**Helicobacter pylori** Infection and Gastric Cancer

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**Abstract**

Twenty-three years have passed since the first isolation and cultivation of *H. pylori*. However, such spiral microorganisms had already been discovered in the 1890s. In Japan, 85 years ago, Rokuzo Kobayashi et al. discovered that a spirochete-like organism (*Helicobacter felis*) colonized the stomachs of dogs and cats. It was not until 70 years later that scientists again began attempting to treat *Helicobacter spp.*-associated gastroduodenal diseases. *H. pylori* is now believed to be one of the major pathogenetic factors in the development of gastroduodenal diseases. The inflammation associated with *H. pylori* damages the epithelial cells, which probably links this infection to preneoplastic lesions such as gastric atrophy or intestinal metaplasia. Recently, a large-scale randomized controlled study revealed the potent link between *H. pylori* infection and gastric cancer development. However, the molecular pathogenesis of *H. pylori*-associated carcinogenesis has been unknown. According to a recent investigation, sonic hedgehog (Shh), morphogen, is also expressed in normal adult gastric fundic mucosa, and this expression disappears in the fundic glandular cells of the *H. pylori*-colonized stomach, demonstrating for the first time that *H. pylori* infection leads to deregulation of the expression of a morphogen and that its infection could be linked to the misregulation of the gastric regenerative pathway. Research to seek the relation between *H. pylori* and gastric cancer development is consistent with the recent trend in molecular regenerative medicine.

**Key words** *Helicobacter pylori*, Gastric cancer, Preneoplastic lesion, Gastric fundic gland, Intestinal metaplasia, Gastric atrophy

Twenty-three years ago, Marshall and Warren demonstrated *Helicobacter pylori* infection in biopsy specimens from patients with gastritis and proved its pathogenicity. Later, chronic atrophic gastritis and certain types of gastric cancer came to be recognized as conditions caused by *H. pylori* infection. Important known factors involved in the pathogenicity of *H. pylori* include urease-dependent NH₃, bacterial cytotoxins such as CagA and VacA, and outer membrane proteins such as heat shock protein and OipA. In addition, the host’s immune responses in the form of inflammatory reactions involving the production of cytokines, such as IL-1β, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, and TNF-α, and the enhancement of proliferation of epithelial cells were also found to be involved in the processes of gastric mucosal atrophy and carcinogenesis. This article outlines the updated information concerning *H. pylori* infection and inflammation of the gastric mucosa, as well as the processes leading from atrophy to carcinogenesis.

**History of *H. pylori* Research**

Marshall and Warren, the discoverers of *H. pylori*, reported the association between chronic gastritis and *H. pylori* infection in 1984.¹,² This year, they were awarded the 2005 Nobel Prize in physiology or medicine. In an infection experiment using oral administration of *H. pylori* suspension, Marshall et al. reported the development of a condition consistent with acute gastritis shortly after infection,³ and Morris et al. proved that the

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Persistence of *H. pylori* infection resulted in a condition consistent with chronic active gastritis. Later animal experiments using various species confirmed the induction of acute gastric mucosal lesions and chronic gastritis by *H. pylori* infection. However, the presence of spirochete-like bacteria in the stomach has been known since the late 19th century. Surprisingly, it was in the 1910s that a Japanese team at the Kitasato Institute led by Rokuzo Kobayashi (Professor Emeritus, Keio University) and Katsuya Kasai isolated spirochete-like bacteria conceivably belonging to *Helicobacter* species from animal stomachs. They proved that infection with these bacteria induced gastric mucosal lesions and used arsaminol in the world’s first attempts to eradicate *Helicobacter* species. However, the report of Palmer in the 1950s completely disproved the presence of bacteria in the stomach, and the gastric lumen was believed to be aseptic for about 30 years thereafter.

As for the relationship between gastric cancer and *H. pylori* infection, the report of Parsonnet in 1991 and many other epidemiological studies demonstrated close association between these two conditions and the WHO/IARC recognized the infection as a definite carcinogen in 1994. An animal model of carcinogenesis using Mongolian gerbils was developed in Japan in 1998. A recent large-scale meta-analysis clearly showed that *H. pylori* infection is an important factor causing gastric cancer. According to this study, the association is stronger for cancers occurring in the non-cardiac part of the stomach than those in the cardiac part, and the risk of carcinogenesis is similar between well-differentiated and poorly-differentiated gastric cancers. Although comparison among various cancer stages indicates stronger association for early-stage gastric cancer than at the later stages, this result seems to reflect the fact that the presence of *H. pylori* was evaluated solely based on serum antibody titers in this study, because we can reasonably consider that the progression of gastric cancer is accompanied by the progression of gastric mucosal atrophy, resulting in a decrease in the antibody titer as an index of *H. pylori* infection. Recent prospective clinical studies also support the causal relationship between *H. pylori* and carcinogenesis.

One of these studies is a 9-year prospective cohort study conducted by Yamagata et al. as a part of the Kyushu University Hisayama Town Study. While *H. pylori*-positive males showed a significant excess in the occurrence of gastric cancer, females showed no significant difference in gastric cancer depending on the status of *H. pylori* infection. Although this result suggests the involvement of female hormones in the

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<tr>
<th>Year</th>
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<tr>
<td>1892</td>
<td>Bizzozero; Discovery of snake-line bodies in a dog stomach</td>
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<td>1906</td>
<td>Krienitz; Discovery of spirochete-like bodies in human stomach</td>
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<td>1919</td>
<td>Kasai &amp; Kobayashi: Establishment of an animal model for <em>H. felis</em> infection and first <em>Helicobacter</em> eradication experiment</td>
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<td>1938</td>
<td>Doenges; Isolation of spirochete-like organism from a human stomach</td>
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<td>1954</td>
<td>Palmer; Denied the existence of microorganism in the stomach</td>
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<td>1976</td>
<td>Lieber; Reduction of gastric NH₃ by ampicillin</td>
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<td>1982</td>
<td>Warren &amp; Marshall; Detection and isolation of <em>Campylobacter</em>-like organism from gastric biopsied specimen (<em>Campylobacter pyloridis</em>)</td>
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<td>1994</td>
<td>NIH Consensus Development Conference; Recommendation of <em>H. pylori</em> eradication therapy for all patients with peptic ulcer disease</td>
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<td>1994</td>
<td>WHO/IARC; Recognition as a definite carcinogen</td>
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<td>1997</td>
<td>Tomb; Total genome analysis of <em>H. pylori</em></td>
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<td>2002</td>
<td>Marshall; Awarded the Keio Medical Prize</td>
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<td>2005</td>
<td>Warren &amp; Marshall; Awarded the 2005 Nobel Prize in physiology or medicine</td>
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development of well-differentiated adenocarcinoma from atrophic gastritis, this does not explain the mechanism for the development of poorly-differentiated adenocarcinoma.

On the other hand, Uemura et al. used more precise diagnosis of *H. pylori* infection combining microscopic examination of multiple gastric biopsy specimens, rapid urease test, and serum antibody titer measurement. In this study, *H. pylori*-positive subjects developed gastric cancer at a rate of 36 patients among 1,246 patients (2.9%) during the follow-up period of 8 years on average, while there were no cases of gastric cancer among the 280 subjects without *H. pylori* infection. This report is very important in that *H. pylori* infection was diagnosed precisely using multiple methods, and strict exclusion of false negative cases enabled the accurate evaluation of gastric cancer risk associated with *H. pylori* infection. In addition, this study analyzed the pattern of gastritis among *H. pylori*-positive subjects, revealing frequent development of well-differentiated adenocarcinoma among *H. pylori*-positive subjects with corpus-predominant gastritis.

However, the results of a large-scale randomized comparative study in China published last year reported that 1,630 *H. pylori*-positive subjects (817 patients receiving and 813 patients not receiving *H. pylori* eradication therapy) were followed for 7 years, and no significant difference in gastric cancer occurrence was found between treatment arms (P = 0.33). A subanalysis of the subjects showing no atrophic gastritis or other preneoplastic lesions demonstrated a significant prophylactic effect of *H. pylori* eradication against gastric cancer (P = 0.02), detecting 6 cases of gastric cancer in the subjects receiving eradication and none in the subjects not receiving eradication. Although this study was conducted as an RCT, the study population was limited to a part of China. Another problem with this study was the fact that the definition of preneoplastic lesions included so-called “early gastric cancer” in the category of dysplasia, which meant that all cases of gastric cancer counted in this study were cases of advanced cancer. These problems prevent the direct comparison of data from this study and other studies. When only the subjects with no preneoplastic lesions such as intestinal metaplasia and dysplasia were compared in this study conducted by Wong et al., eradication treatment resulted in significant reduction in gastric cancer risk. This fact suggests that eradication alone might not be effective in avoiding the risk of carcinogenesis in *H. pylori*-positive subjects who have already had preneoplastic lesions.

In addition, Uemura et al. reported that eradication resulted in significantly less frequent recurrence of gastric cancer after endoscopic mucosal resection (EMR) for early gastric cancer. Animal experiments also suggest that early eradication reduces cancer risk. These observations, considered together, support the possibility that eradication treatment may lead to prevention of gastric cancer.

The Japan Interventional Trial of *H. pylori* (JITHP), conducted to clarify the causal relationship between *H. pylori* and gastric cancer, completed the follow-up period in 2004, and the results of analysis covering the subjects followed for 4 years or more were partly reported by Saito et al. of the Central Hospital, National Cancer Center. In JITHP, *H. pylori*-positive subjects were randomly allocated to eradication and no-eradication groups, and the study was started using the endpoints of (i) emergence or progression of preneoplastic conditions including atrophic gastritis and intestinal metaplasia and (ii) frequency of gastric cancer development. However, the study plan was changed to use only endpoint (i), because the number of subjects turned out to be insufficient. At the time of closure of enrollment in December 2000, there were 751 subjects enrolled (379 in the eradication group and 372 in the no-eradication group). The number of subjects that were followed for 4 years or more and received histological evaluation was 186 in the eradication group and 206 in the no-eradication group. Among the subjects with baseline presence of atrophy, the percentage of subjects showing improvement of atrophy was higher in the eradication group than in the no-eradication group, irrespective of the location of the lesions: greater curvature of the gastric corpus, lesser curvature of the gastric corpus, or greater curvature of the pyloric antrum. No sexual difference was found in the improvement of atrophy. Eradication improved the condition in all age groups and even when atrophy had progressed to a certain degree. On the other hand, intestinal metaplasia improved at a higher rate in the eradication group than in the
no-eradication group, and this improvement was observed even in the subjects that had extensive intestinal metaplasia at baseline. These results suggest that eradication is effective even when the gastric mucosa had atrophy and intestinal metaplasia.

Recent studies in Japan have shown that gastric cancer may develop during the long-term follow-up of patients after successful eradication. At the 11th symposium of the Japanese Society for Helicobacter Research, Murakami et al. reported that gastric cancer develops in 0.8% of cases after eradication and suggested that the development of gastric cancer after eradication may depend on the presence of upper gastrointestinal disorders other than duodenal ulcer, the patient’s age at the time of eradication, and the extent of endoscopic atrophy. In this study, gastric cancer developed in 12 subjects out of the 1,554 subjects that were observed for 26 months on average. An analysis using Kaplan-Meier curves revealed that development of gastric cancer from duodenal ulcer was significantly less frequent than that from gastric ulcer, and the development from duodenal ulcer was significantly lower than after EMR. Furthermore, the patient’s age at the time of eradication tended to be higher among the patients developing cancer than that in the patients developing no cancer. The age at the time of eradication was suggested to be an important factor determining the later development of gastric cancer (personal communication).

Factors Involved in the Pathogenicity of H. pylori

Various factors involved in the pathogenicity of \(H. pylori\) have been reported. First, neutrophils infiltrating into the foci of \(H. pylori\) infection produce reactive oxygen species and myeloperoxidase-dependently produce hypochlorous acid, which reacts with the ammonia resulting from the action of urease produced by \(H. pylori\). This reaction results in the generation of monochloramine, which exerts strong cytotoxicity. This monochloramine further induces apoptosis of the gastric epithelial cells, significantly affecting the cellular turnover of the gastric mucosa. Attention has also been paid to the damage to the host cells inflicted by nitric oxide (NO) produced by the action of inducible nitric oxide synthetase (iNOS) expressed in the macrophages and other inflammatory cells, as well as peroxynitrite (\(\text{ONOO}^-\)) resulting from the reaction between NO and superoxide (\(\text{O}_2^-\)).

In addition, heat shock protein 60 (HSP60) localized in the outer membrane contributes to the adhesion of \(H. pylori\) to the gastric epithelial cells and induces the production of IL-8. Autoimmune reaction has been reported to occur via IL-8-mediated immunoreactivity to self-antigens. Outer membrane protein OipA also promotes the infiltration of inflammatory cells and the production of inflammatory mediators.

Other typical pathogenic factors include CagA, which is injected directly from the bacterial cell to the host cell via Type IV secretion apparatus, and Vac A, a vacuolating cytotoxin secreted to the extracellular space. \(H. pylori\) strains possessing CagA are known to be particularly virulent, and nearly 100% of strains detected in Japan have the \(cag\) pathogenicity island (cagPAI). Once injected into gastric epithelial cells, CagA is considered to undergo tyrosine phosphorylation, bind to Src homology 2-containing protein tyrosine phosphatase
(SHP-2), and enhance its activity, to the effect of promoting cellular proliferation and carcinogenesis. The part of the CagA molecule that undergoes tyrosine phosphorylation and binds to SHP-2 is characterized by the Glu-Pro-Ile-Tyr-Ala sequence (EPIYA motif), and the Tyr residue in this sequence is phosphorylated. The number of instances of this EPIYA motif, as well as the amino acid sequence around them, differs in different strains isolated from clinical specimens. In particular, the difference in amino acid sequence between a strain in East Asia (EPIYATIDF) and a strain in Western countries (EPIYATIDD) is reflected in the difference in the biological activity of CagA. The CagA of the East Asian strain shows stronger biological activity compared with that of the Western strain, suggesting the closer involvement of the former in the development of gastric mucosal atrophy and gastric cancer.

H. pylori and the Cytokine Network

The gastric mucosal tissues with H. pylori infection have been reported to show enhanced production of cytokines, including IL-1β, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, and TNF-α. These cytokines and chemokines not only act as mediators in the inflammation caused by H. pylori infection, but also affect gastric acid secretion and other gastric functions and inflict damage on the gastric mucosa. Many of the components of bacterial cells act on gastric mucosal cells and macrophages, promoting the secretion of IL-8, IL-1β, IL-6, TNF-α, etc. This results in the activation, differentiation, and proliferation of immune cells responding to these chemical mediators, and the excessive generation of reactive oxygen species and cytokines inflicts mucosal damage. IL-1 is produced by monocytes and macrophages; acts on endothelial cells; promotes the adhesion of lymphocytes, monocytes, and neutrophils to the endothelium; and promotes neutrophil migration. It is particularly important that IL-1β induces apoptosis of the gastric epithelial cells. IL-1β is involved in the progression of atrophic gastritis via its strong action to inhibit gastric acid secretion, and El-Omar et al. reported the association between IL-1β polymorphism and gastric cancer. The intron 2 of IL-1RN gene involved in the activity of IL-1β has 5 different patterns of 86-bp tandem repeats. Their study showed that people possessing IL-1RN*2 (2 repeats) are at higher risk of developing gastric cancer and more frequently have hypoacidity. Hsu et al. reported that IL-1RN*2 is a risk factor for gastric and duodenal ulcer. However, IL-1β polymorphism involves considerable ethnic difference, and the results in Japanese populations do not always agree with those in other countries.

H. pylori Infection and Gastric PreneoplasticLesions

In 1975, Correa et al. proposed a theory that gastric cancer develops from atrophic gastritis via intestinal metaplasia. Although this theory preceded the advent of H. pylori in 1982, it accurately described the natural history of gastric lesions resulting from H. pylori infection. In 1994, Gilvarry et al. reported that persistence of H. pylori infection lead to the progression from chronic gastritis to chronic atrophic gastritis. Sakaki et al. studied the relationship between H. pylori infection and atrophic gastritis through endoscopic follow-up of 43 subjects for 10 years. According to their study, the subjects that remained H. pylori negative during follow-up had histologically normal gastric mucosa and no atrophic changes were observed throughout the observation. On the other hand, the 35 subjects that were H. pylori positive showed gastric glandular atrophy in 45.7%, progression of intestinal metaplasia in 48.6%, and the shift of the endoscopic atrophic border toward the oral side in 42.9%.

Progress in research on the molecular mechanism for atrophy has also been remarkable. Recently, we reported that the expression of sonic hedgehog (shh), a morphogen involved in the differentiation and control of fundic glands, is down-regulated in the presence of H. pylori gastritis. Shh is a morphogen belonging to the hedgehog family, and is crucially involved in the determination of the craniocaudal axis during embryogenesis, as well as in controlling the morphogenesis of the central nervous system and various other organs. It has been demonstrated that Shh is also expressed in the fundic glandular mucosa of adults, and this expression disappears in intestinal metaplasia. In the H. pylori gastritis induced in Mongolian gerbils, the expression of Shh dwindled synchro-
nously with the expansion of gastritis from the antrum to the corpus, corresponding to the disappearance of parietal cells and the abnormality of the axis of differentiation from mucous neck cells to chief cells. This observation supports the possible involvement of Shh inhibition in the mechanism for atrophy. The regions showing this disappearance of parietal cells show not only a change in the distribution of Muc 6 (class III mucin)-positive cells but also the presence of glands that are positive for trefoil factor 2 (TFF-2). TFF-2-positive atrophic glands are recently called SPEM (spasmolytic polypeptide-expressing metaplasia), and are attracting much attention as preneoplastic lesions. In fundic glandular mucosa, Shh has been suggested to play an important role in the differentiation from mucous neck cells to chief cells or the differentiation from immature parietal cells to parietal cells. Disruption of these processes is considered to result in preneoplastic changes such as atrophy, SPEM, and intestinal metaplasia. On the other hand, runt domain transcription factor (RUNX) 3 is a transcription factor situated downstream of the TGF-β/BMP signal cascade. In the gastric mucosa of adults, this gene is strongly expressed in cells with well differentiated, such as gastric surface mucous cells and chief cells, and is working as a tumor suppressor gene. It has been reported that the expression of RUNX3 is frequently suppressed in intestinal metaplasia and gastric cancer, suggesting that mutation of the RUNX3 gene can trigger tumor generation and growth.

On the other hand, ghrelin is a peptide produced by endocrine cells called A-like cells, which are located in the mucosa of the gastric corpus, and has activity to promote appetite, acid secretion, and movement of the digestive tract. It is a physiologically active peptide consisting of 28 amino acids, which was purified by Kangawa et al. from rat and human stomachs as an endogenous ligand of growth hormone secretagogue receptor (GHS-R). We studied the kinetics of ghrelin in H. pylori-positive Mongolian gerbils, and reported that H. pylori infection caused gradual decrease in ghrelin in the stomach. We showed that the secretion of ghrelin decreased with the progression of atrophy, and the distribution of ghrelin-immunoreactive cells in the gastric corpus was also decreased by H. pylori infection. In addition, we conducted endoscopy in patients with chronic gastritis to evaluate the relationship between the extent of atrophy and plasma ghrelin level, and reported that plasma ghrelin could be used as a marker for gastric mucosal atrophy, as it was significantly lower in the subjects with severe atrophy. A study using close observation before and after eradication demonstrated that ghrelin and pepsinogen reacted to treatment differently from each other. While no endoscopic improvement of atrophy was observed and serum pepsinogen II dropped with the increase in the pepsinogen I/II ratio shortly after eradication, plasma ghrelin did not change for a while and started to increase gradually 1.5 years after eradication in concordance with the endoscopic improvement of atrophy. This difference is considered to reflect different aspects of the process following eradication. These findings support the prospect that plasma ghrelin can be used as a marker for individuals at high risk of atrophic gastritis or gastric cancer (Japanese Patent Application 2003-155212 by Hidekazu Suzuki, et al.).

**H. pylori Infection and Malignant Transformation**

**H. pylori** has been recognized by WHO/IARC as a definite carcinogen for gastric cancer. The currently accepted mechanism leading from H. pylori infection to the development of gastric cancer can be summarized as follows: The persistence of H. pylori infection causes the persistence of histological gastritis, in which exfoliation and repair of the mucosa take place repeatedly. With additional involvement of genetic and environmental factors, this leads to the development and progression of gastric mucosal atrophy, followed by the changes from intestinal metaplasia to dysplasia. The cascade of these changes is considered to represent the preneoplastic condition leading to well-differentiated gastric cancer. In addition, reactive oxygen species released from inflammatory cells infiltrating the gastric mucosa with H. pylori infection cause DNA damage and enhancement of cellular turnover. As mentioned above, a pathogenic factor specific to H. pylori infection is monochloramine, which is an oxidant with high reactivity and strong cytotoxicity. It is probable that the oxidative stress from these and other factors may impair the DNA repair mechanism, resulting in...
in the occurrence and accumulation of various gene abnormalities. In other words, the development of gastric cancer may be preceded by the mutation of cancer-related genes, which may trigger the development and growth of tumors. The reason that only some \textit{H. pylori}-positive individuals develop gastric cancer is now being studied, considering the differences in other environmental factors, host-side factors, \textit{H. pylori} strain diversity, and the timing of infection.

The mechanism for carcinogenesis due to \textit{H. pylori} involves complicated interactions among factors inherent to \textit{H. pylori} and a multitude of factors related to \textit{H. pylori}. Future studies are expected to provide further insight into this problem.

References


