Stomach

Digestive Diseases

Dig Dis 2016;34:510–516
DOI: 10.1159/000445231

New Biology to New Treatment of *Helicobacter pylori*-Induced Gastric Cancer

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Key Words
Bacterial secretion system · Cytokines · Diet · Gastric adenocarcinoma · *Helicobacter pylori*

Abstract
Background: *Helicobacter pylori* is a bacterial carcinogen that is supposed to have the highest known level of risk for the development of gastric cancer, a disease that claims hundreds of thousands of lives per year. Approximately 89% of the global gastric cancer burden and 5.5% of malignancies worldwide are attributed to *H. pylori*-induced inflammation and injury. However, only a fraction of colonized persons ever develop neoplasia, and disease risk involves well-choreographed interactions between pathogen and host, which are dependent upon strain-specific bacterial factors, host genotypic traits, and/or environmental conditions. ***Key Messages:*** One *H. pylori* strain-specific virulence determinant that augments the risk for gastric cancer is the *cag* pathogenicity island, a secretion system that injects the bacterial oncoprotein CagA into host cells. Host polymorphisms within genes that regulate immunity and oncogenesis also heighten the risk for gastric cancer, in conjunction with *H. pylori* strain-specific constituents. Further, conditions such as iron deficiency and high salt intake can influence *H. pylori* phenotypes that lower the threshold for disease. ***Conclusions:*** Delineation of bacterial, host, and environmental mediators that augment gastric cancer risk has profound ramifications for both physicians and biomedical researchers as such findings will not only focus prevention approaches that target *H. pylori*-infected human populations at increased risk for stomach cancer, but will also provide mechanistic insights into inflammatory carcinomas that develop beyond the gastric niche.

Introduction

Helicobacter pylori is a bacterial carcinogen that is found to have the highest known level of risk for the development of gastric cancer. With an estimated 1 million new cases/year, gastric adenocarcinoma claims >700,000 lives each year and approximately 89% of the global gastric cancer burden and 5.5% of all malignancies worldwide are attributable to *H. pylori*-induced inflammation and injury [1].

Two histologically distinct forms of gastric adenocarcinoma have been well-defined, diffuse-type and intestinal-type, both of which are linked to *H. pylori* infection. Diffuse-type cancer is characterized by widespread neoplastic cell infiltration throughout the gastric mucosa, while intestinal-type cancer progresses through a series of well-defined pathological steps from normal gastric mucosa to superficial gastritis, chronic gastritis, atrophic gastritis, intestinal metaplasia, and finally dysplasia and adenocarcinoma (fig. 1) [2, 3]. Chronic *H. pylori* infection induces gastric inflammation and thus increases the risk of progression through each of these well-defined steps of gastric transformation, including atrophic gastritis, intestinal metapla-

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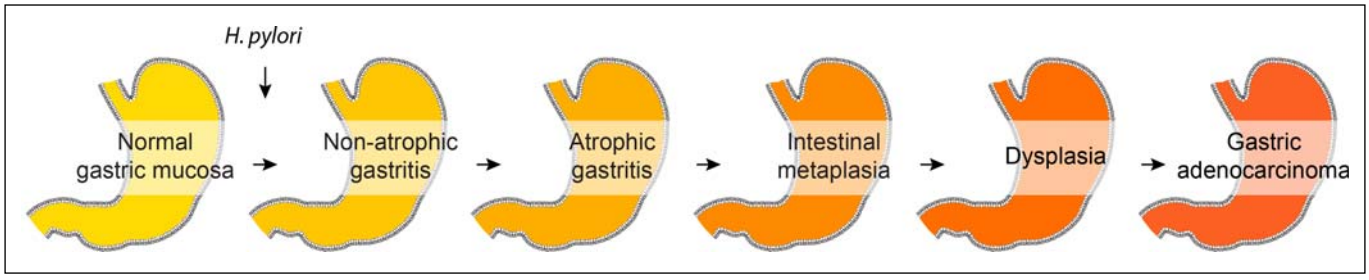

Fig. 1. Histologic cascade to intestinal-type gastric adenocarcinoma associated with *H. pylori* infection.

sia, and dysplasia, which are considered precursors to the development of gastric adenocarcinoma (fig. 1). In contrast to histologic stratification of gastric cancers, a recent study conducted comprehensive molecular analyses of gastric cancer cases to further classify gastric cancer into subtypes specifically associated with distinct clinical outcomes [4]. This classification yielded 4 subtypes, including MSI, TP53$^+$, TP53$^-$, and EMT [4]. MSI or microsatellite-unstable tumors exhibited the best overall prognosis and the lowest frequency of recurrence [4]. The TP53 or tumor protein 53 classifications of active (TP53$^+$) or inactive (TP53$^-$) yielded intermediate prognosis and recurrence rates, with the inactive form yielding worse prognosis than the active form [4]. Finally, the EMT or epithelial-to-mesenchymal transition type classification exhibited the worst prognosis and the highest recurrence frequency [4]. These new molecular classifications of gastric cancer will likely improve future screening practices.

However, only a percentage of *H. pylori*-colonized persons ever develop neoplasia, and enhanced risk is related to bacterial strain differences, inflammatory responses governed by host genetic diversity, and/or specific interactions between host and microbial determinants [3]. These observations, in conjunction with evidence that carriage of certain strains is inversely related to esophageal adenocarcinoma and atopic diseases [3], underscore the importance of identifying mechanisms that regulate interactions of *H. pylori* with its host, which promote carcinogenesis.

H. pylori Virulence Factors That Influence Gastric Cancer Risk

H. pylori strains exhibit a high level of genetic diversity [5]. The *cag* pathogenicity island is a strain-specific locus that encodes a type IV secretion system (TFSS) [6] and *cag*$^+$ strains markedly augment the risk for gastric cancer compared to *cag*$^-$ strains [7]. The product of the *cagA* gene (CagA) is translocated by the TFSS into epithelial cells, undergoes tyrosine phosphorylation [8–10], and activates a eukaryotic phosphatase (SHP-2), leading to cellular responses that may lower the threshold for transformation [8–10]. Our group and others have demonstrated that non-phosphorylated CagA also exerts pathologic effects including the activation of β-catenin, disruption of apical-junctional complexes, and a loss of cellular polarity [11–14], alterations that play a role in carcinogenesis. In addition to CagA, the *cag* TFSS delivers peptidoglycan into host cells where it is recognized by nucleotide-binding oligomerization domain 1, an intracytoplasmic sensor of bacterial peptidoglycan components [15]. Disruption of *H. pylori* peptidoglycan synthesis attenuates β-catenin activation, autophagy, and the development of pre-malignant lesions in response to *cag*$^+$ strains [16].

VacA, a toxin secreted by *H. pylori*, is another microbial constituent linked to the development of gastric cancer [17]. All *H. pylori* strains possess *vacA*, but there are considerable differences in *vacA* sequences, with the regions of greatest diversity localized to the 5′ region of the gene, which encodes the signal sequence and amino-terminus of the secreted toxin (allele types s1a, s1b, s1c, or s2), an intermediate region (allele types i1 or i2), and a mid-region (allele types m1 or m2). Strains containing type s1, i1, and m1 alleles are strongly associated with gastric cancer [18], and in 2 recent studies, *vacA* s1 and i1 genotypes were determined to be better markers of intestinal metaplasia or gastric cancer than *cagA* genotypes [19, 20].

Host Factors and Gastric Cancer

Several studies have utilized high-level genetic analyses to identify host factors linked to *H. pylori* infection and gastric cancer. A recent GWAS study linked poly-

morphisms within a TLR-encoding locus with the presence of *H. pylori* infection, although the specific relationship to gastric cancer was not determined [21]. Another group utilized MLST and SNP analysis to assess *H. pylori* and human genetic variation, respectively, in 2 unique Colombian populations with disparate risks for gastric cancer in order to assess co-evolutionary relationships within the context of pathogenic outcomes [22]. Specific interactions between microbial and human genetic ancestries clearly predicted disease risk, and all persons who fell within the lowest decile of African host ancestry content but who were infected with an *H. pylori* strain containing >19.8% African ancestry, harbored a premalignant lesion. Thus, interactions between host and pathogen ancestries completely accounted for differences in the severity of gastric injury in these populations, suggesting that neither human nor *H. pylori* genetic variation confers disease susceptibility in isolation, but only within the context of a genetic mismatch.

Specific proinflammatory cytokine polymorphisms can greatly increase the risk of gastric cancer among *H. pylori*-infected persons [23, 24]. In fact, odds ratio estimates for distal gastric cancer conferred by IL-1, IL-10, or TNF-alpha genetic polymorphisms are further amplified in persons infected with *cag*$^+$ strains when compared to the total *H. pylori*-infected population [25]. Persons infected with *H. pylori* isolates that possess type s1/m1 *vacA* alleles are more likely to develop hypochlorhydria, a phenotype linked to high-expression alleles of IL-1β and gastric cancer. The combination of high-risk host genotypes and cancer-associated *vacA* alleles or *cag* genotype similarly increases the risk for gastric cancer markedly, up to 87-fold over baseline [26]. Thus, evaluating human genetic variation in conjunction with genetic analyses of infecting *H. pylori* strains can identify colonized persons who harbor the highest risk for gastric cancer and who may be optimal candidates for antimicrobial intervention.

Another promising area for gastric cancer research is defining the specific interactions that occur between *H. pylori* and stem cells. Stem cells are critical for regulating the self-renewing gastric epithelium and maintaining homeostasis, and evidence from mouse models and human tissue has indicated that *H. pylori* can interact with stem-cell populations [27]. In vivo lineage tracing of the gastric epithelium has demonstrated that Lgr5 (leucine-rich repeat-containing G protein-coupled receptor 5) positive cells are self-renewing and multipotent stem cells are capable of generating an entire antral gastric gland [28]. In the human stomach, *H. pylori* infection in patients with gastric cancer leads to an increased population of Lgr5-positive epithelial cells in the antrum compared to uninfected persons with cancer [29]. Furthermore, in *H. pylori*-infected persons with cancer, Lgr5-positive cells are more susceptible to DNA damage than Lgr5-negative cells [29]. A recent novel study using 3D-confocal microscopy has demonstrated that *H. pylori* can specifically interact with Lgr5$^+$ progenitor and stem cells, leading to their functional activation [27].

Leucine-rich repeats and immunoglobulin-like domains 1 (Lrig1) is a transmembrane protein that marks a distinct population of quiescent stem cells and functions as a tumor suppressor [30]. Lrig1 is expressed in the gastric epithelium, and expression of Lrig1 is increased in the gastric epithelium of *H. pylori*-infected vs. uninfected mice suggesting that infection with *H. pylori* increases this stem cell population as well [31].

Bone marrow derived cells (BMDCs) are a heterogeneous population of cells with the ability to differentiate into cells of diverse lineages. Studies in mice infected with *Helicobacter* have demonstrated that BMDCs home to and engraft in sites of chronic gastric inflammation, and repopulate the endogenous tissue stem cells [32]. Within an inflamed stomach, BMDCs degenerate into adenocarcinoma, suggesting that gastric epithelial carcinomas can originate from marrow-derived sources [32].

Dietary Risk Factors

The risk of gastric cancer is not only influenced by *H. pylori* strain-specific constituents and host genetics, but also by environmental factors such as diet. Diets that are rich in salted, pickled, smoked or poorly preserved foods, those with high meat content, and those with low fruit and vegetable content are most commonly associated with an increased risk for developing gastric cancer [33]. Within the context of *H. pylori* infection, high dietary salt intake and low iron levels are most highly associated with increased gastric cancer risk.

High dietary salt intake is associated with increased gastric cancer risk and has been reported in numerous studies [33], and *H. pylori* infection in combination with a high salt diet further increases cancer risk. In Mongolian gerbils, the combination of *H. pylori* infection and a high salt diet exerts synergistic effects on the development of premalignant lesions or gastric cancer [34, 35]. The mechanisms by which salt increases the risk for developing gastric cancer in humans are incompletely understood; however, recent data indicate that salt may modu-

late virulence gene expression in *H. pylori*. Expression of *cagA* is significantly upregulated when certain strains of *H. pylori* are cultured in high salt concentrations [36], and salt-responsive strains more frequently harbor 2 copies of a 5′-TAATGA motif located within the *cagA* promoter [37].

In addition to salt, host iron levels have also been found to modulate the virulence potential of *H. pylori*, and iron deficiency is associated with an increased risk for gastric cancer. Long-term colonization with *H. pylori* can further exacerbate iron deficiency through the development of gastric atrophy, which leads to decreased acid secretion, reduced ascorbic acid levels and decreased iron absorption [38]. Our group has demonstrated that iron deficiency in *H. pylori*-infected persons accelerates the development of carcinogenesis by increasing the virulence potential of *H. pylori* [39]. We also performed mechanistic studies to determine the effects of dietary iron depletion on the development of *H. pylori*-induced carcinogenesis in gerbils [39]. *H. pylori* infection significantly increased the occurrence of dysplasia and gastric adenocarcinoma in iron-depleted gerbils compared to iron-replete gerbils [39], but these effects were present only after infection with CagA-positive *H. pylori*. To investigate the molecular mechanisms underlying these changes, the proteomes of *H. pylori* strains cultured from gerbils fed on an iron-depleted diet or on an iron-replete diet were compared. Proteins involved in function of the *cag* TFSS were differentially regulated when comparing *H. pylori* strains isolated from iron-depleted vs. iron-replete gerbils [39] and levels of phosphorylated CagA (reflecting translocated CagA), the number of *cag* T4SS pili, and induction of pro-inflammatory cytokines were significantly higher following co-culture with strains isolated from iron-depleted gerbils compared with strains isolated from iron-replete gerbils [39]. Collectively these data demonstrate that dietary iron depletion significantly increases the severity of gastric inflammation and accelerates the development of *H. pylori*-induced disease via augmentation of the *cag* secretion system.

Treatment Recommendations for *H. pylori*

The goal of anti-*H. pylori* therapy is to eliminate the organism from the stomach and successful eradication is defined as a negative test for the bacterium ≥4 weeks after completion of therapy. Treatment regimens should be straightforward, well tolerated, and cost-effective. There are several different options for treating *H. pylori* infections, and selection of a particular therapy is dependent on many factors, including drug availability, antimicrobial resistance patterns of *H. pylori* strains, and cost. Two first-line therapies have been currently endorsed by the American College of Gastroenterology: triple therapy using a proton pump inhibitor (PPI) and 2 antibiotics or bismuth-containing quadruple therapy [40]. Triple therapy consists of a PPI plus clarithromycin (500 mg) and either amoxicillin (1 g) or metronidazole (500 mg), each ingested twice daily. Bismuth-based quadruple therapy consists of a PPI dosed twice daily plus bismuth, metronidazole, and tetracycline for 10–14 days. However, these therapies fail to eradicate *H. pylori* in up to 20–25% of patients, likely due to an increased global prevalence of antibiotic-resistant *H. pylori* strains (table 1) [41].

Table 1. *H. pylori* resistance rates in Europe and the United States

Antibiotic	*H. pylori* resistance, %	
	Europe[1]	United States[2]
Clarithromycin	17.5	17.8
Levofloxacin	14.1	31.9
Metronidazole	34.9	21.5

[1] Resistance determined as reported in [47] in Europe.
[2] Resistance determined as reported in [48] in the United States.

Sequential therapy in its original formulation consists of a proton-pump inhibitor daily plus amoxicillin 1 g twice a day for 5 days, followed by PPI therapy, clarithromycin 500 mg, and tinidazole 500 mg, each twice a day, for an additional 5 days. In a pooled analysis of 22 trials evaluating 2,388 patients, collective eradication rates were 91.3 and 93.7% (intention to treat and per protocol, respectively) [41]. An important caveat is the importance of the nitroimidazole used in this regimen. In patients receiving tinidazole (currently unavailable in the United States), eradication rates were 97.4% compared to 84.1% when metronidazole was substituted in the regimen, which may reflect the longer half-life of tinidazole [41]. Sequential therapy has been directly compared to standard triple therapy in several randomized trials and has achieved higher eradication rates with no difference in side effect profiles. Another advantage of sequential therapy is its ability to achieve high eradication rates for clarithromycin-resistant *H. pylori* strains and levofloxacin can be successfully incorporated into sequential therapy (levofloxacin replacing clarithromycin) [42]. However, geographic locale may influence eradication rates. A recent study in Latin America demonstrated that sequential

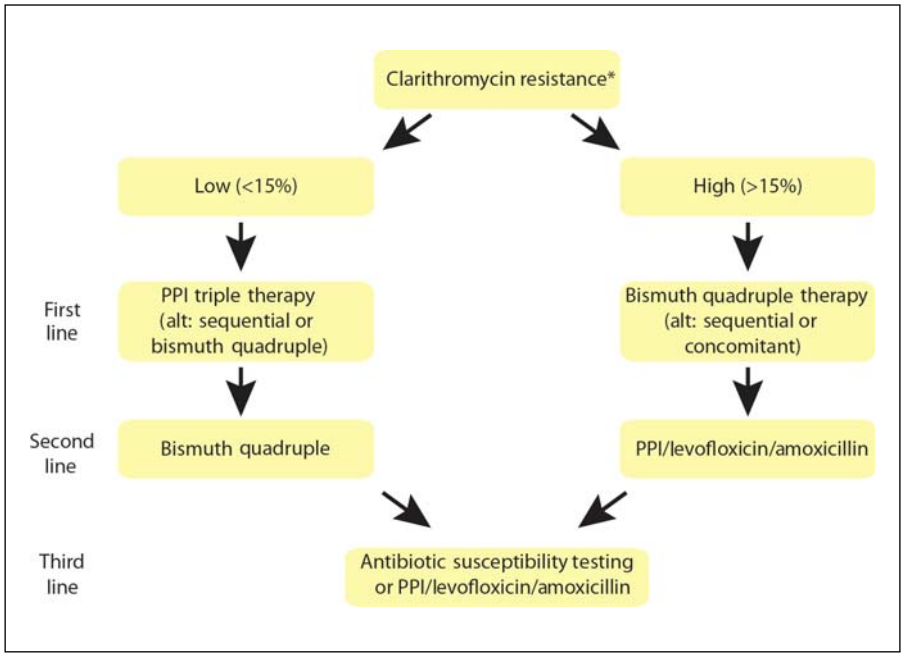

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Fig. 2. Anti-microbial therapies recommended for *H. pylori* eradication. * Or recent use of clarithromycin.

therapy was not superior to standard triple therapy, although metronidazole was used instead of tinidazole and antibiotic susceptibility patterns of infecting strains were not analyzed [43]. This study has raised questions regarding the use of sequential therapy as a first-line therapy for *H. pylori* eradication in the United States. Other concerns include the fact that the efficacy of sequential therapy may be reduced when metronidazole is used in place of tinidazole, and that sequential therapy contains amoxicillin as a major constituent, which limits its use in patients allergic to penicillin.

Another potential alternative to currently recommended first-line therapies is concomitant therapy, which combines a PPI with clarithromycin, metronidazole, and amoxicillin [44]. Although eradication rates using concomitant therapy have been reported to exceed 90%, randomized trials evaluating this regimen have shown it to be less effective [43].

The most recent Maastricht IV/Florence Consensus Conference recommended the following strategy for treatment of *H. pylori* [45]. In areas with low rates of *H. pylori* clarithromycin resistance (<15%), PPI-clarithromycin-containing triple regimens are recommended as first-line treatment; however, bismuth-containing quadruple therapy is an alternative (fig. 2). In areas of high clarithromycin resistance, bismuth-containing quadruple regimens are recommended as first-line empirical treatment. If this regimen is not available, sequential therapy or non-bismuth quadruple therapies such as concomitant therapy (PPI with clarithromycin, metronidazole, and amoxicillin) are recommended. Since local antibiotic resistance rates may not always be readily available, patients with recent or repeated exposure to clarithromycin should be assumed to harbor a resistant *H. pylori* strain (fig. 2).

In patients that fail eradication with a PPI-clarithromycin containing regimen, either bismuth-containing quadruple therapy or levofloxacin-containing triple therapies (PPI, levofloxacin, amoxicillin) are recommended (fig. 2). Levofloxacin-containing triple therapy is also recommended as a second-line therapy if bismuth-quadruple or non-bismuth quadruple therapies fail as first-line treatments. A meta-analysis that included 4 randomized trials of levofloxacin as a component of triple therapy reported higher eradication rates compared with a 7-day course of bismuth-based quadruple therapy, as well as fewer side effects [46]. Resistance to levofloxacin is a major limiting factor with resistance rates exceeding 16% in East Asia, Canada, certain regions of Europe, and the United States [47, 48]. After failure of second-line therapies, treatment should be guided by antimicrobial susceptibility testing if possible. Any of the above regimens may be considered for second-line eradication, depending on the first-line therapy that was utilized [45]. One component of salvage regimens is rifabutin, an antibiotic used to treat mycobacterial infections. Eradication rates are typically inferior

compared to regimens that employ levofloxacin and side effects can be severe, including myelosuppression.

Eradication rates in clinical practice are frequently <90%, primarily due to differences in patient populations, genetic diversity of infecting *H. pylori* isolates, and antibiotic resistance patterns. It is therefore recommended that urea breath testing or stool antigen tests be performed post intervention to confirm successful cure. Follow-up testing should be performed at least 4 weeks after the completion of therapy.

Conclusions

Gastric cancer leads to a high number of cancer-related deaths and understanding the risk factors for this disease is of utmost importance in identifying individuals who are most likely to develop malignancy. The risk of developing gastric cancer is dependent on numerous factors including *H. pylori* strain-specific virulence factors, the host genotype, and environmental factors such as diet. Interactions among these factors affect the outcome of long-term colonization of *H. pylori*; therefore, it is important to utilize results from mechanistic studies to identify persons who are most at risk of developing gastric cancer.

Acknowledgments

Grant support: NIH CA-116087, DK-58404, DK-58587, and CA-77955.

Disclosure Statement

The author has no conflicts of interest.

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