR-200b		ZEB1	EMT	[65, 66]
miR-200c		ZEB1	EMT	[28, 65]
		ND	ND	
miR-222		RECK	Metastasis, angiogenesis	[67]
miR-223	Yes	EPB41L3	Invasion, metastasis	[24, 60]
miR-584		PPP2a, FOXA1	EMT	[42]
miR-1290		NKRF, FOXA1	EMT	[42]

Modified from Noto et al. [16]
EMT epithelial-to-mesenchymal transition, *ND* target mRNA or biological process not determined

response, (2) promoting cell cycle progression, (3) inhibiting apoptosis and promoting proliferation, and (4) promoting invasion and metastasis of gastric cancer [16].

23.3.1.1 Modulation of Host Inflammatory Immune Response

H. pylori can dysregulate miRNA expression to evade host defenses and successfully persist in the gastric niche. For example, *H. pylori* upregulates the expressions of miR-146a and miR-155, both of which modulate the innate and adaptive immune responses in a nuclear factor (NF)-κB-dependent manner [18, 57, 61, 62]. MiR-146a targets the Toll-like receptor (TLR) signaling adaptor molecules, interleukin-1 receptor-associated kinase (IRAK1), and TNF receptor-associated factor (TRAF6) [18, 57]. MiR-155 targets myeloid differentiation primary response gene (MyD88), the universal adaptor protein used by TLRs to activate NF-κB [61, 62]. As a result, both miR-146a and miR-155 overexpressions negatively regulate *H. pylori*-induced IL-8, TNF-α, IL-1β, growth-related oncogene (GRO)-α, and macrophage inflammatory protein (MIP)-3α expression, all key components to the pro-inflammatory innate and adaptive immune responses [16].

23.3.1.2 Promotion of Cell Cycle Progression

Several miRNAs dysregulated by *H. pylori* infection promote cell cycle progression by upregulating cyclin expression and/or downregulating expression of cyclin-dependent kinase (CDK) inhibitors (p15, p16, p18, p19, p21, p27, p28, p57) in gastric cancer [16]. The cell cycle consists of 4 distinct phases: G1, S, G2, and M. Two key classes of regulatory molecules, cyclins and CDKs, determine a cell's progress through the cell cycle. CDK inhibitors prevent the progression of cell cycle and function as tumor suppressors. miRNAs such as miR-106b and miR-449 target cyclins and CDKs as well as CDK inhibitors to disrupt normal cell cycle progression [36, 51, 52]. *H. pylori* is believed to modulate the expressions of cyclins, CDKs, and CDK inhibitors through dysregulation of host miRNAs. Thus it may induce gastric carcinogenesis [16].

23.3.1.3 Inhibition of Apoptosis and Promotion of Proliferation

miRNA dysregulation induced by *H. pylori* infection inhibits apoptosis and promotes cell survival. For example, miR-21 is overexpressed in gastric cancer tissues and cell lines, as well as in *H. pylori*-infected noncancerous gastric mucosae. It was also upregulated in cultured gastric epithelial cell lines cocultured with *H. pylori* [54]. Overexpression of miR-21 promoted cell proliferation and migration and inhibited apoptosis in this cell line. Activator protein (AP)-1 and the signal transducer and activator of transcription 3 (STAT3) can induce the expression of miR-21. *H. pylori* infection induces NF-κB and IL-6 secretion in gastric mucosae, which activate AP-1 and STAT3, respectively, which explains the upregulation of miR-21 during *H. pylori* infection. In addition, miR-21 targets phosphatase and tensin homolog (PTEN) and programmed cell death protein 4 (PDCD4) [26, 75–77].

23.3.1.4 Promotion of Tumor Invasion and Metastasis

H. pylori-induced dysregulation of specific miRNA may play a role in angiogenesis, invasion, and metastasis of gastric cancer. For example, *H. pylori* infection negatively regulates miR-449 during gastric carcinogenesis, which leads to the upregulation of Met, a known proto-oncogene. Upregulation of Met is known to promote not only proliferation but also angiogenesis, invasion, and metastasis of cancer [51, 52]. MiR-218 is known to be inhibited in gastric cancer, in relation to invasion and metastasis [78]. It might be attributed to roundabout homolog 1 (ROBO1) signaling pathway that has been implicated in the regulation of cell migration [46]. As mentioned above, *H. pylori* infection induces overexpression of miR-21. miR-21 has been reported to enhance the invasiveness of gastric cancer cells, which is attributed to inhibit reversion-inducing cysteine-rich protein with Kazal motifs (RECK), a tumor and metastasis suppressor that inhibits tumor

metastasis and angiogenesis through modulation of matrix metalloproteinases (MMPs) [54]. *H. pylori* infection is known to induce the expression of MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9 [79]. So *H. pylori* has the potential to modulate expression of MMPs through dysregulation of host miRNAs [16].

Conclusions

Aberrant DNA methylation and the dysregulation of numerous miRNAs by chronic active *H. pylori* infection play an important role in gastric carcinogenesis. Alterations of these epigenetic changes in gastric mucosae or biological fluids have diagnostic and therapeutic potentials in gastric cancer, but further studies are necessary to validate these potentials in large clinical trials.

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