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REVIEW

Epigenetic modifications induced by *Helicobacter pylori* infection through a direct microbe–gastric epithelial cells cross-talk

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Abstract One of the most fascinating aspects of the field of epigenetics is the emerging ability of environmental factors to trigger epigenetic changes in eukaryotic cells, thus contributing to transient or stable, and potentially heritable, changes in gene expression program in the absence of alteration in DNA sequence. Epigenetic response may result in cell adaptation to environmental stimuli or, in some instances, may contribute to generation or progression of different kind of diseases. A paradigmatic case of disease that is accompanied by multiple epigenetic alterations is gastric cancer, among other relevant examples. In turn, *Helicobacter pylori* (*Hp*) infection has been associated as a leading cause of gastric cancer. One possible hypothesis is that *Hp*–gastric cell interaction initiates an epigenetic reprogramming of host cell genome that may favor tumorigenesis. Accordingly, an abundance of experimental evidence indicates that several epigenetic alterations underlie the gastric cancerogenesis process and that these alterations represent one of the major hallmarks of gastric cancer. However, several critical questions remain unanswered: Does *Hp* directly provoke epigenetic alterations? Which mechanisms underlie these phenomena? Based on currently available data, it is often arduous to discriminate between the epigenetic modifications directly triggered by *Hp*–gastric cell interaction and those alterations that are mediated by inflammation process or by many other molecular and genetic events occurring during the gastric cancer progression. We will review our present knowledge of epigenetic modifications and alterations proven to occur in host cells as a direct consequence of *Hp* infection.

Keywords *Helicobacter pylori*–gastric cell interaction · Epigenetic alterations · Gastric cancerogenesis · DNA methylation · Chromatin modifications

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Epigenetic modifications

Epigenetic mechanisms may operate at gene-specific level and include both chromatin modifications, orchestrated by chromatin-remodeling complexes and histone-modifying enzymes, and DNA methylation, directed by DNA methyltransferases. Histone acetylation is in general associated with an active state of the chromatin, while the effects of histone methylation may be associated with either transcriptional activation or repression, depending on which lysyl residue is modified [1, 2] and whether this residue is mono, di or trimethylated. Among the best studied H3 lysine modifications are di- and trimethylation of H3 on lysine 9 and lysine 27 (H3K9me2 and H3K27me3), associated with closed chromatin, and dimethylation of H3 on lysine 4 (H3K4me2) that marks active chromatin state. Cellular stimuli may be integrated through signal transduction pathways leading to activation of histone-modifying

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enzymes acting at gene-specific level. Among such mechanisms, the most widely studied is the mitogen-activated protein kinase (MAPK) cascade which upon activation leads to phosphorylation of histone H3 on serine 10 (H3S10). This is a mark of transcriptional activation since it predisposes to histone acetylation [2]. DNA methylation of CpG sites at gene regulatory regions is mostly related to transcriptional repression. This is believed to be a more stable epigenetic mark than histone modifications [3–5]. In fact, DNA methylation profiles are sculpted during development, establishing the cell-specific gene expression program; the established DNA methylation profiles are then believed to be quite stable during the adult life, with some exceptions related to environmental stimuli or diseases [6, 7]. Maintenance of properly established DNA methylation profiles is essential for mammalian development and for the normal functioning of the adult organism. DNA methylation is a potent mechanism for silencing gene expression and maintaining genome stability, especially considering the large amount of repetitive DNA sequences which can otherwise mediate illegitimate recombination events and cause transcriptional deregulation of neighboring genes. Chromatin modifications and DNA methylation are strictly linked and can associate or interfere with each other during the course of life [4, 8]. It is worth noticing that other epigenetic marks, including 5-hydroxymethylcytosine, have been recently recognized and their biological significance is currently under deep investigation [9]. The involvement of microRNAs (miRNAs), short RNA molecules that play a role in post-transcriptional silencing of multiple target genes, has been widely documented in gastric cancer and *Hp* infection (reviewed in [10, 11]). The role of miRNA in response to *Hp* infection will be not addressed in this review.

Helicobacter pylori, inflammation, gastric cancer and epigenetic modifications

Helicobacter pylori (*Hp*) is a Gram-negative, spiral-shaped microaerophilic bacterium that is involved in several gastric diseases. Individuals infected by *Hp* develop gastritis, and up to 10 % of infected individuals develop duodenal ulcer disease [12]. Persistent infection with *Hp* may cause chronic atrophic gastritis, with the development of intestinal metaplasia, dysplasia and gastric carcinoma [13–18]. A lot of information about this carcinogenesis pathway has been acquired by experiments in animal models [18, 19], and in 1994, the International Agency for Research on Cancer classified *Hp* as a carcinogenic agent class I [20].

The mechanism of *Hp* pathogenicity is not well understood. Both bacterial virulence and host susceptibility factors have been associated with the development of chronic gastric inflammation and gastric carcinogenesis [18–23]. VacA is a major virulence factor of *Hp* and has pleiotropic effects in target host cells. *H. pylori* strains containing a cluster of approximately 30 genes known as the 'cag' pathogenicity island (cagPAI) are more frequently associated with severe gastric inflammation, ulceration and an increased risk of gastric cancer. The intracellular protein Nod1 is a major pathogen-recognition molecule involved in epithelial cell sensing of cagPAI-positive *H. pylori* strains [24, 25]. In addition, activation of the pattern recognition receptors TLR2, TLR9, Rig-I and, to a lesser extent, TLR4, by *H. pylori*, leading to proinflammatory signaling, has been reported [25]. Other effects induced by *Hp* involve bacterial components such as HP0175 [26], presumed TLR4 ligand, or adhesins BabA and OipA, which directly interact with host tissues [27, 28]. These virulence factors may activate multiple intracellular pathways in epithelial cells such as MAPK, NF-κb, Wnt/β-catenin, PI3K pathways, signal transducers and activators of trascription, such as STAT3, and may alter cellular function and/or trigger inflammatory processes. [29–32].

During the inflammation process, the *Helicobacter pylori* itself as well as intrinsic mediators of inflammatory responses, such as proinflammatory cytokines and reactive oxygen species, can induce genetic and epigenetic changes. These molecular events combine to alter important pathways involved in normal cellular function, thereby accelerating inflammation-associated cancer development. Epigenetic alterations have been suggested to play a key role early in gastric tumorigenesis. There is plenty of experimental evidence linking *Helicobacter* infection to aberrant epigenetic signatures of gastric cells observed during gastric cancerogenesis [33–36]. However, it is reasonable to believe that most of the epigenetic modifications observed in gastric tumors might have been established in any phase of the carcinogenesis process including *Hp*–gastric cell interaction, early inflammatory response to infection, chronic inflammation and cancer progression. In this review, we will first summarize evidences of epigenetic modification generally related to *Hp* infection in human tumors and animal models. We will then review advances obtained in recent works by experimental models consisting in cultured cells in the presence or absence of *Hp* or *Hp* extracts, in which any epigenetic modification observed in host cells may be *bona fide* attributed to, or demonstrated to be, a consequence of a signaling cascade triggered by the direct interaction of *Hp* components with host cell and not by indirect signaling, for example, via cytokines produced by other sources within the infected tissue.

Gene-specific DNA methylation alteration

An early observation was that E-cadherin gene (CDH1) underwent hypermethylation in *Hp*-infected normal gastric

Table 1 Genes differentially methylated in relation to gastric *Hp* infection

Genes	Major functions	Reference
p16 (INK4α)	CDK inhibitor	35
p14 (ARF)	MDM2 inhibitor	35
APC	Cell adhesion regulator	41
hMLH1	DNA mismatch repair	36
BRCA1	DNA repair	42
MGMT	DNA repair	43
E-cadherin	Calcium-dependent adhesion	44
LOX	Lysyl oxidase	45
FLNc	Muscle-specific filamin	46
HRASLS	HRAS-like suppressor	46
HAND1	Heart- and neural crest derivative-expressed protein 1	46
THBD	Endothelial cell receptor	46
p41 ARC	Centrosomal homeostasis (activator and substrate of Aurora kinase)	47
RUNX3	TGF beta signaling	48
TFF2	Trefoil factor 2, epithelial repair	49
WWOX	Putative oxidoreductase, plays a role in apoptosis	50
COX2	Mediator of inflammation	51
GSTP1	Glutathione S-transferase P1-1	51
RASSF1A	Ras association domain family 1A	51

mucosa compared to non-infected individuals [37, 38]. Then, *Hp*-related altered DNA methylation state of several other genes, including those related to cell growth control, (p16, p14 and APC), DNA repair (hMLH, BRCA1, MGMT) and cell adhesion (CDH1), has been described [39–51]. A list of some representative ones is presented in Table 1. By studying 48 promoter CpG islands using methylation-specific PCR, Nakajima et al. [52] found that 26 genes were consistently hypermethylated in individuals with current or past *Hp* infection. In addition to several markers associated with protein coding genes, CpG islands within microRNA genes were found hypermethylated in the presence of *Hp* infection (reviewed in [10, 11]).

Global epigenetic alteration

Epigenetic alterations at global level were also associated with *Hp*-related gastric cancer [53–57] most of which are listed in Table 2. Park et al. [53] found that the high global levels of H3K9me3, and not H3K9 acetylation, correlated with tumor stage, lymphovascular invasion, cancer recurrence and thus with poor survival rate. In another study, Weichert et al. [54] found that the expression levels of HDAC isoforms 1, 2 and 3 correlate with poor prognosis.

Table 2 *Hp*-related global epigenetic alterations in gastritis and gastric cancer

Epigenetic marks	Alteration	Reference
H3K9me3	Increased levels	53
HDAC1, 2, 3	Overexpression	54
H3S10 phosphorylation	Increased levels	55
H3K27me3	Increased levels	56
Global DNA methylation	Decreased levels	35
LINE-1 methylation	Decreased levels	57

Thus, the authors suggested that HDACs expression may be considered a prognostic marker and a potential target of epigenetic therapy for gastric cancer. Furthermore, Takahashi et al. [55] by investigating 122 patients observed that overexpression of phospho-H3S10 correlated with poor prognosis for gastric cancer.

Gene-specific chromatin alteration

Chromatin alterations were found in *Hp*-related gastric cancer also at gene-specific level (Table 3). Hypoacetylation of histones H3 and H4, associated with CpG hypermethylation, was found at the tumor suppressor genes *HLTF* [58], $p21^{(WAF1/CIP1)}$ [59, 60] and *SLC5A8* [61] in *Hp*-related gastric cancer.

Epigenetic modifications after Hp infection eradication

Animal models were very useful to study the dynamic of epigenetic changes during *Hp* infections. Mongolian gerbils experimentally infected with *Helicobater pylori* exhibited hypermethylation at several promoters from the gastric mucosa [62, 63]. Methylation levels increased during the first weeks after infection and, although remaining higher than in uninfected animals, decreased significantly some weeks after eradication of infection. Other interesting studies [44, 64] evaluated promoter methylation of CDH1 and other genes related to gastric carcinogenesis (p16, APC, MLH1 and COX2) in patients with active or eradicated *Hp* infection. Perri et al. [64] found that while CDH1 methylation was an early event in *Hp* gastritis, MLH1 methylation occurred late along with intestinal metaplasia, and that *Hp* eradication was able to significantly reduce gene methylation, thus delaying or reversing *Hp*-induced gastric carcinogenesis [64].

Epigenetic changes and gastric inflammation

It appears very likely that many epigenetic changes in *Hp*-infected tissues may be mediated by the inflammation process. The inflammatory molecules TNFα, IL-1β and NOS2 have been considered as the main mediators of a

Table 3 Gene-specific *Hp*-related chromatin modifications in gastric cancer

Genes	Gene function	Epigenetics modifications	References
HLTF	Chromatin-remodeling enzyme	H3-H4 hypoacetylation	58
$p21^{(WAF1/CIP1)}$	Cyclin-dependent kinase inhibitor 1	H3 hypoacetylation	59
		H4 hyperacetylation	60
SLC5A8	Sodium co-transporter	Histone hypoacetylation	61

signaling cascade that may lead to aberrant hypermethylation in gastric cells [65]. Interestingly, cyclosporine A, which suppresses inflammation without affecting bacterial colonization, abolished the aberrant methylation of several genes, indicating that in these cases inflammatory process mediated gene-specific hypermethylation [66].

However, to reach a more comprehensive view of early molecular events and epigenetic dynamics associated with *Hp* infection, it is critical to identify the specific epigenetic modifications and alterations directly triggered by *Hp* in gastric host cells. In particular, it is important to distinguish the epigenetic modifications not mediated by inflammatory molecules produced by other sources within the infected tissue.

Chromatin modification directly triggered in host cells by *Helicobacter pylori*

The exciting recent emerging evidences of bacterial impact on host epigenetics emphasize a novel strategy used by bacterial pathogens to interfere with key cellular processes. Recent illuminating studies demonstrate that bacteria or bacterial components (e.g., LPS) are able to provoke histone modifications and chromatin remodeling in infected cells, thereby altering the host's transcriptional program [67–71]. The coming era of pathoepigenetics of microbial infections has been therefore foreseen [72].

Chromatin modifications directly induced by *Helicobacter pylori* have been recently reported (Table 4). One of the first evidence was obtained by studying IL-6 gene in macrophages and the signaling cascade that caused chromatin changes at IL-6 promoter [67]. IL-6 is overexpressed in the mucosa at the margin of gastric ulcers and may be responsible for *Hp*-induced tissue invasion by macrophages. Recently, Pathak et al. [67] identified a *Hp* factor, HP0175, responsible for IL-6 gene activation causing gene-specific histone modifications. The authors showed that HP0175, a TLR-4 interacting protein [26], induced NF-κB, ERK and p38 MAPK activation, which in turn activated IL-6 expression. Furthermore, IL-6 expression correlated with phosphorylation of H3S10 at the IL-6 promoter. This modification occurred upon the induction of ERK and p38 that in turn activated MSK1, a serine kinase responsible for phosphorylating H3S10 [67]. H3S10 phosphorylation was shown to be required for NF-κB-dependent IL-6 expression

Table 4 Gene-specific chromatin alterations proven to be directly provoked by Hp–host cell interaction

Genes	Gene function	Epigenetic modifications	Reference
$p21^{(WAF1/CIP1)}$	Cyclin-dependent kinase inhibitor 1	H3 hypoacetylation	59
		H4 hyperacetylation	60
		HDAC1 release	
IL-6	Pro-inflammatory and anti-inflammatory cytokine	H3S10 phosphorylation	67
COX-2	Mediator of inflammation	H3K4me2 increase	70
		H3K9me2 decrease	
		H3K27me3 increase	
		H3 acetylation increase	
iNOS	Inducible nitric oxide synthase	H3 acetylation increase	71
		H3K4me2 increase	
		H3K9me decrease	
		MeCP2 release	
c-jun	Transcription factor	H3S10 dephosphorylation	73
hsp70	Heat shock proteins	H3S10 dephosphorylation	73

upon TLR4 activation by HP0175. In addition, direct exposure of gastric epithelial cells to *Helicobacter pylori* caused upregulation of p21WAF1 protein expression both in NCI-N87 cell line and in primary gastric cells [60]. The increased p21WAF1 expression is associated with release of HDAC1 from the *p21WAF1* promoter and hyperacetylation of histone H4 [60].

Ding et al. [73] demonstrated that *Helicobacter pylori* provoked a *cag*PAI-dependent dephosphorylation of histone H3S10 and a slight deacetylation of H3K23 in gastric epithelial cells, but did not affect seven other specific histone modifications. *Hp*-induced H3S10 dephosphorylation was associated with changes in host gene expression such as upregulation of *c-Jun* and downregulation of *hsp70*. Feheri et al. [74] demonstrated that *Hp*-induced modification of the histone H3 phosphorylation status in gastric epithelial cells may have an impact not only on transcriptional regulation but also on cell cycle regulation. In this work, it was shown that infection of gastric epithelial cell lines with *Hp* leads to type IV secretion system

(T4SS)-dependent decreases in H3 phosphorylation levels at serine 10 (pH3Ser10) and threonine 3 (pH3Thr3). At the same time, *Hp* caused a strong decrease in the cell division cycle 25 phosphatase (CDC25C). As a consequence, mitotic histone H3 kinases (vaccinia-related kinase 1, VRK1) was not fully activated in infected cells. The authors found that VRK1 activity was reduced after *Hp* infection and that overexpression of VRK1 compensated for the *Hp*-induced decrease in pH3Ser10. Increased phosphorylation of H3Ser10 was IkappaB kinase alpha (IKKalpha)-dependent and occurred at later time points after infection. These observations highlighted the impact of bacterial pathogens on host cell chromatin state and revealed that this modulation reflects the subversion of key cellular processes such as cell cycle progression.

Recently, a direct impact of *Hp* exposure on epigenetic control of COX-2 gene has been demonstrated [70]. Through TLRs receptors, *Hp* induces expression of host inflammatory genes such as TNF-α, which in turn activates NF-*k*B, a transcription factor whose target genes include COX-2 [75, 76]. COX-2 gene dysregulation is believed to play a critical role in gastric cancerogenesis. Therefore, COX-2-oriented therapy was proposed and showed promising anticancer efficacy [77]. Epigenetic status of COX-2 has been associated with clinical outcome of gastric cancer [78]. In particular, it was shown that COX-2 gene hypomethylation correlates with COX-2 overexpression and poor prognosis [78]. Moreover, transcriptional activity of COX-2 gene was associated with increased histone H3 and H4 acetylation in macrophage and smooth muscle cells study systems [79, 80]. Pero et al. [70] investigated the epigenetic changes occurring at the COX-2 locus during the first 48 h after exposure of human MKN28 gastric cells to *Hp*. The expression levels of COX-2 gene increased dramatically upon *Hp* stimulation concomitantly with the occurrence of several chromatin and DNA methylation changes. It was shown that the main drivers of COX-2 activation upon *Hp* exposure were the increased levels of H3K4me2 and the decreased H3K9 methylation state. Conversely, H3K27me3 levels significantly increased at COX-2 promoter shortly after *Hp* exposure, despite the concomitant detection of the highest COX-2 mRNA levels. Interestingly, the H3K4me2 and H3K9me2 basal levels were restored within 24 h, while the higher H3K27me3 levels persisted for longer time after *Hp* infection. These phenomena possibly reflected a more refractory state of the COX-2 gene in cells exposed to *Hp* compared to unexposed cells. The stable repressive mark, H3K27me3, is associated with the Polycomb repressor complex (PrC). Because PrC includes DNA methyltransferase activity [81], it was suggested that high H3K27me3 levels could increase the susceptibility of COX-2 promoter to alterations in DNA methylation profiles.

More recently, Angrisano et al. [71] described in detail the specific chromatin dynamics at iNOS gene early after *Hp* infection of cultured gastric cells. In this work, the authors investigated the epigenetic changes occurring at iNOS locus during the first 48 h after exposure of MKN28 gastric cells to *Hp*. MKN28 cells expressed low levels of iNOS mRNA and protein under the unstimulated condition. The expression levels of iNOS mRNA increased early and dramatically upon *Hp* stimulation concomitantly with the occurrence of several chromatin changes including transient histone H3 hyperacetylation. Modulation of histone deacetylase expression in *Hp*-exposed gastric cells was previously described in an animal model [82]. Angrisano et al. [71] also demonstrated that the main epigenetic drivers of iNOS activation upon *Hp* exposure were the increase in H3K4me2 levels and the decrease in H3K9 methylation state. Because LSD1 has both repressive and activating effects through H3K4 and H3K9 demethylation capability [83, 84], the *Hp*-dependent histone modification occurring in gastric cells pretreated with different LSD1 inhibitors was investigated. LSD1 inhibitors had no effects on iNOS basal levels, while a strong influence on *Hp*-induced iNOS activation was observed [71]. In fact, LSD1 inhibitors repressed *Hp*-dependent iNOS activation during the first 4 h after *Hp* infection; however, they could not prevent the iNOS induction at later times (6 h). The authors' interpretation of these data was that early *Hp*-dependent iNOS activation requires LSD1 (or other monoamineoxidase) activity, while the later iNOS peak (6 h) may be the result of different putative enzymatic activity. Interestingly, MeCP2, a methyl-binding protein frequently associated with a chromatin repressor complex including HDAC1 and Sin3A [85], was present at iNOS promoter in non-infected cells and then displaced together with repressor complex upon infection [71]. The authors proposed a model in which LSD1, limited to the first phases of infection, and MeCP2 may play an intermediary role between repressive and active histone marks by removing methylation and providing the naked lysine residue to be acetylated [71] (Fig. 1).

DNA methylation changes directly induced by *Helicobacter pylori* in host cells

While the mechanisms leading to chromatin changes induced by *Helicobacter pylori* have been in some cases investigated in detail, much less is known about the molecular events leading to specific changes in DNA methylation patterns upon *Hp* infection of gastric cells. Increased expression of DNA methyltransferases DNMT1 and DNMT3a [50], mediation by PcG complex [56], injection of bacterial DNA methyltransferases homologs

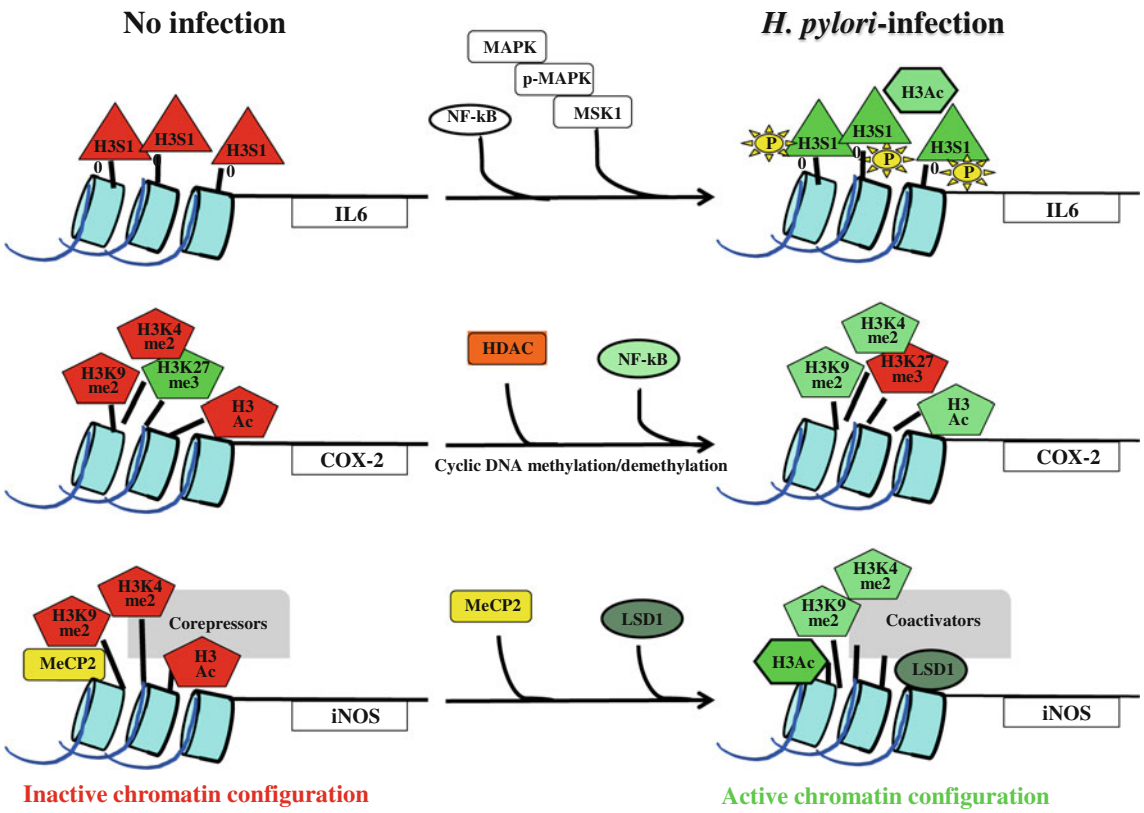

Fig. 1 Schematic models illustrating possible sequence of epigenetic events at IL6, COX-2 and iNOS genes upon *Hp*-induced activation. See also text for further explanation and references. *MeCP2* Methyl-CpG-binding protein 2, *LSD1* lysine-specific demethylase 1

Table 5 Gene-specific DNA methylation alterations proven to be directly provoked by Hp–host cell interaction

Genes	Gene function	Epigenetics modifications	References
TFF2	Epithelial repair	DNA hypermethylation	49
WWOX	Putative oxidoreductase, plays a role in apoptosis	DNA hypermethylation	50
		DNMT1 increase	
		DNMT3b increase	
COX-2	Mediator of inflammation	Cyclic DNA methylation/demethylation	70
RUNX3	TGF beta signaling	DNA hypermethylation (mediated by macrophages)	87
E-cadherin	Calcium-dependent adhesion	DNA hypermethylation (via IL-1β stimulation)	88
USF1	Transcription factor	DNA hypermethylation	89
	E-box binding protein		
USF2	Transcription factor	DNA hypermethylation	89
	E-box binding protein		

[86] or involvement of inflammatory molecules [87, 88] are some of the mechanisms that have been proposed to date. However, evidence that altered DNA methylation may be induced by *Hp* has been recently reported [49, 50, 70, 87–90] included those summarized in Table 5. Katayama et al. [87] using gastric cells co-cultured with macrophages, focused on a cancer suppression gene, RUNX3, and demonstrated the following: (a) *Hp* induces nitric oxide (NO) production in macrophages. (b) NO causes methylation of RUNX3 in epithelial cells. (c) *Hp* induces the methylation of RUNX3 in epithelial cells in the presence of macrophages, which is reversed by an NO-specific inhibitor. These results indicated that *Hp*-induced RUNX3 methylation is mediated by NO which in turn is produced by macrophages in response to *Hp* exposure.

Hypermethylation of E-cadherin gene is a well-established early event in gastric cancerogenesis [38, 39, 44]. Huang et al. [88] indicated that E-cadherin gene

methylation could be achieved by *Hp* infection of gastric cells even in the absence of co-cultured macrophages; nevertheless, such hypermethylation was mediated by IL-1β and activation of nitric oxide production in gastric cancer cell [88]. Increased expression of NFκB was accompanied by upregulation of iNOS and production of NO in treated cells. Reversal of all these phenomena in cells pretreated with IL-1 receptor antagonist suggests a model in which *H. pylori*-induced E-cadherin methylation occurs via IL-1β stimulation of the NFκB transcriptional system and leads to the activation of DNMT activity by NO production [88].

Peterson et al. [49] investigated the epigenetic silencing of *Trefoil* Factor 2 (TFF2), a tumor suppressor gene, in gastric biopsy specimens from individuals with *Hp*-positive gastritis, intestinal metaplasia, gastric cancer and disease-free controls and found that DNA methylation at the TFF2 promoter began at the time of *Hp* infection and increased throughout gastric tumor progression.

TFF2 methylation levels were inversely correlated with TFF2 messenger RNA levels, and the authors proposed that this methylation marker could be used to distinguish between disease-free controls, *Hp*-infected and tumor tissues. Genome demethylation restored TFF2 expression in gastric cancer cell lines, so TFF2 silencing requires DNA methylation. Moreover, experimental *Hp* infection in wild-type mice reduced antral expression of TFF2 by increased promoter methylation. The authors also concluded that TFF2 gene product and *Hp*-related alteration in TFF2 gene DNA methylation state may play an active role in gastric cancerogenesis. In fact, TFF2 negatively regulates preneoplastic progression and subsequent tumor development in the stomach, a role that is subverted by promoter methylation during *Hp* infection [49].

Upstream stimulatory factors 1 and 2 (USF1 and USF2) are transcription factors and tumor suppressor genes that bind E-box of target genes potentially in a competitive manner with c-myc oncogene product [89, 90]. A decrease in USF1 and USF2 expression is observed in different human gastric epithelial cell lines infected with *Hp*, associated with a lower binding to their DNA E-box recognition site. The treatment of cells with 5′-azacytidine, an inhibitor of DNA methylation, restored the USF1 and USF2 gene expression in the presence of infection [89]. By promoter methylation PCR assays, the promoter regions of both USF1 and USF2 genes were found hypermethylated in infected cells and in gastric tissues derived from infected mice [89]. Because these epigenetic alterations were found in metaplastic lesions, the authors suggested that alteration in USF1 and USF2 levels may play a role in the tumor promotion during *Hp* infection.

Another recent study addressed the role of WW domain-containing oxidoreductase (WWOX) gene methylation during *Hp*-related gastric cancerogenesis [50] since WWOX gene was reported to be a tumor suppressor gene downregulated in gastric cancer and hypermethylated in other tumors [91].

The results of this study [50] showed that WWOX hypermethylation was frequently detected in gastric cancer, and also significantly correlated with *Helicobacter pylori* infection. WWOX thus behaves as a specific tumor suppressor gene in *Hp*-related gastric cancerogenesis. Promoter methylation of WWOX, as well as expression of DNMT1 and DNMT3A, was induced in both BCG823 and AGS gastric cells co-cultured with *Hp* a few days after infection, suggesting a direct role of *Hp* in determining the alteration in WWOX gene methylation state.

Pero et al. [70] used mass spectrometry (MALDI-TOF) to study DNA methylation changes at several CpG sites on both strands of COX-2 regulatory region. The novelty of this work was that the methylation analysis was performed by collecting the cells at short intervals of 5 min for a total of 120 min after *Hp* infection of gastric cells. The results showed that COX-2 gene activation, as well as most of the chromatin events occurring at COX-2 gene in response to *Hp* exposure, is preceded by rapid strand-specific DNA methylation/demethylation events at 8 specific CpG sites (−176, −136, +25, +36, +57, +82, +198, +231) surrounding the COX-2 gene transcriptional start site. DNA methylation has been considered for long time a simple epigenetic mark generally associated with transcriptional silent, condensed chromatin. Only very recently, evidence of an unanticipated dynamic role of DNA methylation in gene regulation in human cells has been reported for estrogen-responsive genes [92, 93]. The mechanisms underlying the methylation/demethylation cycles at estrogen-responsive genes have been investigated in-depth and revealed that the demethylation process is initiated by the same enzymes that establish the methylation mark, the DNA methyltransferases DNMT3A and DNMT3B [92, 93]. Thus, it is likely that a similar phenomenon may be directly initiated at the COX-2 gene promoter upon *Hp* infection of gastric cells. Because in MKN28 cells as well as in normal gastric mucosa most of CpG sites at the COX-2 promoter display about 70 % methylation degree, the observed transient changes in DNA methylation could be critical for COX-2 derepression. The authors propose a model in which the DNA methylation/demethylation events are triggered by the binding of NF-kB to the COX-2 promoter, allowing chromatin to reach an open conformation through modification of histone acetylation and methylation [70].

Conclusions

There is plenty of experimental evidence showing a correlation between *Helicobacter pylori* infection, host cell

epigenetic alterations and gastric cancer. Several of such alterations could be mediated by the inflammatory process or can be achieved in late steps of gastric cancerogenesis. However, the precise nature and sequence of these molecular events remain largely unknown. Nevertheless, it is now emerging that the interaction between *Helicobacter pylori* and gastric cells may have a direct impact on host cell epigenetics possibly contributing to initiation of the cancerogenesis process. The study of microbe-induced epigenetic alterations in host cells represents a new, rapidly emerging field of investigation, and the study of the direct epigenetic effects of *Hp* infection is going to gain ground. However, to date, only a few studies have addressed this topic and a complete map of early epigenetic alterations and of the underlying molecular mechanisms is far to be drawn. Possibly, future genome-wide approaches able to give a comprehensive view of *Hp*-induced epigenetic modifications, including the newly discovered 5-hydroxymethylcytosine, could give a great contribution to unravel the causal role of *Hp*-induced epigenetic reprogramming. It is likely that this knowledge may have a critical impact on future epigenetic-based therapy or prevention strategies for *Hp*-related gastric diseases.

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