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Helicobacter pylori infection, oncogenic pathways and epigenetic mechanisms in gastric carcinogenesis

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Abstract

Chronic colonization of the human stomach by *Helicobacter pylori*, a Gram-negative bacterium, is the major cause of chronic gastritis, peptic ulcers and gastric cancer. Recent progress has elucidated important bacterial and host factors that are responsible for *H. pylori*-induced gastric inflammation and gastric malignancy. *H. pylori* cytotoxin-associated antigen A is the major oncogenic factor injected into host cells from bacteria and it disrupts epithelial cell functions. Together with *H. pylori cag* pathogenicity island, it causes general inflammatory stress within gastric mucosa and activates multiple oncogenic pathways in epithelial cells. A growing list of these pathways includes NF-κB, activator protein-1, PI3K, signal transducers and activators of transcription 3, Wnt/β-catenin and cyclooxygenase 2. *H. pylori* induces epigenetic alterations, such as DNA methylation and histone modification, which play critical roles in oncogenic transformation. In addition, investigations into gastric stem cell or progenitor cell biology have shed light on the mechanisms through which gastric cancer may originate. Continued investigation in these areas will yield novel insights and help to elucidate the mechanisms of bacteria-induced carcinogenesis.

Keywords

cancer; chromatin; epigenetics; gastric epithelial cell; *Helicobacter pylori*; histone; oncogene

The past 25 years have witnessed impressive progress in the study of the human stomach pathogen, *Helicobacter pylori*, a spiral-shaped Gram-negative bacterium, which infects half of the world's population and is the major cause of chronic gastritis, peptic ulcers and gastric malignancies, including gastric noncardia adenocarcinoma and mucosal-associated lymphoid tissue lymphoma [1]. *H. pylori* infection induces both acute and chronic gastritis, which present as superficial mucosal inflammation in the gastric mucosa. Although most infected individuals have no clinical presentation, approximately 10–20% will develop peptic ulcers and 1% will develop gastric cancer [2]. Gastric cancer continues to be the second leading cause of death among all cancers; in the year 2000, there were approximately 876,000 cases of primary gastric cancer, accounting for approximately 647,000 deaths worldwide, second only to lung cancer [3].

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Upon infection, *H. pylori* activates multiple intracellular pathways in epithelial cells, such as MAPK, NF-κB, activator protein (AP)-1, Wnt/β-catenin, PI3K pathways and signal transducers and activators of transcription (STAT)3 [1,2,4-7]. These affect various cellular functions, leading to increased inflammatory cytokine production, altered apoptosis rate, epithelial cell proliferation and differentiation, and finally resulting in epithelial cell oncogenic transformation. Bacterial virulence factors, such as cytotoxin-associated antigen A (CagA), the *cag* pathogenicity island (PAI), vacuolating cytotoxin (VacA) and outer membrane proteins (OMPs), are responsible for many of these effects [8].

Effects of *H. pylori* CagA & *cag*PAI in gastric epithelial cells

The first *H. pylori* genome sequenced (strain 26695) is 1.67 million bp in size and has 1590 predicted coding sequences [9]. The *cagPAI* of *H. pylori* is an approximately 40-kbp region of the *H. pylori* genome that encodes approximately 30 genes, some of them form a type IV secretion system, which is important for pathogenesis and is responsible for delivery of CagA protein and peptidoglycan (PGN) into the host cells [10,11]. The type IV secretion system injects CagA protein by forming a syringe-like structure capable of penetrating into host cell. The CagL protein binds to and activates host cell integrins and is required for subsequent delivery of CagA across the host cell membrane [12]. Infection with *cagA*-positive, which is a marker for *cag*PAI-positive, *H. pylori* strains is linked to increased inflammation and gastric cancer risk [1,2].

H. pylori CagA is encoded by the *cagA* gene within the *cag*PAI, has a molecular weight of approximately 120–145 kDa and profoundly affects host cell function (Figure 1) [13]. Following *H. pylori*'s adherence to gastric epithelial cells, CagA is injected into the host cell cytoplasm. It is then tyrosine phosphorylated by Src family kinases or Abl kinases in its glutamic acid–proline–isoleucine–tyrosine–alanine (EPIYA) motif [14,15]. The phosphorylated CagA binds to and activates SHP-2 [16]. SHP-2 acts upstream of the RAS/RAF/MEK/ERK cascade and has important functions in regulating cell proliferation, morphogenesis and motility [13]. In addition, CagA interacts with and disrupts epithelial cell barrier function through dephosphorylation of cortactin and induction of actin rearrangement by Src inactivation [17], dephosphorylation of cell junction molecules, such as ezrin [18], and associates with the epithelial tight-junctional scaffolding protein, ZO-1 [19]. These effects together damage normal cell contact, cell polarity and epithelial cell permeability. Interaction of CagA with the c-Met receptor or phospholipase C-γ and Crk enhances motogenic responses [20]. Grb-2 is the key mediator of CagA activities via its binding to the EPIYA motif that stimulates growth factor-like signaling, leading to cell morphological changes and proliferation [21]. Thus, CagA disrupts normal cellular signaling pathways and confers the cell oncogenic potential that is critical for *H. pylori* pathogenesis.

CagA also interacts with VacA, another major virulence factor for *H. pylori*, to deregulate nuclear factor of activated T cell signaling [22]. VacA causes cellular vacuolization, inhibits cell proliferation and ulcer healing. Deregulation of nuclear factor of activated T cell signaling induces cell cycle gene *p21* expression, affecting the cell cycle and differentiation [22]. More recently, CagA has been shown to disrupt cell polarity through its binding to the PAR1/MARK complex and inhibit its kinase activity [23]; interaction with E-cadherin deregulates β-catenin signaling, which induces expression of genes downstream of β-catenin, such as *CDX1*, and promotes cell intestinal transdifferentiation [24].

Previous studies indicated that *cagPAI*-dependent but *cag*A-independent host signaling was induced by *H. pylori;* of note is the activation of Rho-GTPase Rac1 and CDC42 that regulate host cell cytoskeleton rearrangement [25], introduction of PGN into the cytoplasm and activation of NF-κB by Nod1 signaling [11], which is especially important because NF-κB

activation mediates expression of multiple genes involved in *H. pylori*-induced host responses. *cagPAI* is also shown to mediate c-Met-induced gastric cancer cell invasiveness, independent of *cagA*, during *H. pylori* infection in gastric epithelial cells [26]. These results provide a critical insight and the molecular basis for *H. pylori-induced* cellular dysfunction and potential for oncogenic transformation.

H. pylori activates multiple oncogenic pathways in host cells

In addition to causing inflammatory stress and disrupting the cell cycle and cell polarity, *H. pylori* activates multiple oncogenic pathways, such as PI3K/AKT/GSK3β, STAT3 and β-catenin pathways and induces aberrant expression of activation-induced cytidine deaminase (AID), all of which are important in promoting gastric oncogenesis (Figure 1) [4-7,27]. Several critical pathways, including MAPK, NF-κB and AP-1, which are well known for their role in these processes and are activated by *H. pylori*, will not be reviewed here.

The PI3K/AKT/GSK3β pathway regulates various cellular functions, including cell growth, proliferation, differentiation and motility. Its aberrant activation is associated with various types of cancers, including gastric cancer. AKT is activated as a result of PI3K activity and regulates its downstream targets, including GSK3β, which has important functions in regulating cell proliferation, inflammation, metabolism and apoptosis. *H. pylori* activates the PI3K pathway, including AKT and GSK3β, in a *cagPAI*-dependent manner. OMP outer inflammatory protein A (OipA) also activates AKT and induces GSK3β phosphorylation; mutation of the *H. pylori cag*-PAI or *oipA* gene decreases the effects on both AKT and GSK3β [4]. In addition, Nagy *et al.* reported that *H. pylori* activates PI3K and AKT in a Src- and EGF receptor-dependent manner, which is mediated by a functional *cag*PAI and PGN delivery; mutation of *cagA* does not affect the activation of AKT by *H. pylori* [5]. Therefore, constant PI3K activation induced by *H. pylori* may contribute to cellular transformation and the development of gastric cancer.

Signal transducers and activators of transcription 3, a transcription factor and a member of the STAT protein family, regulates cell growth, apoptosis and differentiation. Several inflammatory cytokines and growth factors, such as IL-6, interferon and EGF, trigger STAT3 activation, which has been related to carcino genesis [7]. *H. pylori*-induced STAT3 activation depends on a functional *cag*PAI and CagA, but not CagA phosphorylation. Pre-incubation of cells with an IL-6 receptor antagonist or inhibition of gp130 prevented *H. pylori*-mediated STAT3 activation. In addition, wild-type *H. pylori* but not a *cagA*-negative mutant activates STAT3 in gastric epithelial cells in a *H. pylori* gerbil infection model [7]. These results provide an additional mechanism by which *H. pylori* promotes the development of gastric cancer. In addition, STAT3 overexpression is also associated with cancer stages and poor prognosis of gastric cancer [28].

Recently, a clear relationship has emerged on the oncogenic mechanisms of Wnt/β-catenin, cyclooxygenase 2 (COX-2)/prostaglandin E2 (PGE_2) and *H. pylori*-induced inflammation in gastric carcinogenesis. The Wnt/β-catenin pathway is implicated in multiple types of human malignancies, including gastric cancer [29], and overexpression of COX-2, which increases PGE_2 production, is critical in gastric tumorigenesis. *H. pylori* infection activates both pathways [6,30].

Oshima *et al.* reported that the simultaneous overexpression of Wnt1 and COX-2 induces dysplastic gastric tumors in transgenic mice [29]. This process involves sequential changes from metaplasia and dysplasia to carcinoma in mice gastric mucosa. *Wnt1* transgenic mice have increased β-catenin activity, suppressed epithelial differentiation and preneoplastic lesions, while the generation of tumors in these mice requires the cooperative activation of both Wnt and COX-2/PGE_2 pathways. Furthermore, Oguma *et al.* demonstrated that

macrophage-derived TNF-α promotes Wnt/β-catenin activation through the inhibition of GSK3b, which contributes to tumor development [31]. *Helicobacter felis* infection in *Wnt1* transgenic mice induced increased gastric mucosal macro-phage infltration, which is the source of TNF-α and causes epithelium nuclear β-catenin accumulation. Since *H. pylori* infection both induces TNF-α production [32] and activates the GSK3b pathway [4], these results provide an important link between these signaling events.

In addition, the role of β-catenin in gastric carcinogenesis has been reported [6]. *H. pylori* strain 7.13 induces gastric dysplasia and adeno-carcinoma in a gerbil model. It selectively activates β-catenin in gerbil gastric epithelia, which is dependent on the translocation of CagA into host epithelial cells. β-catenin nuclear accumulation is also increased in gastric epithelium from *H. pylori*-infected gerbils and from people carrying *cag*PAI strains [6].

To further explore the molecular mechanisms of *H. pylori* CagA in regulating β-catenin activity, Kurashima *et al.* found that the CagA EPIYA-repeat region is required for β-catenin membranous translocalization [33]. Sequential mutational ana lysis reveals residues 1009–1086 of ABCCC type of Western CagA and residues 908–1012 of ABD type of East-Asian CagA, a 16-amino-acid CagA multimerization sequence, is required for this effect [33]. CagA also physically interacts with E-cadherin and disrupts E-cadherin and β-catenin complex formation, which causes cytoplasmic and nuclear accumulation of β-catenin. This subsequently activates downstream genes such as *cdx1* and $p21^{(WAF1/Cip1)}$ and induces aberrant expression of the intestinal-differentiation marker, goblet-cell mucin (MUC2) [24]. Together, these results provide important insight into the mechanism of *H. pylori*, and in particular CagA, in the deregulation of the Wnt/β-catenin pathway and the promotion of gastric cancer.

The AID protein is a member of cytidine deaminase family that acts as a DNA- and RNA-editing enzyme. It was originally demonstrated to produce immune diversity in B cells by inducing somatic hypermutations and class-switch recombinations in human immunoglobulin genes [27]. The generation of somatic mutations in various host genes of non-lymphoid tissues contributes to tumorigenesis. Pathogenic factors, including *H. pylori* infection and proinflammatory cytokine stimulation, induce AID expression in epithelial cells. *H. pylori*-induced upregulation of AID depends on the *cag*PAI/NF-κB pathway. Activation of AID results in the accumulation of nucleotide alterations in the *TP53* tumor suppressor gene, which may be an important mechanism of mutation accumulation in the gastric mucosa during *H. pylori*-associated gastric carcinogenesis [27].

Helicobacter-induced gastric cancer in animal models

Animal models provide critical insights to the mechanisms of *H. pylori*-induced oncogenesis. In a transgenic mouse model, Ohnishi *et al.* demonstrated that expression of CagA in mice induced multiple malignancies, including gastric epithelial hyperplasia, hyperplastic polyps, gastrointestinal carcinomas and hematological malignancies, such as myeloid leukemia and B-cell lymphoma [34]. The mice did not show any signs of gastritis or systemic inflammation and the cancer was cell autonomous. Since CagA alone is able to induce cancer without any inflammation, the experiment identifies its critical role in tumorigenesis as an oncoprotein.

Mongolian gerbils infected with wild-type *H. pylori* strain 7.13 or its *cagA*, *vacA*, *oipA* mutants all developed gastritis; inflammation was generally attenuated in animals infected with *cagA* mutant but not *vacA*- or *oipA*-negative strains [35]. Gastric dysplasia and cancer developed in more than 50% of the gerbils infected with either wild-type or *vacA*-negative strain, but none in those infected with *cagA* mutant strains. Inactivation of *H. pylori oipA* gene decreased β-catenin nuclear localization and reduced cancer incidence in gerbils. Eradication of *H. pylori* decreasesd the incidence and severity of lesions with carcinogenic potential, but the effectiveness of this eradication to prevent malignancy was dependent on the timing of

intervention, resembling that seen in human clinical studies [36]. These results uncover a critical role of the major *H. pylori* virulence factors in the induction of gastric malignancies.

Using a primate oral carcinogen and *H. pylori* infection model (rhesus monkeys), Liu *et al.* found that the nitrosating carcinogen, ethyl-nitro-nitrosoguanidine and a virulent *H. pylori* strain (*cagA*, *vacA* and *babA* positive) together induced gastric cancer and neoplastic gene expression in non-neoplastic mucosa [37]. Transcriptional analysis of biopsies of infected animals revealed neoplasia-specific gene-expression profiles that were characterized by changes in multiple cancer-associated genes. The neoplastic profile was also evident in non-neoplastic mucosa, indicating that gene changes may represent early events preceding tumor formation and that consumption of carcinogen and *H. pylori* infection synergistically induces gastric neoplasia.

It has been known that chronic hypergastrinemia in insulin–gastrin transgenic mice synergizes with *Helicobacter* infection and contributes to eventual parietal cell loss and progression to gastric cancer [38]. Recently, this same group reported that stomach-specific expression of human IL-1β in transgenic mice lead to spontaneous gastric inflammation and cancer, which correlated with early recruitment of myeloid-derived suppressor cells to the stomach. Antagonism of IL-1 receptor inhibits the development of gastric pre-neoplasia and suppresses myeloid-derived suppressor cell mobilization [39]. Therefore, these results together suggest that, in addition to bacterial factors, host factors, including gastrin and inflammatory cytokines, participate in the gastric oncogenesis.

H. pylori-induced epigenetic changes: DNA methylation in gastric cancer

Epigenetic changes are generally categorized into four areas: DNA methylation, histone modification, chromatin remodeling and miRNAs [40]. Disruption of epigenetic mechanisms leads to abnormal development and malignant transformation. Both DNA methylation and histone modification have altered patterns of distribution in cancer cells; epigenetic alterations may occur at different stages of tumorogenesis and, thus, contribute to cancer development [40-42]. Connections between *H. pylori* infection, gastric cancer and epigenetic changes have been noted in just the past few years, and many unanswered questions remain to be explored, including the role of *H. pylori* virulence factors CagA, VacA, *cag*PAI and OMP, as well as their effects on stem cells. The results of future investigations will greatly expand our understanding in these areas.

DNA methylation is introduced by addition of a methyl group to the fifth carbon of a cytosine pyrimidine ring of DNA, which typically occurs in a CpG dinucleotide. CpG islands are genomic regions that contain a high frequency of CpG sites, usually near the 5′ transcription-start site of genes. In normal cells, approximately 80% of all CpGs are methylated. DNA of cancer cells is generally hypomethylated, while promoters of certain genes are hypermethylated, both of which are implicated in carcinogenesis. Promoter-specific increased methylation leads to silencing of the affected genes that may function as tumor suppressors and result in heritable transcriptional silence. Aging and chronic inflammation can induce methylation in CpG islands [40,41].

H. pylori infection induces aberrant methylation in a number of gene promoters in gastric mucosa, including cell growth-related genes *p16(INK4a)*, *p14(ARF)* and *APC;* DNA-repair genes, *hMLH1*, *BRCA1* and *MGMT*; the cell adherence gene E-cadherin; as well as *LOX*, *FLNC*, *HRASLS*, *HAND1*, *THBD* and *p41ARC*, which are known to be methylated in gastric cancer patients [43-46]. The gene methylations in *H. pylori*-infected individuals are increased and the methylations decrease after bacteria eradication, suggesting that the effects are induced by bacteria infection [43-46]. Gastric cancer tissue also shows higher levels of methylation

than noncancerous tissues [44], providing a mechanistic explanation towards *H. pylori*-induced carcinogenesis.

There are a number of genes that are methylated in gastric cancer, but their correlation with *H. pylori* infection has not been reported, including those listed in Table 1. Their functions range from regulation of apoptosis and cell growth to tumor suppression. Treatment with a methyltransferase inhibitor, 5-aza-deoxycytidine, reverses the methylation status and usually restores gene expression. Therefore, aberrant methylation is regarded as major event during the early stages of malignant transformation as well as in the progression of cancer, and higher levels of methylations are found more frequently in late stages of gastric cancer [47].

Histone modification in gastric cancers & its induction by *H. pylori*

Recent advances also underscore the importance of histone modifications in the pathogenesis of gastric cancer. Histones are the basic unit of the nucleosome, consisting of two copies of each of the core histones, H2A, H2B, H3 and H4 [41,42]. H3 and H4 histones have long tails protruding from nucleosome that can be covalently modified. This allows regulatory proteins to access DNA and regulate transcription. Modifications of histone tails include methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, citrullination and ADP-ribosylation. Histone modifications act in diverse biological processes, such as gene regulation, DNA repair and cell growth [42]. Disruption of certain histone modifications is associated with birth defects, age-related diseases and cancer [41,42].

The acetylation and deacetylation of key lysine residues of histone H3 and H4 are controlled by histone acetyltransferases and histone deacetylases (HDACs) [64]. Transcription becomes active when histones are acetylated, silenced when histones are deacetylated and silenced or activated when methylated by histone methyltransferases [41,42]. Both histone (H)3 lysine (K) 9 and H3K27 trimethylation (triMe) are associated with gene silencing and histone H3K27triMe causes gene silencing independent of promoter DNA methylation [65].

Global histone modification patterns are suggested to be an independent predictor for gastric cancer recurrence and survival. Park *et al.* evaluated the patterns of histone H3 and H4 acetylation and trimethylation in gastric cancer, including expression of acetylated H3K9, acetylated H4K16, H3K9triMe and H4K20triMe in gastric adenocarcinoma samples [66]. The results indicated that only H3K9triMe positively correlated with tumor stages, lymphovascular invasion and cancer recurrence. Higher levels of H3K9triMe expression correlated with a poor survival rate. Methylation dominance, which contains two trimethylated histone scores, is associated with lymphovascular invasion, cancer recurrence and poor survival rate.

Histone deacetylase overexpression results in histone hypoacetylation and is involved in multiple types of cancer, including gastric and breast cancers [67]. Weichert *et al.* investigated the expression status of class I HDAC isoforms 1, 2 and 3 in gastric cancer in a retrospective analysis [67]. They found that 32 of the 150 (21%) gastric tumors have nuclear expression of all three HDAC isoforms. General HDAC expression levels were higher when patients had lymph node metastases, and the 3-year survival rate decreased to 21% when HDAC1 was positive, 16% when HDAC2 was positive and 5% when all isoforms were positive. These data suggest that HDAC can be an independent prognostic marker for gastric cancer and an indication of the potential of HDAC inhibitors as therapeutics [67].

In certain cancer patients, overexpression of phosphorylated histone H3 Ser 10 (H3S10) has been reported to be an indicator of poor prognosis for gastric cancer. Takahashi *et al.* studied H3S10 expression and its relation to cancer progression [68]. In 30 out of 122 cancer cases, H3S10 showed overexpression (24.6%), while in the rest of the cases, the expression was low

(75.4%). The cancer patients that showed overexpression of phosphorylated histone H3 had a poorer prognosis than those with low expression.

In addition to the general histone protein and HDAC expression, certain histone modifications are associated with specific gene promoters in gastric cancer and participate in the regulation of gene transcription. For example, DNA hypermethylation and histone hypoacetylation of the *HLTF* gene are linked to its reduced expression in gastric cancer [53]. *HLTF* is a homolog to the *SWI*/*SNF* genes, which encode chromatin-remodeling enzymes and serves as a tumor suppressor. In tumor cell lines, acetylation levels of histones H3 and H4 in the 5′ CpG islands of *HLTF* gene are inversely associated with DNA methylation status. Treatment with both DNA methytransferase and HDAC inhibitors, aza-2′-deoxycytidine and trichostatin A, restore *HLTF* mRNA expression, suggesting both mechanisms contribute to the loss of gene expression.

Histone H3 in the $p21^{(WAF1/CIP1)}$ promoter is hypoacetylated in gastric cancer [69]; this hypoacetylation is associated with reduced $p21^{(WAF1/CIP1)}$ expression in gastric cancer specimens. Treatment of gastric carcinoma cell lines with HDAC inhibitor, trichostatin A, increases the acetylation level and restores $p21^{(WAF1/CIP1)}$ expression. Aberrant DNA methylation and histone deacetylation are also linked to the silencing of the *SLC5A8* gene in gastric cancer [51]. *SLC5A8* is a sodium co-transporter, solute carrier family 5 member 8 gene and a putative tumor suppressor. Aberrant methylation of *SLC5A8* gene is detected in both cell lines and in primary gastric cancers and acetylation of histone H3 correlates directly with *SLC5A8* expression and inversely with DNA methylation.

The aforementioned results demonstrate that histone modification and DNA CpG island methylation are important epigenetic events in gastric cancer. Reduced histone acetylation correlates with the extent of tumor invasion and nodal metastasis of gastrointestinal cancers [70]. It has also been suggested that dysregulated epigenetic modifications, especially in early neoplastic development, may be just as significant as genetic mutations in driving cancer development and growth [40]. However, the role of *H. pylor*i infection status during these processes is not clear, although it has been proven to be a crucial factor in triggering inflammation and cancer. We anticipate that future studies will advance our understanding in this important field.

In fact, relatively little information is available about the effects of *H. pylori* infection on histone modifications (Figure 2). As expected, specific histone modification in host cells is cell type and promoter specific. In mouse macrophages, *H. pylori* peptidyl prolyl *cis*-, *trans*-isomerase (HP0175) induces H3S10 phosphorylation at the *IL-6* promoter, and this is associated with increased IL-6 mRNA and protein expression [71]. Direct exposure of *H. pylori* to gastric epithelial cells causes upregulation of $p21^{WAF1}$ protein expression in both the gastric epithelial cell line NCI-N87 and in primary gastric cells. The increased $p21^{WAF1}$ expression is associated with increased HDAC1 recruitment from the $p21^{WAF1}$ promoter and hyperacetylation of histone H4 [72].

Our recent work [73], as well as the work by *Fehri et al.* [74], demonstrate that *H. pylori cag*PAI-dependently induces dephosphorylation of histone H3S10, H3 threonine 3 and deacetylation of H3K23 in gastric epithelial cells, but does not affect nine other distinct histone modifications. *H. pylori*-induced H3S10 dephosphorylation is associated with changes in host gene expression, including upregulation of the oncogene *c-Jun* and downregulation of *hsp70*. These results indicate novel mechanisms in *H. pylori* pathogenesis via histone modification. Future studies are required to clarify the correlation of *H. pylori*-induced histone modification in gastric cancer.

The origin of gastric cancer cells & future directions

Epithelial cells that line the gastric mucosa are formed by four major cell types: pit, parietal, neck and zymogenic cells, as well as their progenitor cells. Studies of the transcriptome of gastric epithelial cells from the stomach of *H. pylori*-infected mice demonstrate that gastric pit cells or mucus-producing cells are the major targets of *H. pylori* [75]. However, because these differentiated pit cells are regenerated so quickly, they may not carry genetic or epigenetic lesions for very long before they are replaced. Therefore, the cells located in the isthmus, the multipotent stem cells that are responsible for generating the four major cell types, may have the potential to carry molecular damage to the next generation of cells [76].

It has been reported that gastric cancers can originate from bone marrow-derived cells that repopulate the gastric mucosa, and *H. felis* infection induces gastric cancer in these cells [77]. Studies in a gnotobiotic mouse model of chronic atrophic gastritis have shown that loss of parietal cells results in the amplification of multi- and oligo-potential gastric stem cells that express sialylated glycan receptors, which are recognized by *H. pylori* adhesions, and *H. pylori* interacts with and resides within a subset of these progenitor cells [78].

Since sporadic gastric cancer has increased risk with age, one hypothesis is that stem or progenitor cells accumulate enough molecular lesions to evade homeostatic control, resulting in cancer. The damages that originate from genetic and/or epigenetic processes are progressively accumulated during aging and directly contribute to cell transformation [79]. *H. pylori* has been shown to invade epithelial cells, intracellularly, inter-cellularly and interstitially [80]. It is believed that *H. pylori* also interacts with gastric progenitor cells, therefore introducing the molecular damage to these cells. These results support the previous hypotheses that the mechanisms of carcinogenesis are based on atrophy (loss of differentiated cells) followed by redifferentiation of stem cells, which may be damaged or abnormal, therefore causing cancer [81].

Virtually all types of cancers require several critical steps during tumorigenesis. These include: self-sufficiency in growth signals, insensitivity to growth inhibition, evasion of apoptosis, immortalization, sustained angiogenesis and tissue invasion and metastasis [82]. *H. pylori* infection is able to activate several oncogenic pathways, as previously described, to fulfill these requirements. Since oncogenic transformation does not require activation of all the oncogenic pathways, nor are these pathways required for all stages of cancer development and progression, deregulation of a few relevant pathways may initiate transformation, while activation of other genes may contribute to progression or metastasis. As *H. pylori* has been observed to invade gastric progenitor cells [78], two interesting questions to answer in the future will be: does *H. pylori* induce oncogenic, genetic and epigenetic changes in progenitor cells, and how does this effect their differentiation? Furthermore, how are these alterations introduced into daughter cells, therefore accumulating toward oncogenesis? Future studies addressing these questions will be of great interests to expand our understanding of gastric cancer.

In summary, *H. pylori* infection and chronic inflammation linked to cancer is probably owing to the activation of multiple oncogenic pathways and facilitating tumorigenesis. In this context, inflammatory stress, activation of oncogenic proteins, epigenetic mechanisms, local micro-environment and host genetic susceptibility together determine and promote the tumorigenesis and cancer progression. Interactions of *H. pylori* with gastric stem cells or progenitor cells may, therefore, provide important clues to uncover the mechanism of carcinogenesis and, thus, are the targets of extensive investigations. Continued study of *H. pylori*-induced molecular pathogenesis will be critical to understand the basis and origin of gastric cancer and will also provide us with options for future prevention and intervention in combating this deadly disease.

Future perspective

Although a great deal of progress has been made over the past two decades in understanding the pathogenesis and carcinogenesis of *H. pylori*-induced gastric cancer, the detailed mechanisms still remain elusive. It is generally accepted that chronic inflammation induced by *H. pylori* is important for the promotion of oncogenesis. This general inflammatory micro-environment, which includes increased inflammatory cell infiltration, reactive oxygen species levels, inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, growth factors and hormones, such as gastrin, impacts on the epithelial cell and facilitates oncogenic transformation. It is important to consider the interaction of these factors and their net effects on host cells in future investigations, especially how these environmental factors might affect the local stem cells or progenitor cells and their differentiation.

Inside the host cell, various oncogenic pathways are activated by *H. pylori* or by its virulence factors, especially CagA and *cag*PAI. Deregulation of these pathways and disruption of normal cell–cell contact confer the epithelial cell oncogenic transformation potential. Therefore, study of the pathogen–host interaction from a platform of stomach stem cells or progenitor cells will greatly extend our understanding about the patho physiology of chronic inflammation and oncogenesis. This will be helpful to understand not only the *H. pylori*-induced inflammation, but also the pathogenesis of other chronic inflammatory diseases, such as chronic hepatitis and inflammatory bowel disease as well.

Epigenetic alternation, including DNA methylation and histone modification, during chronic inflammation or *H. pylori* infection represent another layer of gene transcription control; however, relatively little information is currently available, and we anticipate future studies addressing these important issues will uncover their effects in pathogenesis and carcinogenesis and, more importantly, provide possible options for prevention and intervention.

Executive summary

■ Chronic infection with *Helicobater pylori* in the human stomach lasts decades and causes persistent inflammation. Most>*H. pylori*-infected patients have no clinical symptoms. Approximately 10–20% of *H. pylori*-infected patients will develop peptic ulcers and 1% will develop gastric cancer.

■ Upon infection, *H. pylori* activates multiple oncogenic pathways, such as activator protein-1, NF-kB, Wnt/β-catenin, signal transducers and activators of transcription 3, PI3K and cyclooxygenase-2, all of which have been shown to contribute to the oncogenic transformation processes.

■ Bacterial virulence factors, such as cytotoxin-associated antigen A and *cag* pathogenecity island, are critical for causing inflammation and the activation of oncogenic pathways. In addition, cytotoxin-associated antigen A protein also disrupts the epithelial cell normal contact and cell polarity, which confer the epithelial cell oncogenic potential.

■ *H. pylori*-induced epigenetic alternation, including DNA methylation and histone modification, as well as its interaction with gastric stem cells or progenitor cells are important topics that will greatly enhance our understanding of the bacteria-induced carcinogenesis; therefore, they are currently the target of extensive investigation.

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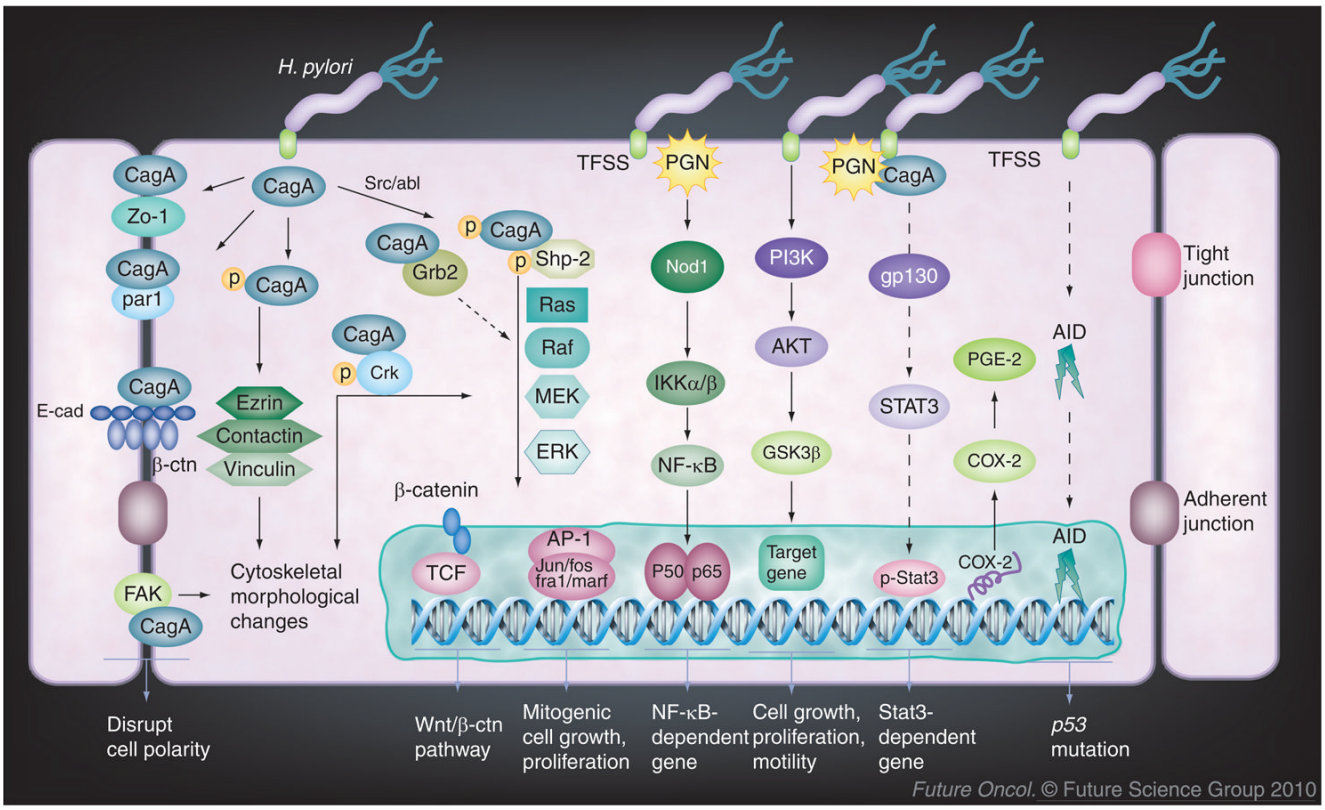

Figure 1. *Helicobacter pylori*-induced host cell response and oncogenic signaling in gastric epithelial cells

*H. pylori cag*PAI-positive strains, which possess TFSS, translocate the bacterial effectors CagA and PGN into the host cells; these effector molecules activate multiple epithelial cell signaling pathways and confer oncogenic potential to the epithelial cell. Once inside the cells, CagA is tyrosine phosphorylated by Src or Abl kinases and targets host proteins to induce the cell responses in a phosphorylation-dependent or -independent manner. The effects range from activation of oncogenic pathways to cytoskeletal rearrangements. PGN is also injected by TFSS and activates NF-κB, STAT3 and PI3K pathways. Persistent deregulation of these pathways provides an important molecular mechanism toward the *H. pylori*-induced carcinogenesis. AP: Activator protein; CagA: Cytotoxin-associated antigen A; COX: Cyclooxygenase; PGN: Peptidoglycan; STAT: Signal transducers and activators of transcription; TFSS: Type IV secretion system.

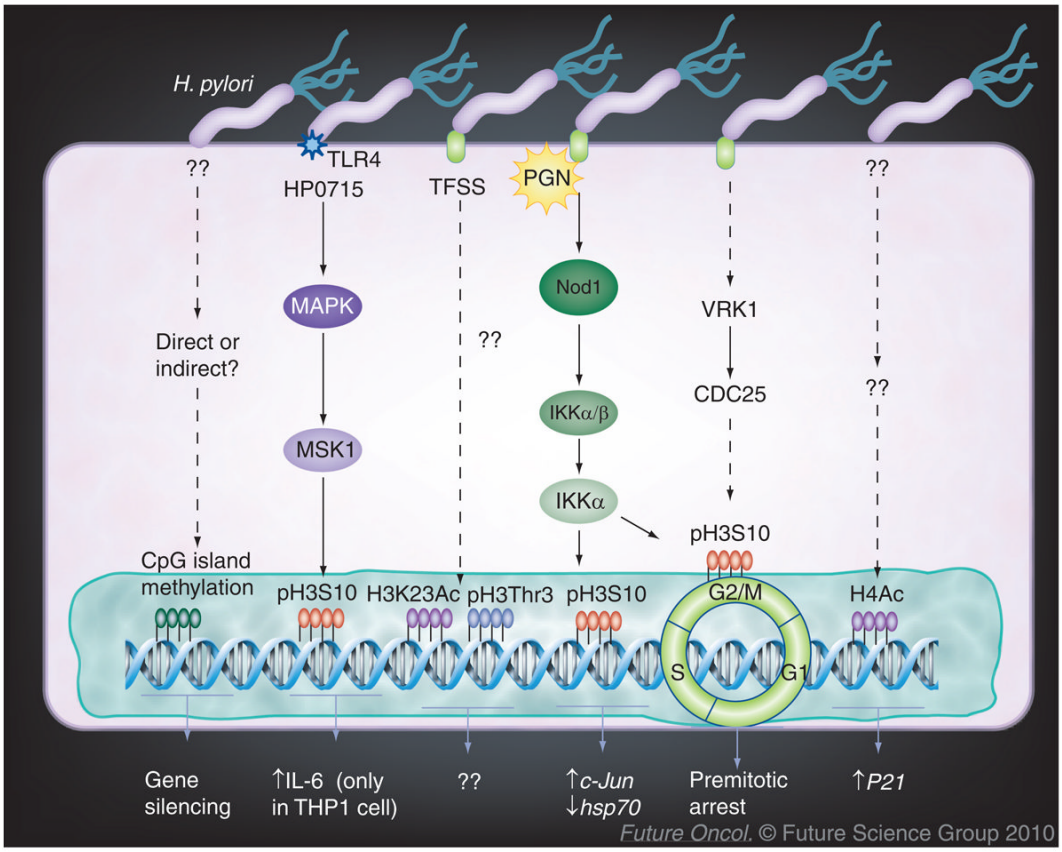

Figure 2. *Helicobacter pylori*-induced epigenetic changes

Several studies have investigated the effects of *H. pylori* on histone modifications. Infection with *H. pylori* causes acetylation of H4, and this is associated with *p21* gene expression in gastric epithelial cells. *H. pylori* also induces *cag* pathogenicity island-dependent pH3S10, which is associated with *c-Jun* upregulation and *hsp70* downregulation and cell cycle premitotic arrest. However, the function of H3Thr3 and H3K23, and whether *H. pylori* directly induces DNA methylation in gastric epithelial cells, remain to be studied.

Ac: Acetylation; H3K23: Histone 3 lysine 23; pH3S10: Phosphorylation of histone 3 serine 10; PGN: Peptidoglycan; pH3Thr3: Phosphorylation of histone 3 threonine 3; TLR: Toll-like receptor; TFSS: Type IV secretion system.

Table 1

Genes that are methylated in gastric cancer and their function.

Gene name (abbreviation)	Protein function	Ref.
BCL2/adenovirus E1B-interacting protein 3 (*BNIP3*)	Proapoptotic Member of Bcl-2 family	[48]
Harakiri, BCL2-interacting protein (*HRK*)	Proapoptotic	[49]
Death-associated protein (*DAP*)	Serine/threonine kinase Proapoptotic	[50]
Solute carrier family 5 (iodide transporter), member 8 (*SLC5A8*)	Sodium cotransporter tumor suppressor	[51]
Inhibitor of DNA binding 4 (*ID4*)	Transcriptional regulator	[52]
Helicase-like transcription factor (*HLTF*)	Tumor suppressor	[53]
Retinoblastoma-interacting zinc-finger protein 1 (*RIZ1*)	Zinc finger protein	[54]
Runt-related transcription factor 3 (*RUNX3*)	Tumor suppressor	[55]
Deleted in liver cancer 1 (*DLC1*)	Tumor suppressor	[56]
Retinoic acid receptor β (*RARB*)	Retinoid signaling	[57]
TSPY-like 5 (*TSPYL5*)	Cell growth	[58]
Ras association (RalGDS/AF-6) domain family member 1 (*RASSF1A*)	Putative tumor suppressor	[59]
XIAP-associated factor 1 (*XAF1*)	Putative tumor suppressor	[60]
ADAM metallopeptidase domain 23 (*ADAM23*)	Putative tumor suppressor	[61]
Lysyl oxidase (*LOX*)	Tumor suppressor	[62]
Checkpoint with fork head-associated (*FHA*) and ring finger (*CHFR*)	Mitotic checkpoint protein	[63]