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Toshiro Sugiyama

Development of gastric cancer associated with *Helicobacter pylori* infection

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Abstract *Helicobacter pylori* infection is associated with histological gastritis, gastric atrophy, gastric cancer and mucosa-associated lymphoid tissue lymphoma in the stomach. However, gastric cancer only develops in a minority of infected individuals. Such clinical diversity is caused by variations in the interactions between *H. pylori* pathogenicity, host susceptibility, and environmental factors. Based on evidence from three prospective epidemiological studies, the International Agency for Research on Cancer and the World Health Organization (IARC/WHO) concluded in 1994 that *H. pylori* has a causal linkage to gastric carcinogenesis and is a definite carcinogen in humans. Two large-scale, prospective, epidemiological studies have recently been reported in Japan and have confirmed that *H. pylori* infection constitutes a high risk factor for the development of gastric cancer, at least in males. In order to obtain evidence that eradication of *H. pylori* leads to a reduction in the occurrence of gastric cancer, reversibility of precancerous lesions, gastric atrophy or intestinal metaplasia should be proven after eradication treatment. A biopsy specimen from the lesser curvature of the corpus is the most sensitive for evaluating the regression of gastric atrophy on histology, and the evaluation needs be conducted at least 13 months after treatment. In a Mongolian gerbil model with or without low-dose chemical carcinogens, it has been demonstrated that *H. pylori* can lead to the development of gastric cancer. Experimental studies have elucidated that virulence factors of *H. pylori* interact with gastric epithelial cell signaling related to carcinogenesis. The cag pathogenicity island (cagPAI) is a major virulence gene cluster; it encodes the type IV secretion machinery system forming a cylinder-like structure. The CagA protein is translocated into target cells via this secretion system and induces a hummingbird phenotype, a growth factor-like effect. The other gene products are probably translocated into target cells and accelerate cellular proliferation and apoptosis. The molecular mechanism of the interaction between *H. pylori* and gastric epithelial cells may provide a new strategy for effective prevention of the development of gastric cancer induced by *H. pylori* infection.

Keywords *Helicobacter pylori* · Gastric cancer · Cag pathogenicity island · Type IV secretion machinery system · Apoptosis

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T. Sugiyama
Department of Gastroenterology,
Hokkaido University Graduate School of Medicine,
North 15, West 7, Kita-ku, 060-8638
Sapporo, Japan
E-mail: tsugi@med.hokudai.ac.jp
Tel.: +81-11-7161161
Fax: +81-11-7067867

Introduction

Helicobacter pylori is one of the most widespread infections in humans worldwide and its cure prevents recurrence of gastroduodenal ulceration. Consequently, several worldwide consensus statements (US National Institutes of Health consensus, European Maastricht consensus, Asian-Pacific consensus and Guideline by the Japanese Society for *Helicobacter* Research) have recommended an attempted cure of *H. pylori* infection in patients with peptic ulcer [1, 7, 15, 21]. In addition, *H. pylori* infection has a causal relationship with histological gastritis, atrophic gastritis, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma in the stomach. All patients with *H. pylori* infection have histological gastritis and most of them are asymptomatic. Only a minority of infected individuals will develop gastric ulceration, duodenal ulceration, gastric cancer or MALT lymphoma. Such clinical diversity is considered to be caused by variations in interactions between *H. pylori* pathogenicity, host susceptibility and environmental factors, including foods.

Numerous epidemiological studies have shown *H. pylori* infection to be associated with an increased risk of gastric cancer. In 1994, the International Agency for Research on Cancer and the World Health Organization (IARC/WHO) concluded that *H. pylori* has a causal link with gastric carcinogenesis and is a definite carcinogen in humans [12]. In addition, these epidemiological studies demonstrated that *H. pylori* infection is closely associated with both intestinal and diffuse types of gastric cancer according to the Lauren classification in North America and Europe, which corresponds to well-differentiated and poorly-differentiated types in the Japanese classification, respectively. Recently, evidence that *H. pylori* infection leads to the development of gastric cancer has been obtained from animal models as well as from in vitro experimental studies. In the present paper, recent clinical and experimental evidence associated with the development of gastric cancer is reviewed.

Recent epidemiological evidence in Japan

The IARC/WHO statement in 1994 was based on the results of three prospective cohort studies conducted in California, Hawaii and the UK [8, 22, 23], in which patients who developed gastric cancer were compared to matched controls who did not. Anti-*H. pylori* antibodies were measured in blood samples collected up to 24 years prior to the diagnosis of gastric cancer. These studies showed significantly elevated odds ratios (between 1.8 and 6.0) for the development of gastric cancer following *H. pylori* seropositivity. Recently, two large-scale prospective studies have been reported in Japan. In 2001, Uemura et al. [32] reported the development of gastric cancer in 36 of 1246 *H. pylori*-positive patients (714 male and 532 female) during a 7.6-year follow-up, but in none of the 280 *H. pylori*-negative patients (155 male and 125 female; 2.9% vs. 0%). The relative risk for development of gastric cancer was dependent on the distribution of gastritis, that is 34.5 (95% CI 7.1–166.7) in corpus-predominant gastritis and 15.6 (95% CI 6.5–36.8) in pangastritis, in comparison with antrum-predominant gastritis. The results from the patients positive or negative for *H. pylori* in this study were not adjusted for age, sex or concomitant disease, and patients were enrolled at one hospital; consequently, there might have been some sampling bias. In this study, *H. pylori* status was confirmed by three diagnostic tests—histological examination, rapid urease test and serological test (HM-CAP test).

In 2000, Yamagata et al. [35] observed the development of gastric cancer in 40 of 765 *H. pylori*-positive males (5.2%) and in 11 of 956 *H. pylori*-positive females (1.2%) during a 9-year follow-up, and in 5 of 247 *H. pylori*-negative males (2.0%) and in 6 of 484 *H. pylori*-negative females (1.2%). The relative risk for development of gastric cancer was 2.59 (95% CI 1.03–6.50) in *H. pylori*-positive males and 0.99 (95% CI 0.36–2.68) in *H. pylori*-positive females. As this study was based on fieldwork in the Hisayama area in Kyushu and the results were adjusted for age, sex and other factors, sampling bias was excluded. However, the investigators used only one test to diagnose *H. pylori* infection: a serological HM-CAP test. As this serological test has an 8–10% false-negative rate in Japanese patients [16], the relative risk for development of gastric cancer in *H. pylori*-positive individuals may have been underestimated.

To obtain direct epidemiological evidence of a causal relationship between *H. pylori* infection and the development of gastric cancer, it is necessary to conduct clinical intervention trials in which eradication of *H. pylori* is shown to lead to a reduction in the occurrence of gastric cancer in humans. However, such clinical intervention trials require long-term follow-up as well as a large study population. As a result, substituted clinical trials are in progress worldwide where the endpoint is a reversibility of precancerous lesions for gastric cancer, gastric atrophy or intestinal metaplasia, after eradication treatment.

Issues on reversibility of precancerous lesions

Helicobacter pylori infection causes persistent inflammation in the gastric mucosa and histological gastritis, characterized by marked infiltration of neutrophils and lymphocytes, which is improved and is reversible after eradication treatment. Since persistent *H. pylori* infection is not resolved without eradication treatment, long-term inflammation in the gastric mucosa results in the progression of gastric atrophy, intestinal metaplasia and, finally, the development of gastric cancer. Therefore, an atrophic gastric mucosa with or without intestinal metaplasia is a high-risk factor for the development of gastric cancer and is precancerous. If such a precancerous mucosa is reversible after eradication of *H. pylori* infection, the treatment might reduce the progression to gastric cancer and prevent its development. To date, the question as to whether there is reversal of gastric atrophy or intestinal metaplasia after eradication treatment remains controversial (Fig. 1) [4, 30]. Gastric atrophy is

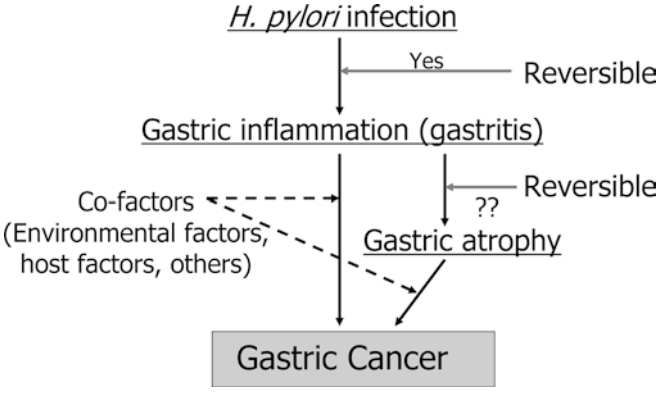

Fig. 1 Reversibility of gastritis–atrophy–cancer sequence associated with *H. pylori* infection

generally evaluated using the updated Sydney system [6] worldwide. Five biopsy sites are recommended in this system for histology. Reports on the reversibility of gastric atrophy vary depending on the histological evaluation sites as well as the time of the evaluation after treatment. These are important reasons as to why such different results have been reported. Which biopsy site is the best for evaluation of regression of gastric atrophy after eradication and when can we confirm it histologically?

We conducted a retrospective study to determine the appropriate sensitive biopsy site and evaluation time for evaluating the regression of gastric atrophy by histology after treatment. Biopsy specimens were collected from 38 patients who showed definite histological regression of gastric atrophy after treatment in five Japanese hospitals (Sapporo, Sendai, Utsunomiya, Tokyo and Oita). The updated Sydney system was used for pathological evaluation before and after treatment. To avoid personal bias, all members of the study group (Helicobacter Pylori Forum Gastritis Study Group) viewed all biopsy specimens and determined a final pathological diagnosis. Four biopsy specimens, from the lesser and greater curvature of the antrum and corpus (LA, GA, LC, GC) were evaluated at baseline and at 3, 6, 12 and 18 months after eradication treatment. As shown in Fig. 2, the biopsy specimen from the lesser curvature of the corpus (LC) was the most sensitive for evaluating the regression of gastric atrophy after treatment. Moreover, at least 13 months was required for the regression of atrophy to be evaluated. Therefore, we recommend that a biopsy specimen from the lesser curvature of the corpus should be tested for the reversal of gastric atrophy at least 13 months after eradication treatment [29].

Although we speculate that gastric atrophy is reversible after eradication treatment, large-scale clinical trials are needed to confirm this under these conditions.

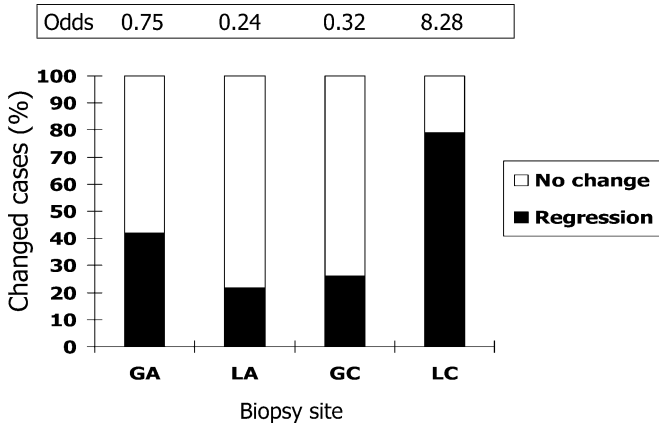

Fig. 2 The most sensitive biopsy site for evaluating the regression of glandular atrophy in histology after *H. pylori* eradication treatment. Modified with permission of Blackwell Publishing from Sugiyama et al. [29] (*LA* lesser curvature of the antrum, *GA* greater curvature of the antrum, *LC* lesser curvature of the corpus, *GC* greater curvature of the corpus)

Following this preliminary trial, the Japanese Intervention Trial of *H. pylori* infection (JITHP) is ongoing under the auspices of the National Cancer Center of Japan. The endpoint of this ongoing trial is whether eradication treatment can prevent the progression of gastric atrophy, compared with untreated control groups as evaluated by histology and endoscopy during 3 years of follow-up.

Development of gastric cancer in animal models

Other direct evidence of a causal relationship between *H. pylori* infection and gastric cancer can be gained by observing the development of gastric cancer in an animal model of *H. pylori* infection. In the first decade following the discovery of this organism, *H. pylori* infection was reported to induce gastritis in gnotobiotic piglets, beagle dogs and Japanese monkeys. These animal models indicated that *H. pylori* infection could induce histological gastritis, characterized by infiltration of numerous inflammatory cells, epithelial erosion and degeneration; the Japanese monkey model demonstrated that the infection is capable of inducing gastric atrophy 1.5 years after inoculation. However, these animals are too large for conducting cancer experiments, and there have been no reports on the development of gastric cancer in these animal models. Although a mouse model resembling human *H. pylori* chronic gastritis has been developed, a specialized *H. pylori* strain, the SS-1 strain, or *H. felis* were used instead of *H. pylori* in studies using this model. Hirayama et al. [10] reported for the first time in 1996 that *H. pylori* could induce gastritis, gastric ulceration and intestinal metaplasia during long-term infection in a Mongolian gerbil (MG) model. In this model, *H. pylori* was able to colonize the stomach and induce gastritis at 12 weeks after inoculation, gastric ulceration at 24 weeks, and intestinal metaplasia at 24–48 weeks. These histological characteristics (infiltration of numerous neutrophils and lymphocytes, deep defect of gastric mucosal tissues leading to the muscular layer and occurrence of intestinal metaplasia) resemble those of *H. pylori* infection in humans. Following this report, the use of the MG model in experiments studying gastric carcinogenesis caused by *H. pylori* infection began [11, 26, 27, 28, 33].

In 1998 and 1999, three papers were published on the development of gastric cancer using the MG model, and the findings strongly suggested that *H. pylori* infection may be responsible for the occurrence of gastric cancer in animal experiments [11, 28, 33]. Sugiyama et al. [28] first demonstrated that *H. pylori* can increase the incidence of gastric cancer induced by N-methyl-N-nitrosourea (MNU) in the MG model: 7 of 19 MGs (36.8%) that had been inoculated with *H. pylori* ATCC type strain and then treated with 10 ppm MNU for 20 weeks developed gastric adenocarcinoma 40 weeks after the study commenced. Five of the seven cancers were signet ring-cell carcinomas, one was poorly differentiated

adenocarcinoma, and one was well-differentiated adenocarcinoma. Of 18 MGs that had first been treated with 30 ppm of MNU for 6 weeks and then inoculated with *H. pylori*, 6 (33.3%) developed gastric adenocarcinomas at 40 weeks. Four of the six cancers were well-differentiated adenocarcinomas. These findings suggest that the timing of inoculation of *H. pylori*, the dose of chemical carcinogen and the order of inoculation of *H. pylori* and administration of chemical carcinogen are critical factors in determining the histological type of gastric cancer. In contrast, 20 MGs infected with *H. pylori* ATCC type strain alone, and 18 MGs treated with 10 ppm MNU alone for 20 weeks or 18 MGs treated with 30 ppm MNU alone for 6 weeks did not develop gastric cancer at all. These findings demonstrate that *H. pylori* is able to induce gastric cancer in an MG model, and that *H. pylori* infection plus administration of very low-dose chemical carcinogen are required for the development of gastric cancer.

In the same year, Watanabe et al. [33] reported that long-term infection with *H. pylori* alone could induce gastric adenocarcinoma in an MG model at 62 weeks after inoculation, without administration of any chemical carcinogen. They reported that 10 of 27 *H. pylori*-infected MGs (37%) developed gastric cancer and that all of the cancers were well-differentiated, intestinal-type carcinomas. Interestingly, they used a unique strain of *H. pylori*, TN2GF4, which was originally isolated from a patient with gastric ulcer and then inoculated into the stomach of an MG several times. This strain had vacuolating cytotoxin and the cagA gene, and it showed an extreme spiral form. Another key point in their study was that no gastric cancers were observed in the infected animals at 39 weeks or at 52 weeks after inoculation of *H. pylori*. However, Hirayama et al. [11] reported that only one gastric cancer developed in 56 MGs infected with *H. pylori* ATCC 43504 type strain at 96 weeks of follow-up (1.8%), and that the pathology was poorly differentiated adenocarcinoma.

In 1999, Shimizu et al. [26] reported that *H. pylori* infection plus administration of N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), another chemical carcinogen, enhanced the incidence of gastric cancer at 50 weeks, compared to the incidence with administration of MNNG alone or *H. pylori* infection alone. Of 25 MGs that had been given 60 mg MNNG first for 10 weeks and then inoculated with *H. pylori* ATCC 43504 type strain, 6 developed gastric cancer. Three of the cancers were well-differentiated adenocarcinomas, one was poorly-differentiated adenocarcinoma, and two were signet ring-cell carcinomas. In contrast, no cancer developed in animals treated with MNNG alone. Of 25 MGs that had been first infected with *H. pylori* ATCC 43504 type strain and then given 20 mg MNNG for 30 weeks, 15 (60%) developed gastric cancer at 50 weeks. Nine of the cancers were well-differentiated adenocarcinomas, two were poorly differentiated adenocarcinomas, and four were signet ring-cell carcinomas. These studies were conducted under different experimental conditions, including different cotreatments with chemical carcinogens, strain differences and different observation periods. These differences may explain the differences in the incidence of gastric cancer and subtypes of gastric cancer (Table 1).

The most troublesome issue is diagnostic criteria for gastric cancer in MGs. In general, gastric cancer in humans is diagnosed on the basis of three histological characteristics: (1) cellular and nuclear atypia; (2) aberrant glandular structure; and (3) invasion. MGs infected with *H. pylori* sometimes show invasion of glands into the muscular layer and aberrant glands, and these histological changes are reversed by *H. pylori* eradication. The histological characteristics in this model may be confused with well-differentiated adenocarcinomas that develop in experiments in which animals are infected with *H. pylori* alone. On the other hand, treatment with low-dose chemical carcinogens sometimes induces cellular and nuclear atypia. Therefore, prudence is needed in the diagnosis of well-differentiated adenocarcinoma in MGs induced by *H. pylori* infection. With regard to these comments, the incidence of well-differentiated adenocarcinomas in animals infected with *H. pylori* alone and those infected with *H. pylori* plus administration of low-dose chemical carcinogens may have been overestimated. To resolve these issues, common criteria for diagnosis of gastric cancer in MGs are required.

With regard to cancer prevention, Shimizu et al. [27] reported that *H. pylori* eradication can decrease the incidence in MGs of gastric carcinomas which had been induced by *H. pylori* inoculation plus administration of low-dose chemical carcinogens. Of 23 MGs given MNU in drinking water at 30 ppm for a total of 5 weeks and then infected with *H. pylori*, 15 (65.2%) had developed gastric cancer at 50 weeks. Another group of 24 MGs that were given MNU and then infected with *H. pylori* underwent eradication treatment at 21 weeks. At week 50, the incidence of gastric cancer in *H. pylori*-eradicated MGs was significantly lower (20.8%) than in *H. pylori*-infected MGs ($P < 0.01$). These findings indicate that *H. pylori* eradication treatment in the early

Table 1 Development of gastric cancer in *H. pylori*-infected Mongolian gerbils (*MNU* N-methyl-N-nitrosourea, *MNNG* N-methyl-N′-nitro-N-nitrosoguanidine)

Reference	Strain	Chemical carcinogen	Time of observation (weeks)	Incidence
28	ATCC	MNU	40	13/37 (35%)
33	TN2GF4	None	72	10/27 (37%)
11	ATCC	None	96	1/56 (2%)
26	ATCC	MNNG	50	15/25 (60%)

phase of infection reduces the occurrence of gastric cancer in MG models. These results suggest that *H. pylori* eradication treatment in early life may be promising for gastric cancer prevention in humans.

In vitro study of gastric carcinogenesis

Major virulence factor of *H. pylori*: CagPAI

Helicobacter pylori strains are classified as type 1 in the presence of the cagA gene (or cag pathogenicity island: CagPAI) or type 2 in the absence of the cagA gene [3]. Type 1 *H. pylori* may be more virulent in its pathogenesis and is frequently isolated from patients with gastric cancer in Western countries [2]. In Asian countries, including Japan where the prevalence of gastric cancer is high, almost all *H. pylori* strains are type 1 [19]. After complete resolution of the full sequence of the *H. pylori* genome [31], a cagA gene was found to be one component within the cagPAI gene cluster; moreover, it was an indirect marker for the presence of cagPAI. CagPAI is a complex of genes encoding approximately 30 proteins and is thought to be acquired by horizontal transfer from other bacteria or *H. pylori* during bacterial evolution, due to the presence of typical transposons, tnpA and tnpB within the cagPAI gene cluster [5]. Interestingly, this lesion codes for the type IV secretion machinery system forming a cylinder-like structure connected to epithelial cells described in other bacteria, such as *Bordetella pertussis* and *Agrobacterium tumefaciens* (Fig. 3). After adhesion of *H. pylori* to gastric epithelial cells and the formation of type IV secretion machinery, many virulence gene products or other interactive proteins including CagA might be transferred into the host cells via this system.

Within the target cells, CagA protein is activated by phosphorylation at defined tyrosine phosphorylation motifs, and SHP-2 (the Src homology 2 domain containing tyrosine phosphatase) is bound to the phosphorylated tyrosine on CagA protein [9]. Activation of SHP-2 occurs and the complex formation results in the induction of the hummingbird morphology of the target epithelial cells, which resembles the morphological changes induced by hepatocyte growth factor (HGF) or platelet-derived growth factor (PDGF). Therefore, CagA protein may function as a growth factor-like effector molecule within the target epithelial cells after transfer from *H. pylori*. Mutant cagA, in which the tyrosines are changed to alanines, cannot bind to SHP-2 proteins resulting in loss of induction of the hummingbird phenotype. On the other hand, Mimuro et al. [18] have recently observed that CagA binds to the other intrasignaling molecule, growth factor receptor bound 2 (Grb2). The CagA–Grb2 complex activates the Ras/MEK/ERK cascade resulting in the induction of

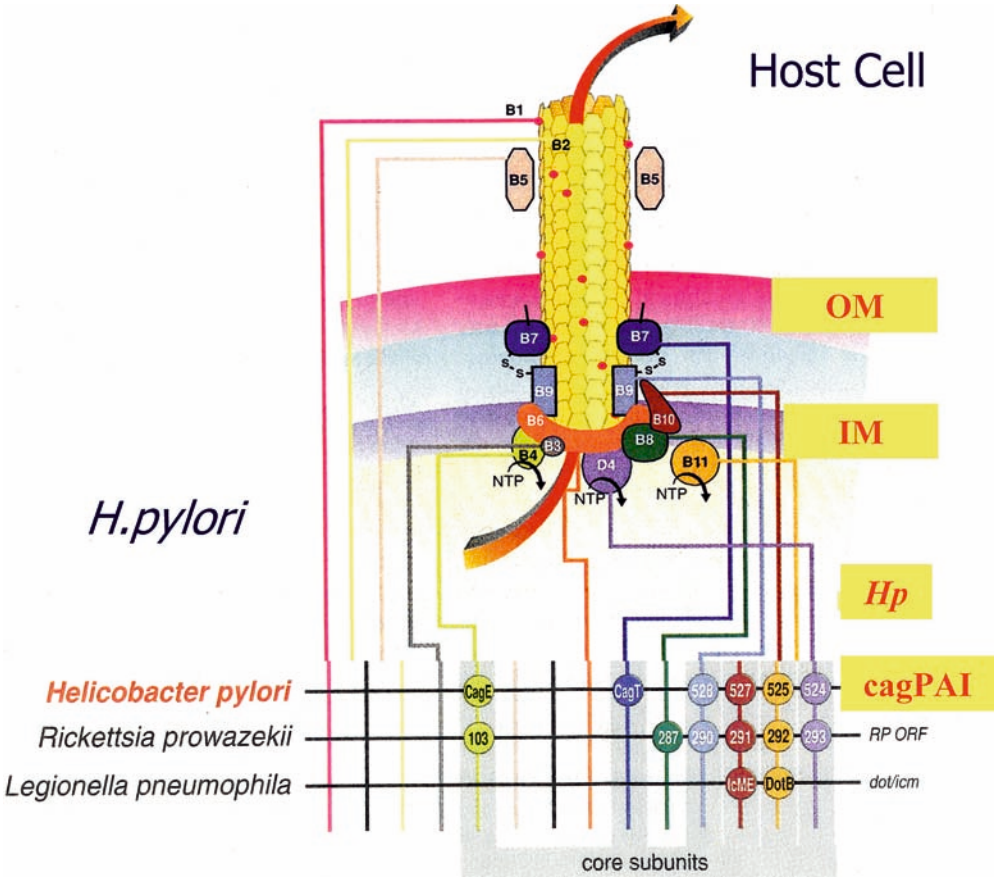

Fig. 3 Type IV secretion system coded in cagPAI. Modified with permission from Covacci et al. [5]. Copyright 1999, AAAS

hummingbird phenotype, as well as that of cellular proliferation. Mutant cagA (tyrosines replaced by phenylalanines on CagA) showed no effects on binding to Grb2 and the formation of CagA–Grb2 complex, or on the induction of the hummingbird phenotype. The authors concluded that tyrosine phosphorylation is not essential for the formation of the CagA–Grb2 complex and the induction of hummingbird phenotype and cell proliferation.

Although it remains to be established whether tyrosine phosphorylation on CagA protein is necessary for the induction of hummingbird phenotype, CagA protein acts as an effector to induce morphological changes in gastric epithelial cells after attachment (Fig. 4). As tyrosine phosphorylation on CagA protein is dependent on the interaction of gastric epithelial cell enzymes (Src family) and tyrosine phosphorylation motifs (TMPs) on CagA, the number of TMPs on CagA should be compared among *H. pylori* strains isolated from patients with various clinical outcomes. If CagA is a sole virulence determinant associated with the development of gastric cancer, the presence of CagA or the phosphorylation of it might be closely associated with the development of gastric cancer. As mentioned above, most *H. pylori* isolated from patients in Japan and other Asian countries are CagA-positive strains, regardless of clinical outcome. Therefore, the presence of CagA itself is not causally linked to the development of gastric cancer but probably has an indirect effect via the progress of gastric atrophy.

Another hypothesis is that the degree of tyrosine phosphorylation on the CagA protein of *H. pylori* isolated from patients with gastric cancer may be different from that isolated from patients with asymptomatic histological gastritis. Lai et al. [14] have recently reported that there is no difference between the number of TMPs on CagA of *H. pylori* isolated from patients with asymptomatic histological gastritis and those with gastric cancer. In general, it is well known that patients with duodenal ulceration are likely to be protected against the development of gastric cancer, although the isolates from patients with duodenal ulceration or gastric cancer are both CagA-positive. According to our findings, there is no difference between the number of TMPs on CagA of *H. pylori* isolated from patients with duodenal ulceration and those with gastric cancer (T. Sugiyama et al.; manuscript in preparation). This evidence suggests that the phosphorylation of CagA is not a sole virulence determinant associated with the development of gastric cancer.

The activating protein-1 (AP-1) family of transcription factors plays a central role in cellular proliferation and neoplastic transformation. AP-1 complexes consist of hetero- and homodimers of the protooncogene families Fos, Jun and ATF. AP-1 complexes are activated by a variety of extracellular stimuli, including growth factors, cytokines and cellular stress signals. The Fos and Jun proteins are sequentially activated by three related kinase cascades: MAP KK kinase, MAP K kinase and MAP kinase. MAP kinase finally transduces to the nucleus, where it phosphorylates a transcription factor. Three distinct and interactive MAP kinase pathways—the ERK1/2 pathway, the SAP kinase/JNK pathway and the p38 pathway—have been identified. AP-1 activity is regulated by all three MAP kinase pathways. Meyer-ter-Vehn et al. [17] have reported that *H. pylori* selectively activates the ERK/MAP kinase cascade. Stimulation of ERK leads to phosphorylation of the transcription factor Elk-1 and markedly increases c-fos transcription; expression of c-Fos protein and phosphorylation of c-Jun is also strongly induced. Keates et al. [13] have reported that *H. pylori* is capable of inducing epidermal growth factor (EGF) receptor phosphorylation, which is responsible for ERK1/2

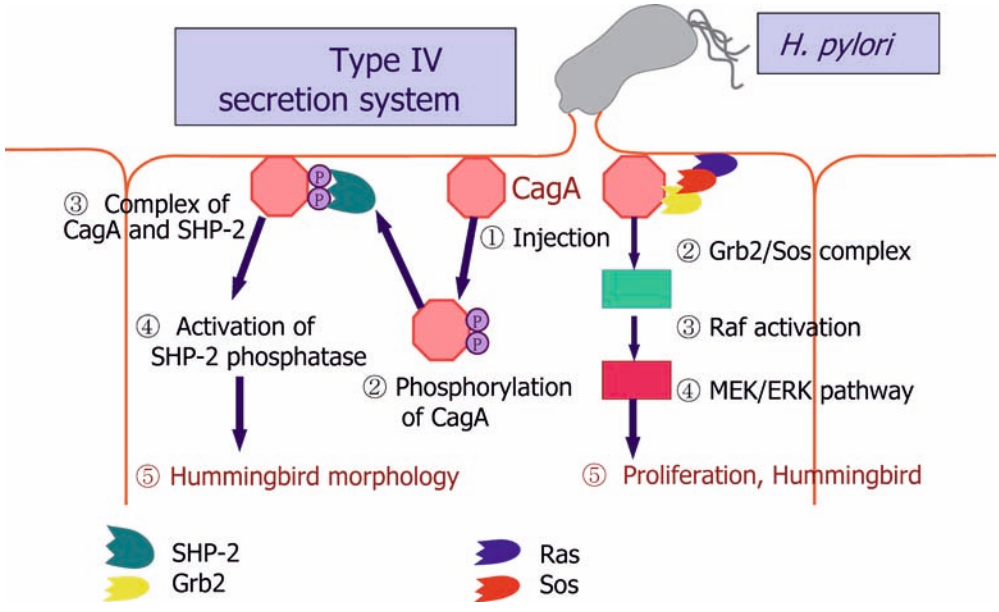

Fig. 4 CagA protein induces a growth factor-like response to gastric epithelial cells

activation via the activation of the small GTP-binding protein Ras. Interestingly, a cagA-negative isogenic mutant does not affect the ability to induce activation of the EGF receptor. In contrast, a cagE-negative isogenic mutant, an essential component in the formation of the type IV secretion machinery system, cannot phosphorylate and activate the EGF receptor. Therefore, the presence of the type IV secretion machinery system and other effectors besides CagA protein might be crucial for cellular proliferation via the Ras-ERK1/2-AP-1 signaling pathway.

Apoptosis and carcinogenesis

Gastric mucosal integrity is maintained by a balance between the rate of cellular loss and the rate of cellular regeneration or proliferation. *Helicobacter pylori* has many virulence factors that are capable of inducing the loss of gastric epithelial cells. A vacuolating cytotoxin produced by *H. pylori*, a cytotoxic monochloramine generated by reactive oxidative radicals produced from infiltrating neutrophils in the gastric mucosa and ammonium of *H. pylori*, and immune-mediated cytotoxic cells are damaging to gastric epithelial cells. The apoptosis of gastric epithelial cells by *H. pylori* has a bipolar role: a cytotoxic effect and a protective effect by removing damaged epithelial cells. Immunohistochemical studies in native gastric mucosa [25] have revealed increased expression of CD95 (Apo-1/Fas) receptor in epithelial cells and high expression of CD95 ligand in lamina propria lymphocytes, which mediates T-cell-dependent cytotoxicity. In addition, *H. pylori* is directly capable of inducing apoptosis in coculture experiments with target epithelial cells via activation of the caspase family, especially caspase-9 and caspase-3 [24].

Besides these conventional mechanisms, we have reported that *H. pylori* upregulates Smad5 expression of gastric epithelial cells and that the Smad 5 upregulation is involved in *H. pylori*-induced apoptosis of gastric epithelial cells [20]. In addition, it has been found that the presence of intact cagPAI is essential for Smad 5-mediated apoptosis of epithelial cells. Eight different Smads have been identified in mammals and can be classified into three subclasses: receptor-regulated Smads (R-Smads); common mediator Smads (Co-Smads) and inhibitory Smads (I-Smads). R-Smads can be further subdivided into two subtypes: those phosphorylated after stimulation by transforming growth factor β (TGF-β) or by bone morphogenetic protein (BMP). Smad 5 belongs to this latter group. BMP actively mediates apoptosis in the embryonic limb, and BMP also induces apoptosis in human medulloblastoma cells, probably via upregulation of R-Smads (Smads 1, 5, 8). Therefore, it is likely that the apoptosis of epithelial cells infected with *H. pylori* is mediated via Smad 5 upregulation. We have also confirmed that only cagPAI-positive *H. pylori* strains are capable of inducing upregulation of Smad 5 mRNA, as well as having apoptotic effects in human gastric epithelial cells. Although CagA is the only *H. pylori* protein known to translocate from the bacterium into the cell via the type IV secretion system, it can be assumed that transfer of unknown genes or gene products through this machinery might be essential for upregulation of the Smad 5 in gastric epithelial cells.

Studies on cell kinetics of *H. pylori*-infected gastric mucosa have shown that both the acceleration of apoptosis and the proliferation of gastric epithelial cells occur according to the grade of gastric atrophy [34]. However, the apoptosis is generally inhibited in gastric cancer cells, although the proliferation is still accelerating. Therefore, the apoptotic signaling might be switched off during progression of gastric atrophy to gastric cancer. After passing through the irreversible step in the sequence of gastritis–atrophy–cancer (point of no return), an eradication treatment against *H. pylori* infection may have no preventive effects on the development of gastric cancer (Fig. 5). Thus, an investigation of the apoptotic mechanisms induced by *H. pylori* infection might offer an important clue for determining which subjects should receive eradication therapy for the

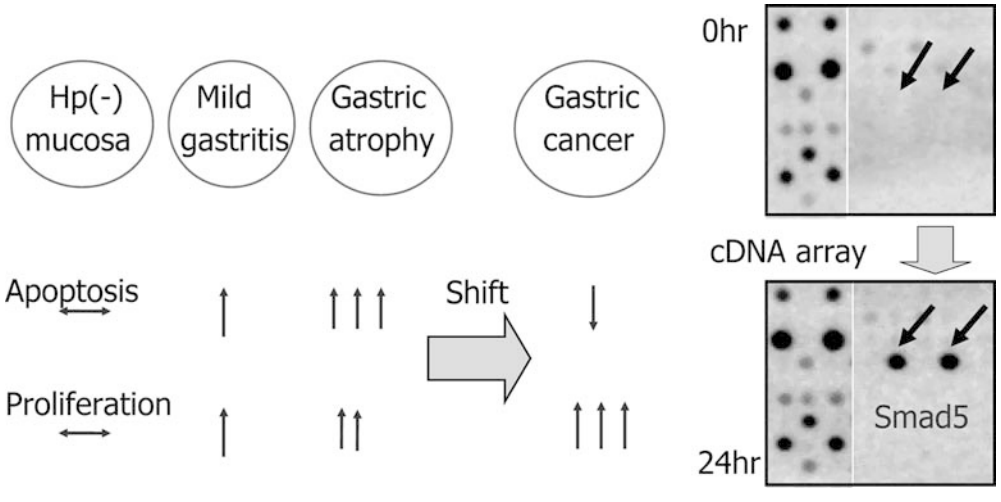

Fig. 5 Cell kinetics of gastric epithelium infected with *H. pylori* during gastric carcinogenesis

effective prevention of the development of gastric cancer. It would be impossible to treat all asymptomatic subjects with *H. pylori* infection in Japan—60 million people.

Conclusions

Helicobacter pylori infection has a causal relationship to histological gastritis, gastric atrophy, gastric cancer and MALT lymphoma in the stomach. Gastric cancer only develops in a minority of infected individuals. Such clinical diversities are considered to be caused by interactions between *H. pylori* pathogenicity, host factors (IL1-β polymorphism) and environmental factors (excess intake of salt, low intake of fresh vegetables, fruits, etc.). Clinical studies and experiments in animal models have clearly demonstrated that *H. pylori* infection has a causal linkage to gastric carcinogenesis. In addition, in vitro experimental studies have shown that virulence factors of *H. pylori* interact with gastric epithelial cell signaling related to carcinogenesis. These in vivo and in vitro studies may provide a new strategy for the effective prevention of the development of gastric cancer induced by *H. pylori* infection.

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