Molecular Pathways: Involvement of *Helicobacter pylori*–Triggered Inflammation in the Formation of an Epigenetic Field Defect, and Its Usefulness as Cancer Risk and Exposure Markers

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**Abstract**

Infection-associated cancers account for a large proportion of human cancers, and gastric cancer, the vast majority of which is associated with *Helicobacter pylori* infection, is a typical example of such cancers. Epigenetic alterations are known to occur frequently in gastric cancers, and *H. pylori* infection has now been shown to induce aberrant DNA methylation in gastric mucosae. Accumulation of aberrant methylation in gastric mucosae produces a field for cancerization, and methylation levels correlate with gastric cancer risk. *H. pylori* infection induces methylation of specific genes, and such specificity is determined by the epigenetic status in normal cells, including the presence of H3K27me3 and RNA polymerase II (active or stalled). Specific types of inflammation, such as that induced by *H. pylori* infection, are important for methylation induction, and infiltration of monocytes appears to be involved. The presence of an epigenetic field defect is not limited to gastric cancers and is observed in various types of cancers. It provides translational opportunities for cancer risk diagnosis incorporating life history, assessment of past exposure to carcinogenic factors, and cancer prevention.

**Background**

Infection-associated cancers account for a large proportion of human cancers. These include gastric cancers induced by *Helicobacter pylori* (1), hepatocellular carcinomas induced by hepatitis C virus (HCV) and hepatitis B virus [HBV (2–4)], cervical cancers induced by human papilloma virus [HPV (5, 6)], and lymphomas and nasopharyngeal cancers associated with Epstein-Barr virus [EBV (7, 8)]. The carcinogenic mechanisms of these infection-associated cancers have been extensively investigated, and although multiple contributing mechanisms have been clarified, they are not yet completely understood.

**General mechanisms of infection-associated cancers**

Virus-associated cancers have complex mechanisms of carcinogenesis. Viral oncoproteins, such as E6 and E7 of HPV and X protein of HBV, can be integrated into host cells and produce aberrant growth signals and inactivate tumor-suppressor genes (6). Also, integration of virus genes into the host genome can alter the expression of nearby tumor-related genes and induce a genomic instability that will eventually contribute to cancer development (4). Even if the virus genes are not integrated, they can be persistently expressed and perturb important cellular signaling, such as cell proliferation, apoptosis, and cytokine expression, as in the case of HCV and EBV (7).

Both bacterial and viral infections can be associated with severe tissue damage and resultant chronic inflammation (4, 6, 7). Tissue damage itself activates cell proliferation and increases the chance that mutations will occur. In addition, chronic inflammation is considered to be deeply involved in cancer development and progression by multiple mechanisms, such as increased production of active oxygen species, induction of inflammation-mediated cell proliferation, and increased cytokine production (9, 10). In addition to this, induction of epigenetic alterations is now recognized as one of the mechanisms underlying induction of cancer by chronic inflammation.

**H. pylori infection and epigenetic alterations in gastric cancers**

Gastric cancer is still the third-leading cause of death from cancer in men and the fifth-leading cause in women worldwide, although its incidence is gradually decreasing (11). The vast majority of gastric cancers are caused by *H. pylori* infection (12), which is a Gram-negative bacterium (13). A minor percentage (~10%) of gastric cancers are associated with EBV infection (14). It is known that when *H. pylori* infects the human stomach, it induces severe inflammation, including ulcers, then chronic inflammation, and finally gastric cancers within tens of years. Investigators have mainly discussed the carcinogenic mechanisms of *H. pylori* from...
the standpoint of induction of cell proliferation, mutations, and direct activation of cellular signaling (15–17).

However, tumor-suppressor genes such as CDKN2A, CDH1, MLH1, and RUNX3 are inactivated more frequently by aberrant DNA methylation than by mutations, indicating that gastric cancer is an epigenetic disease (18). In addition to methylation silencing of driver tumor-suppressor genes, recent genome-wide analyses have revealed that hundreds of passenger genes are also methylated in gastric cancers (19). The fact that H. pylori infection induces epigenetic alterations provides the missing link between the causal role of H. pylori infection in gastric carcinogenesis and the deep involvement of epigenetic alterations in gastric cancers. Gastric cancer is a typical example of a disease in which infection, chronic inflammation, and epigenetic alterations are interconnected.

**Induction of epigenetic alterations by H. pylori and the formation of field defects**

The first hint that the presence of aberrant DNA methylation might be associated with H. pylori infection came from the observation that promoter methylation of CDH1 was detected more frequently in the gastric mucosae of individuals with H. pylori infection than in those without the infection (20). By quantifying the methylation levels of 8 marker CpG islands, Maekita and colleagues (21) convincingly showed that individuals with H. pylori infection have much higher methylation levels in their gastric mucosae (5.4- to 303-fold) than those without (P < 0.0001). In addition to the 8 marker CpG islands associated with protein-coding genes, CpG islands of microRNA genes are also methylated in association with H. pylori infection (22, 23).

In one study, patients with gastric cancer who had previously had an H. pylori infection but were currently not infected had lower methylation levels of the 8 marker CpG islands in the gastric mucosae compared with patients who were currently infected with H. pylori (21). This suggests that the methylation level is very high when active H. pylori infection is present in the stomach and decreases to certain levels when the infection is discontinued. In other studies, various degrees of decrease were observed in individuals who received eradication therapy for H. pylori (24, 25), and the methylation level after the decrease was considered to represent the degree of epigenomic damage to the individual. This decrease of methylation could be due to a turnover of gastric epithelial cells with methylation or to the removal of 5-methylcytosine, which is present in individuals with active H. pylori infection.

Of importance, among individuals without current H. pylori infection, the methylation levels of the 8 marker CpG islands in gastric mucosae were shown to correlate with gastric cancer risk (21, 26). Patients with gastric cancers had 2.2- to 32-fold higher methylation levels in gastric mucosae compared with healthy individuals (21), and patients with multiple gastric cancers had significantly higher methylation levels than those with a single gastric cancer (26). This correlation strongly supports the notion that the accumulation of aberrant methylation in gastric mucosae produces an epigenetic field for cancerization, i.e., a field defect (Fig. 1; ref. 27).

**Epigenetic field for cancerization**

In the epigenetic field for gastric cancers, tumor-suppressor genes that are causally involved in gastric cancer development (i.e., driver genes), such as CDKN2A, CDH1, MLH1, and RUNX3, are methylated only at very low levels, showing that such events are present only in a very small fraction of cells (21). In contrast, many other genes that are unlikely to be causally involved in gastric carcinogenesis (i.e., passenger genes), such as HAND1 (a transcription factor involved in heart morphogenesis), are methylated at high levels, showing that their methylation is present in a large fraction of cells. Most of the genes that are highly methylated in gastric cancers are either unexpressed or expressed only at low levels in normal cells (28). Generally, genes with low expression are susceptible to methylation induction (29), and it is considered that most of the genes that are methylated in the epigenetic field were methylated as a consequence of gastric carcinogenesis. In addition to accumulation of aberrant methylation, an epigenetic field involves hypomethylation of the Alu and Sat2 repeat sequences (30), which potentially can be involved in genomic instability.

Epigenetic field defects are present not only in gastric cancers but in other cancers as well (27). In the case of hepatocellular carcinoma, aberrant DNA methylation was frequently observed in noncancerous tissues of cancer patients compared with normal livers of patients with metastatic liver tumors (31). A quantitative analysis revealed increased methylation of multiple tumor-suppressor genes, such as SOCS1, RASSF1A, and CDH1, in HCV-infected, noncancerous liver tissues (32), suggesting the importance of an epigenetic field for HCV-associated hepatocarcinogenesis. In the case of esophageal adenocarcinoma, the presence of APC and CDKN2A methylation in Barrett’s metaplasia has been reported (33), and such methylation was shown to be associated with progression of Barrett’s metaplasia (34). Also in the case of esophageal squamous cell carcinoma, methylation of specific genes, such as UCHL1 and HOXA9, in esophageal mucosae was associated with the risk of developing esophageal squamous cell carcinoma (35, 36). In ulcerative colitis, the driver gene CDKN2A and passenger genes such as MYOD and ESR were shown to be methylated in colonic mucosae, which are predisposed to colon cancers (37, 38). In addition, in the case of sporadic colorectal cancers, MGMT methylation in cancer tissues was associated with high levels of MGMT methylation in the background colonic mucosae (39). The presence of epigenetic field defects has also been indicated for breast (40), renal (41), and bladder cancers (42, 43).

**Critical roles of specific types of inflammation in methylation induction**

The association between H. pylori infection and high levels of DNA methylation in gastric mucosae in humans strongly indicates that H. pylori infection induces aberrant
DNA methylation. This cause-consequence relationship was shown with the use of Mongolian gerbils, in which *H. pylori* infection-induced gastritis and gastric cancers can be recapitulated (44). Gerbils infected with *H. pylori* developed severe gastritis and had markedly increased methylation levels, showing the causal role of *H. pylori* infection in methylation induction (45). The methylation levels were clearly decreased after eradication of the *H. pylori*, in agreement with the decreased methylation levels observed in patients who received eradication therapy.

In the attempt to determine how *H. pylori* induces methylation, investigators have considered both direct and indirect actions of *H. pylori*. First, because *H. pylori* possesses multiple DNA methyltransferases (46) and a type IV secretion system [a syringe-like structure capable of delivering bacterial materials into a host cell (47)], *H. pylori* itself may...
induce methylation in epithelial cells by injecting its own DNA methyltransferases. Alternatively, studies in patients with ulcerative colitis showed that chronic inflammation played a role in methylation induction (37, 38), and chronic inflammation triggered by \( H. \) pylori infection may have been responsible for the methylation induction. Niwa and colleagues (45) addressed this issue by suppressing inflammation in gerbils with \( H. \) pylori infection using cyclosporin A, an immunosuppressant. Although colonization of \( H. \) pylori was not affected at all, methylation induction was markedly suppressed. This clearly shows that it was the inflammation triggered by the \( H. \) pylori infection, not the \( H. \) pylori itself, that was involved in methylation induction. A temporal analysis of the expression of inflammation-related genes in gastric mucosae of infected gerbils showed that the expression levels of \( C x c l 2, I l 1 b, N o s 2, \) and \( T n f \) paralleled the methylation levels.

Inflammation in the stomach can be induced not only by \( H. \) pylori infection but also by high concentrations of ethanol (EtOH) or saturated sodium chloride (NaCl) solution. A methylation analysis of gastric mucosae exposed to these kinds of inflammation showed that only inflammation triggered by \( H. \) pylori infection was capable of inducing aberrant DNA methylation (48). Histologically, \( H. \) pylori infection induced chronic inflammation with prominent lymphocyte and macrophage infiltration, whereas EtOH and NaCl treatment induced persistent neutrophil infiltration. Cell proliferation, which is known to be important for methylation induction (38), was most strongly induced in the NaCl group and was shown to be insufficient for methylation induction. Among inflammation-related genes, expression of \( I l 1 b, N o s 2, \) and \( T n f \) was increased specifically in gastric mucosae of gerbils with \( H. \) pylori infection. Therefore, it is considered that specific types of inflammation are necessary for methylation induction.

Chronic inflammation is characterized by infiltration of mononuclear cells, i.e., lymphocytes and monocytes. To clarify which cell type(s) plays the major role in methylation induction, Katsurano and colleagues (49) examined SCID mice, which lack both B and T lymphocytes. Because \( H. \) pylori cannot infect mice efficiently, they used a colitis model induced by dextran sulfate sodium (DSS). Even in SCID mice, DNA methylation and colon tumors could be induced at the same levels as in wild-type mice. This shows that lymphocytes are dispensable for methylation induction, and strongly suggests that monocytes are important. Expression of \( I f n g, I l 1 b, \) and \( N o s 2 \) was induced in both wild-type and SCID mice by DSS treatment.

If we hypothesize that the same effectors are working in gerbil stomachs infected by \( H. \) pylori and mouse colons treated with DSS, we can conclude that expression of \( I l 1 b \) and \( N o s 2 \) may be involved in methylation induction. Promoter polymorphisms of \( I l 1 b \) are reported to be associated with human gastric cancer susceptibility by increasing or decreasing \( I l 1 b \) production in response to \( H. \) pylori infection and thus the progression of gastric atrophy (50, 51). Increased production of NO in vitro is reported to increase the enzyme activity of DNA methyltransferases without changing their expression, and to induce DNA methylation of specific genes (52). In the human and gerbil stomachs infected by \( H. \) pylori and mouse colons treated with DSS, no changes in the expression of DNA methyltransferases 1, 3a, and 3b were observed (28, 45, 49). It is possible that a signal from chronic inflammation, possibly IL1\( \beta \), and elevation of NO in epithelial cells lead to inappropriate localization of deregulated DNA methyltransferase(s) to methylation-susceptible CpG islands (see below) and induce aberrant DNA methylation as an infrequent event.

\textbf{Methylation fingerprints produced by \( H. \) pylori infection}

Target genes for methylation induction by \( H. \) pylori infection are present in gastric mucosae (28). Among 48 promoter CpG islands whose methylation was analyzed in gastric mucosae of individuals with and without \( H. \) pylori infection, some were consistently methylated in individuals with current or past infection and others were not methylated at all. Analysis of polyclonal tissues, unlike that of cancers, can provide information about multiple events that have taken place independently, and the presence of target genes was convincingly shown in gastric mucosae (29). Similarly, in the esophagus, specific genes were methylated in association with smoking history (35), and again the presence of target gene specificity for methylation induction was shown.

The target gene specificity is defined by epigenetic factors in the cells where methylation is induced (29, 53, 54) and in the genome architecture (55, 56). Epigenetic factors that promote methylation induction include low transcription and the presence of an H3K27me3 modification. In contrast, the presence of histone acetylation and RNA polymerase II (active or stalled) protects CpG islands from becoming methylated. A multivariate analysis revealed that the most influential factors are the promoting effect of H3K27me3 and the protective effect of RNA polymerase II (54). A genomic factor that promotes methylation induction is a distant location from repetitive elements (55, 56). It is currently speculated that infection by \( H. \) pylori induces H3K27me3 and removes RNA polymerase II at its target genes, and that these genes then become methylated.

\textbf{Clinical-Translational Advances}

\textbf{Cancer risk marker that reflects life history}

The importance of predicting cancer risks has been repeatedly emphasized because the ability to select high-risk individuals enables efficient cancer screening and reduces social costs (57–59). To this end, a massive effort has been made in association studies, and many cancer risk alleles for common cancers have been identified. Most of these risk alleles give odds ratios between 1.5 and 2.0 (51, 58, 59), and can be used to estimate cancer risk when a person is born.

At the same time, a person is exposed to various environmental carcinogenic factors, and the cancer risk of an adult will differ depending on what sort of life he or she has.
spent. Therefore, a cancer risk marker that incorporates information about life-to-date is important. At least some of a person’s life history, such as smoking and infection by *H. pylori*, is imprinted on the epigenome and produces an epigenetic field for cancerization. The severity of the field can be measured as methylation levels of specific marker genes, and correlates with cancer risk. The odds ratios obtained by DNA methylation markers of gastric cancers, such as *THBD, FLNC*, and *miR-124a*, range from 2.4 to 22.1 [calculated based on our previous reports (21, 22)]. Passenger genes can be useful as marker genes because they are consistently methylated and have high methylation levels in noncancerous tissues (21, 27), whereas driver genes are only stochastically methylated and have low methylation levels (Fig. 1). Therefore, for evaluating the degree of genomic damage that has been done in the past, methylation of passenger genes is often superior to that of driver genes.

The presence of an epigenetic field defect is also known for other types of cancers, as mentioned above (27). Therefore, investigators are now developing methods to estimate epigenetic cancer risk, taking life history into account, in various types of cancer. For example, a multicenter study was conducted to evaluate the validity of methylation markers to predict progression of Barrett’s esophagus, and methylation of *HPP1, CDKN2A*, and *RUNX3* were shown to be informative (34).

**Marker for past exposure to specific environmental factors**

*H. pylori* infection is associated with methylation of a specific set of genes, most of which are considered as passengers, in gastric mucosae (28). A history of smoking is associated with methylation of *UCHL1* and *HOXA9*, which are also considered to be passengers, in esophageal mucosae (35, 36). Once the specificity of methylation signatures to various carcinogenic agents is clarified, past exposure to such carcinogenic factors can be estimated by the methylation signature. The methylation signature has advantages over other exposure markers because it persists for a long time and does not require any record by humans. For example, past exposure to *H. pylori* infection can be estimated by serum antibody, but it persists only up to several years after *H. pylori* infection discontinues (60). The ability to estimate past exposure using a methylation signature would be very helpful from an epidemiological viewpoint.

**Epigenetic cancer prevention**

The presence of an epigenetic field for cancerization and the deep involvement of chronic inflammation in its formation provide targets for cancer prevention. Suppression of induction of aberrant DNA methylation is expected to lead to a decreased incidence of cancers. This concept is supported by animal models for macroscopic colon tumors (61, 62), lung tumors (63), and prostate cancers (64, 65). It was shown that in gerbil stomachs, administration of a demethylating agent, 5-aza-2’-deoxycytidine (5-aza-dC), decreased the incidence of gastric cancers induced by *H. pylori* infection and a mutagen, *N*-methyl-*N*-nitrosourea (unpublished). In addition to suppression of DNA methylation induction, suppression of H3K27me3, a premarker for DNA methylation induction, is also an attractive target. The histone methyltransferase that is responsible for this modification, EZH2, is known to be overexpressed in aggressive tumors (66) and precancerous lesions (67), and therefore inhibitors of EZH2, such as 3-deazaneplanocin A (66), may have preventive applications.

Anti-inflammatory drugs, especially nonsteroidal anti-inflammatory drugs (NSAIDs), are effective for prevention of at least some cancers, but their use is still limited to individuals with high risk (68). The use of NSAIDs is limited in part because of possible side-effects, such as peptic ulcer. To avoid such side-effects and suppress the pathways that are responsible for cancer development, researchers are actively investigating the mechanisms of cancer induction by chronic inflammation (69). Because induction of epigenetic alterations is one of these important mechanisms, suppression of specific components of inflammation that are responsible for induction of epigenetic alterations is expected to provide a good target for cancer prevention.

Lastly, DNA methylation is reversible by DNA demethylating agents, such as 5-aza-dC and 5-azacytidine (70). Currently available demethylating agents do not have a high specificity for aberrantly methylated genes, and can demethylate normally methylated sequences. Such sequences include normally methylated CpG islands and repetitive sequences originating from retrotransposons, and it is feared that DNA demethylating agents might induce demethylation of these retrotransposons. Therefore, for cancer prevention using current demethylating agents, we must carefully balance risk and benefit, and probably such agents are not widely indicated. However, many epigenetic drugs are being developed, and it is possible that some of the new demethylating agents will have a specificity or preference for aberrantly methylated promoter CpG islands, and can be used in a wider range of individuals in the future.

**Conclusions**

The fact that *H. pylori* infection induces aberrant DNA methylation in gastric mucosae provides the missing link between the major role of *H. pylori* infection in gastric cancers and the deep involvement of epigenetic alterations in gastric cancers. The severity of infection correlates with gastric cancer risk and can provide a unique cancer risk marker that reflects a person’s life. *H. pylori* infection has been shown to induce methylation of specific genes, and there are underlying mechanisms. The methylation signature has potential as a marker for past exposure to *H. pylori* infection. Specific types of inflammation, such as that induced by *H. pylori* infection, are capable of inducing aberrant methylation, and monocytes appear to be involved in the induction. Suppression of methylation induction,
specific inflammatory processes, and reversal of epigenetic alterations are targets for cancer prevention.

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References


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