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Diet, microbial virulence, and *Helicobacter pylori*-induced gastric cancer

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Gastric adenocarcinoma is a leading cause of cancer-related death worldwide, and *Helicobacter pylori* infection is one of the strongest known risk factors for this malignancy. *H. pylori* strains exhibit a high level of genetic diversity, and the risk of gastric cancer is higher in persons carrying certain strain types (for example, those that contain a *cag* pathogenicity island or type s1 *vacA* alleles) than in persons carrying other strain types. Additional risk factors for gastric cancer include specific human genetic polymorphisms and specific dietary preferences (for example, a high-salt diet or a diet deficient in fruits and vegetables). Finally, iron-deficiency anemia is a risk factor for gastric cancer. Recent studies have provided evidence that several dietary risk factors for gastric cancer directly impact *H. pylori* virulence. In this review article, we discuss mechanisms by which diet can modulate *H. pylori* virulence and thereby influence gastric cancer risk.

Epidemiology of Gastric Cancer

Gastric adenocarcinoma is the second leading cause of cancer-related death worldwide.[1-4] Several different types of cancer can arise in the stomach, including adenocarcinoma, lymphoma and leiomyosarcoma, but adenocarcinoma ifs by far the most common. Two types of gastric adenocarcinoma (intestinal-type and diffuse-type) can be differentiated histologically.[5] The diagnosis of gastric adenocarcinoma often is not established until late in the course of disease, and therefore there is great interest in understanding the factors that contribute to the occurrence of this malignancy, identifying persons who are at highest risk and developing means for gastric cancer prevention.

The incidence of gastric adenocarcinoma varies markedly throughout the world. Incidence rates are currently highest in East Asia, Central America, parts of South America and Eastern Europe.[1,2,6,7] Throughout the world, gastric adenocarcinomas of the distal stomach (body and antrum) occur more commonly in men than in women in a 2:1 ratio.[1,7] There have been marked changes in the incidence of gastric adenocarcinoma over the past 100 y. A century ago, gastric cancer was a leading cause of cancer-related death in developed countries.[8,9] Most gastric adenocarcinomas in the United States in the early 1900s occurred in the distal stomach and were of intestinal-type histology, but the incidence of these tumors in the distal stomach has steadily declined over the past century.[1,8,9] Currently in the United States, distal gastric adenocarcinoma is diagnosed most commonly in elderly persons and occurs more commonly in African-Americans, Hispanic-Americans and Native Americans than in other ethnicities.[10,11] In conjunction with the declining incidence of gastric adenocarcinomas of the distal stomach over the past century, there has been a steady increase in gastric adenocarcinomas of the proximal stomach and gastresophageal junction in the United States and Europe.[12,13]

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Helicobacter pylori as a Risk Factor for Gastric Cancer

Histologic studies performed many decades ago suggested that intestinal-type gastric adenocarcinoma of the distal stomach was usually preceded by gastric inflammation (termed superficial gastritis) and several other histologic alterations, including intestinal metaplasia (presence of intestinal-type epithelium in the stomach), gastric atrophy (loss of specialized cell types such as parietal cells and chief cells), and dysplasia.[5,14] In the early 1980s, the Gram-negative bacterium *Helicobacter pylori* was identified as a causative agent of superficial gastritis,[15] and *H. pylori* colonization of the stomach is now widely recognized as the strongest known risk factor for distal gastric adenocarcinoma.[16,17]

H. pylori is typically acquired early in life and persistently colonizes the human stomach in the absence of antimicrobial treatment.[18,19] *H. pylori* is present in about 50% of the global population worldwide, but its prevalence varies substantially throughout the world.[20] The majority of persons in developing countries are colonized with *H. pylori*, whereas colonization rates are lower in developed countries.[20]

Numerous case-control studies throughout the world have shown that *H. pylori* is associated with an increased risk for intestinal-type adenocarcinoma of the distal stomach,[21,22] but not cancer of the gastric cardia.[23] The risk of gastric cancer conferred by *H. pylori* is similar to the risk of lung cancer conferred by smoking. A prospective study in Japan showed that gastric cancer (both intestinal and diffuse types) developed in a significantly higher number

of *H. pylori*-colonized persons than in uninfected persons,[24] and serologic analyses of stored serum specimens have also provided evidence that *H. pylori* colonization of the stomach precedes the development of cancer.[25,26] Gastric inflammation (superficial gastritis) is consistently detected in *H. pylori*-infected persons,[27] and persistent *H. pylori* colonization of the stomach increases the risk of each of the histologic abnormalities (atrophic gastritis, intestinal metaplasia, and dysplasia) that are considered precursors to gastric adenocarcinoma. There are probably multiple mechanisms by which *H. pylori* infection contributes to the development of gastric cancer, including alterations in DNA induced by chronic inflammation, alterations in cell proliferation or apoptosis, direct effects of *H. pylori* products on host cells and alterations in gastric pH that lead to colonization of the stomach by nitrate-producing bacteria that are not typically found in the acidic stomach.[16,17,28-30]

H. pylori infection is considered one of the most common infectious causes of cancer, but only a small fraction of colonized persons develop gastric cancer. In this review, we consider the factors that are known to influence the risk of *H. pylori*-associated gastric cancer. These include strain-specific variations in *H. pylori* virulence, host genetic factors and diet. We also discuss recently identified relationships among these individual risk factors, focusing in particular on relationships between diet and *H. pylori* virulence.

Microbial Virulence Constituents that Influence Gastric Cancer Risk

There is a high level of genetic variability among isolates of *H. pylori* from unrelated persons.[31] The variability includes strain-specific variation in gene content as well as variation in the sequences of individual genes. Several strain-specific *H. pylori* constituents influence the risk of gastric adenocarcinoma.

One of the *H. pylori* determinants that influences the risk of gastric cancer is a 40 kb chromosomal region known as the *cag* pathogenicity island (PAI).[32,33] This chromosomal region may be either present, present in an incomplete form, or absent in *H. pylori* strains.[34] Genes within the *cag* PAI encode an antigenic effector protein (CagA) as well as proteins that form a type IV bacterial secretion system (T4SS) that exports CagA from adherent *H. pylori* into host cells.[35-38] *H. pylori* strains containing the *cag* PAI (*cag*$^+$ strains) are associated with a significantly higher risk of distal gastric cancer than are *cag*$^-$ strains.[39,40] In contrast to wild-type *H. pylori* strains carrying an intact *cag* PAI, mutant strains lacking *cagA* or defective in *cag* T4SS function fail to cause gastric cancer in rodent models.[41,42]

After CagA is translocated into host epithelial cells, it undergoes tyrosine phosphorylation by Src and Abl kinases at motifs containing the amino acid sequence EPIYA (**Fig. 1**).[35,43] Phospho-CagA interacts with and activates several host cell proteins, including a host cellular phosphatase (SHP-2), leading to morphological alterations such as cell scattering and elongation.[44] Non-phosphorylated CagA also exerts effects within the host cell. For example, non-phosphorylated CagA directly binds PAR1b, a central regulator of cell polarity, and inhibits its kinase activity, an interaction that promotes loss of cell polarity.[45] Non-phosphorylated CagA associates with the epithelial tight-junction scaffolding protein ZO-1 and the transmembrane protein junctional adhesion molecule-A (JAM-A) to cause ineffective assembly of tight-junctions at sites of bacterial attachment,[46] and also activates β-catenin, leading to transcriptional upregulation of genes implicated in cancer.[47,48] The CagA protein of certain *H. pylori* strains also can induce IL-8 expression via NFκB activation,[49] thereby contributing to neutrophil infiltration in the gastric mucosa. Thus, contact between *cag*$^+$ strains and host cells activates multiple signaling pathways that may increase the risk for malignant transformation during the prolonged colonization that is typical of *H. pylori* infection. Compared with wild-type control mice, mice that transgenically express CagA develop increased gastric epithelial cell proliferation and carcinoma in the absence of inflammation.[50] Because of these activities, CagA has been labeled a "bacterial oncoprotein".[51]

Another *H. pylori* constituent linked to the development of gastric cancer is the secreted VacA toxin.[52,53] A 140 kDa precursor VacA protein undergoes proteolytic processing, resulting in an 88 kDa protein that is secreted through an autotransporter pathway.[52,54] Similar to several other autotransporter passenger domains, the secreted VacA protein has a predominantly β-helical structure.[55] VacA forms anion-conductive channels in lipid bilayers and the plasma membrane of cells,[56-58] and therefore, it has been classified as a pore-forming toxin. VacA can cause a wide assortment of alterations in gastric epithelial cells, including cell vacuolation, alteration of plasma membrane permeability, alterations in the permeability of polarized epithelial cell monolayers, increased mitochondrial membrane permeability, autophagy and cell death.[52,53] VacA can also cause alterations in multiple types of immune cells, including T cells, B cells, neutrophils, mast cells and macrophages.[52,54,59-62]

All *H. pylori* strains possess *vacA*, but there is marked variation in *vacA* sequences among strains. The regions of greatest diversity are localized to the 5' region of the gene, which encodes the signal sequence and N-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).[63,64] Type s2 VacA proteins are inactive in most in vitro assays,[63,65,66] and there are detectable differences in the activities of m1 and i1 VacA proteins compared with m2 and i2 VacA proteins, respectively.[64,67,68] Strains containing type s1, i1 and m1 forms of *vacA* are associated with a higher risk of gastric cancer than are strains containing type s2, i2 and m2 forms of *vacA*.[69-71]

Another *H. pylori* constituent that has been linked to gastric cancer risk is an outer membrane protein (BabA) that binds sialyl-Lewis b.[72-75] *H. pylori* strains that contain the *cag* PAI typically contain type s1 forms of *vacA*.[63] Similarly, BabA is expressed more commonly by *cag* PAI-positive strains than *cag* PAI-negative strains.[73,76] Thus, patients can be infected with strains harboring multiple constituents associated with increased gastric cancer risk, with strains lacking most of these constituents, or with strains containing an intermediate assortment of virulence determinants. In East Asia, where the incidence of gastric cancer is much higher than in the United States and Western Europe, most strains contain the *cag* PAI and type s1 *vacA* alleles.[77] Moreover, the CagA proteins produced by East Asian

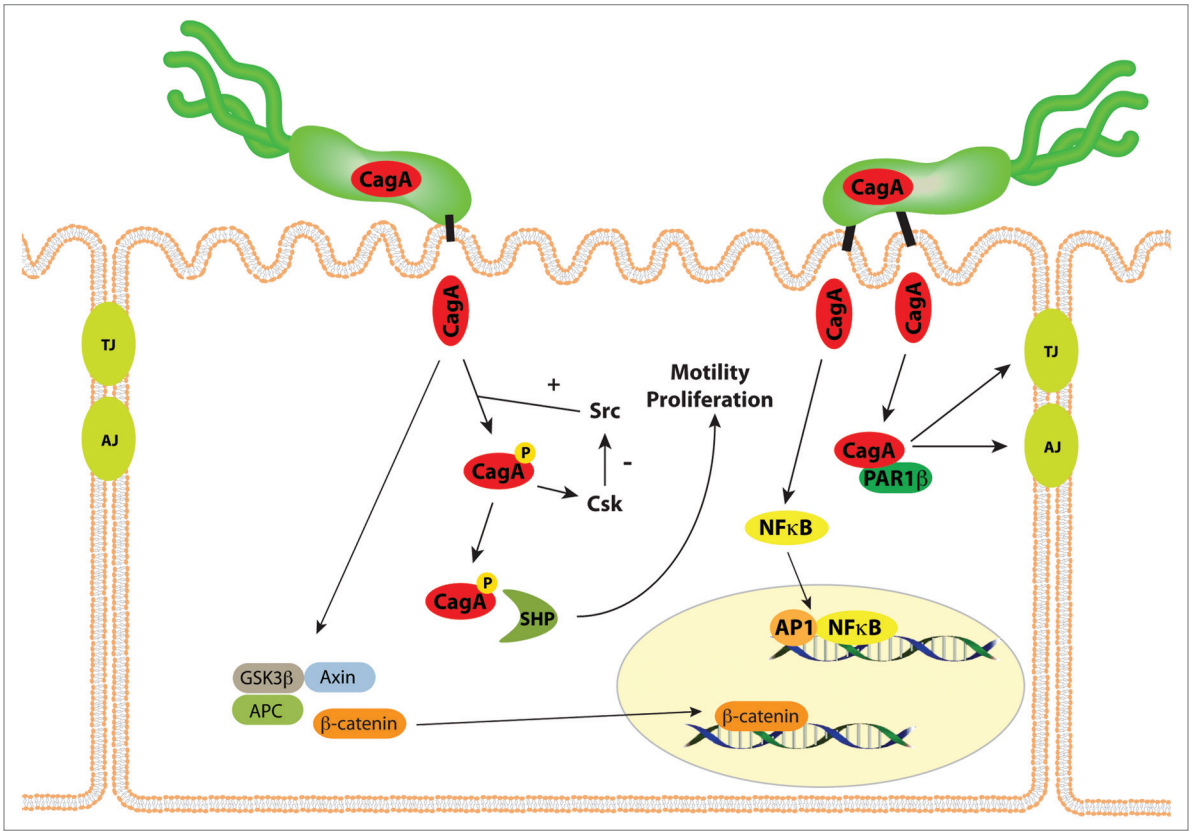

Figure 1. CagA affects multiple signaling pathways within gastric epithelial cells. CagA is translocated into host epithelial cells by the *cag* type IV secretion system and undergoes tyrosine phosphorylation by Src and Abl kinases. Phospho-CagA interacts with and activates several host cell proteins, including a host cellular phosphatase (SHP-2), leading to increased motility and proliferation. Non-phosphorylated CagA directly binds PAR1b, a central regulator of cell polarity and inhibits its kinase activity, an interaction that promotes loss of cell polarity. CagA in an unphosphorylated form activates β-catenin, leading to transcriptional upregulation of genes implicated in cancer. The CagA protein of certain *H. pylori* strains also can induce NFκB activation. Thus, the entry of CagA into host cells activates multiple signaling pathways that may increase the risk for malignant transformation.

strains contain distinctive EPIYA motifs that are associated with increased intracellular tyrosine phosphorylation and activity, compared with Western *H. pylori* strains.[44,78]

Host Genetic Factors that Influence Gastric Cancer Risk

Several host genetic factors influence the likelihood of gastric cancer arising in *H. pylori*-infected persons. IL-1β is a pro-inflammatory cytokine that inhibits gastric acid secretion, and production of IL-1β is increased in the gastric mucosa of *H. pylori*–infected persons compared with uninfected persons.[79] Polymorphisms in the IL-1β gene cluster, specifically *IL-1*β-31 and *IL-1*β-511, are associated with increased IL-1β production and are associated with a significantly increased risk for hypochlorhydria, gastric atrophy and distal gastric adenocarcinoma compared other IL-1β genotypes.[69,80] These relationships are observed among persons infected with *H. pylori,* but not among uninfected persons.[69,80] Another genetic locus harboring polymorphisms that influence gastric cancer risk encodes TNFα, a pro-inflammatory, acid-suppressive cytokine that is present at higher levels in *H. pylori*-colonized human gastric mucosa than in the stomach of uninfected persons.[81] TNFα polymorphisms linked to increased TNFα production are associated with an increased risk of gastric cancer and its precursors.[82]

Genetic polymorphisms associated with increased production of IL-1β and TNFα are associated with increased gastric cancer risk, and conversely, polymorphisms associated with decreased production of the anti-inflammatory cytokine IL-10 are associated with increased risk for distal gastric cancer.[82] Investigations into the combinatorial effects of IL-1β, TNFα and IL-10 polymorphisms on the development of cancer have revealed that the risk of cancer increases progressively with an increasing number of pro-inflammatory polymorphisms; in one study, the presence of three high-risk polymorphisms increased the risk of cancer 27-fold over baseline.[82]

Host genetic factors can influence not only the risk of gastric cancer among *H. pylori*-infected persons, but can also influence the likelihood that individuals become infected with *H. pylori*. Two independent genome-wide association studies (GWAS) and a subsequent meta-analysis showed that the *TLR-1* and *FCGR2A* loci are associated with *H. pylori* sero-prevalence.[83]

The presence of a virulent strain of *H. pylori* in a host with genetic risk factors for gastric cancer results in a particularly high risk for development of gastric cancer. One study analyzed the risk for development of gastric cancer in persons harboring

high- or low-expression *-511* alleles of *IL-1*β, who were infected with *H. pylori* isolates that differed in virulence.[69] Persons harboring a high-expression *IL-1*β allele who were infected with *cagA*$^+$ *H. pylori* strains had a 25-fold increased risk for gastric cancer compared with uninfected persons. The risk for cancer in persons harboring a high-expression *IL-1*β allele who were colonized with an *H. pylori vacA* s1-type strain was increased 87-fold compared with uninfected persons.[69]

Tobacco Use and Gastric Cancer Risk

Tobacco use has been linked to an increased risk for gastric cancer.[84] A meta-analysis of prospective studies concluded that the cumulative risk of gastric cancer risk in male smokers was 1.62 and in female smokers was 1.20, compared with persons who never smoked.[85] The risk for gastric cancer increases in a linear fashion with tobacco exposure, whether measured by cigarettes smoked per day, pack-years, or smoking duration.[85] Moreover, gastric cancer risk is lower in former smokers compared with current smokers. When analyzed in conjunction with *H. pylori* and CagA status, the risk for gastric cancer conferred by tobacco increases synergistically; the relative risks for smokers infected with *H. pylori* were 16.6 and 9.2 for persons harboring CagA$^+$ or CagA$^-$ strains, respectively, when compared with non-smokers who were not infected.[86] In *H. pylori*-infected non-smokers, the same relative risks for persons harboring CagA$^+$ or CagA$^-$ strains were 6.1 and 2.4 respectively, compared with uninfected non-smokers.[86]

Dietary Risk Factors for Gastric Cancer

Epidemiologic studies throughout the world have shown a relationship between diet and gastric cancer risk. The diets that are most commonly linked to high gastric cancer risk are those that are rich in salted, pickled, smoked or poorly preserved foods, those with a high meat content and those with low fruit and vegetable content.[87-93]

A link between high salt consumption and increased gastric cancer risk has been reported in numerous studies.[87,94,95] Dietary salt intake varies widely among humans, and in some populations with a high incidence of gastric cancer, median dietary salt intakes of 46 g per day have been reported.[96,97] One study analyzed urinary sodium excretion in persons from 24 different countries and showed a strong correlation between salt intake and gastric cancer mortality rates.[98] In Colombia, the consumption of high levels of salt (as measured by high urinary sodium-to-creatinine ratios) was associated with an increased risk for precancerous gastric lesions (chronic atrophic gastritis, intestinal metaplasia and dysplasia) compared with what is observed in persons who consume lower levels of salt.[99] Additionally, a prospective study of a Japanese population, conducted over a 14 y period, reported that *H. pylori*-infected subjects consuming a high-salt diet had an increased risk of gastric cancer when compared with *H. pylori*-infected subjects who consumed lower levels of salt.[100]

Meta-analyses of case-control studies have shown that a high intake of fruits and non-starchy vegetables confers a significant benefit against the development of stomach cancer, an effect that is stronger in Asia than in the United States or Europe. Specifically, flavonoid intake is associated with a significant reduction (20%) in gastric cancer risk in women.[101] A recent meta-analysis demonstrated that dietary fiber intake is inversely associated with gastric cancer risk.[102] Vitamin C has also been studied as a potential protective factor against the development of gastric cancer, likely through its antioxidant effects. Higher plasma levels of vitamin C have been associated with a lower risk for gastric cancer, irrespective of anatomic site[103,104]; however, dietary intake of vitamin C has not been associated with a significantly reduced risk for cancer of the stomach.[101] A weak positive association has been found between ingestion of alcohol and the development of distal, but not proximal, gastric cancer.[105] Finally, non-steroidal anti-inflammatory drug use has been associated with a decreased risk of gastric cancer.[106]

Over the past century, there have been numerous changes in methods for storing and preserving food in developed countries. The availability of refrigeration has resulted in increased consumption of fresh fruit and vegetables, decreased reliance on older preservative methods (salt, curing or smoking) and a reduction in the consumption of spoiled food.[107] The gradual decrease in gastric cancer rates in many populations over the last century may be at least partially attributable to the changes in diet that have accompanied refrigeration.

Iron Deficiency and Gastric Cancer Risk

Iron deficiency is associated with an increased risk for gastric cancer, as well as neoplasms that arise elsewhere in the gastrointestinal tract.[108-113] There are multiple mechanisms through which iron deficiency may arise, including blood loss and dietary deficiency of iron. Among the many possible causes of blood loss, colonization by certain *H. pylori* strains has been associated with hemorrhagic gastritis and a resulting loss of iron.[114] Long-term *H. pylori* infection can also lead to the development of gastric atrophy, which results in hypochlorhydria, lower ascorbic acid levels and a concomitant reduced absorption of iron.[115] Case-control studies have demonstrated an inverse relationship between dietary iron intake and gastric adenocarcinoma,[108,109,111,116] which suggests that not only iron deficiency resulting from blood loss but also iron deficiency resulting from a low-iron diet is relevant.

Analysis of Diet in Animal Models of *H. pylori* Infection

Epidemiologic studies of diet in humans are subject to many limitations, including a reliance on patient reporting and difficulty in ascertaining the foods that were consumed decades prior to the development of gastric cancer. Moreover, it is difficult to determine whether dietary parameters are causally linked to the development gastric cancer or merely markers for other factors that are important in gastric cancer pathogenesis. To further investigate potential relationships between diet and gastric cancer

risk, several studies have tested the role of diet in rodent models of *H. pylori*-induced gastric cancer.

Salt

The effects of a high-salt diet on *H. pylori* infection and gastric cancer have been investigated using both mouse and gerbil models. One study in mice showed that a high salt diet enhanced levels of *H. pylori* colonization in the stomach and resulted in increased parietal cell loss.[117] Other studies reported that a high salt diet had relatively little effect on disease outcomes in *H. pylori*-infected mice.[118,119] Most of these studies in mice were conducted using *H. pylori* strains that have a non-functional *cag* type IV secretion system.

Several studies have provided evidence indicating that a high salt diet contributes to gastric carcinogenesis in Mongolian gerbil models.[120,121] One study reported that *H. pylori* infection and a high-salt diet could independently induce atrophic gastritis and intestinal metaplasia in Mongolian gerbils.[122] Other studies provided evidence that the presence of *H. pylori* and a high-salt diet have a synergistic effect on gastric carcinogenesis in a Mongolian gerbil model when the animals also receive a chemical carcinogen.[123,124]

To further investigate the effects of a high-salt diet on *H. pylori*-induced carcinogenesis in a gerbil model, a recent study infected Mongolian gerbils with a wild-type *cagA*$^+$ *H. pylori* strain and maintained the animals on a regular diet or a high-salt diet.[42] At 4 mo postinfection, gastric adenocarcinoma was detected in a significantly higher proportion of the infected animals on a high-salt diet than in infected animals on a regular diet.[42] Infected animals that were fed a high-salt diet had more severe gastric inflammation compared with those on a regular diet.[42] Hypochlorhydria, parietal cell loss and high levels of gastric IL-1β were detected in the animals that developed gastric cancer. Animals infected with a *cagA* mutant strain and fed a high salt diet had low levels of gastric inflammation and did not develop hypochlorhydria or gastric cancer. Similarly, a high salt diet did not cause the development of gastric cancer in uninfected animals.[42] These results indicate that a high-salt diet potentiates the carcinogenic effects of *cagA*$^+$ *H. pylori* strains.

The high-salt diets used in these studies (containing 6% to 8% sodium chloride) approximate the concentration of sodium chloride in some foods consumed by humans. For example, dried fish is often preserved in 3 to 20% salt, pickled foods contain up to 25% salt and soy sauce contains 19% salt.[96,117] In contrast to human diets, which can vary considerably from day to day, the animals in these studies were fed a high-salt diet with no variation throughout the experiments.

Iron

Iron deficiency in rats accelerates carcinogen-induced gastrointestinal cancer and metastasis.[125] To evaluate whether *H. pylori* infection is sufficient to induce iron deficiency, several studies have examined the effects of chronic *Helicobacter* infection on iron status in rodents.[126,127] One system that has been extensively utilized to study *Helicobacter*-induced pathogenesis is the transgenic INS-GAS mouse model. INS-GAS mice overexpress human gastrin and spontaneously develop gastric cancer, but this requires the virtual lifetime of the animal (2 y).[118] Concomitant infection with *Helicobacter felis* accelerates this process, suggesting that persistently elevated gastrin levels synergize with *Helicobacter* to augment cancer progression. *Helicobacter* infection in INS-GAS mice leads to corpus-predominant gastritis and parietal cell loss,[30] similar to changes that develop in humans; thus this model recapitulates many features of gastric cancer in humans. A recent study demonstrated that infection of INS-GAS mice with *H. felis* results in decreased serum iron concentrations, reduced numbers of parietal cells and altered expression of host genes important in iron physiology such as hepcidin, ferroportin 1 and transferrin receptor 1.[128] Notably, *H. felis* does not contain many of the virulence factors present in *H. pylori*, such as the *cag* pathogenicity island, which limits its applicability to studies of *H. pylori* pathogenesis.

A recent study analyzed the effect of dietary iron depletion on the development of *H. pylori*-induced cancer in gerbils infected with a *cagA*$^+$ *H. pylori* strain.[129] *H. pylori* infection induced more severe gastritis in iron-depleted gerbils than in iron-replete gerbils. Gastritis also developed earlier in *H. pylori*-infected iron-depleted gerbils compared with infected iron-replete gerbils.[129] Consistent with the increased severity of gastric inflammation, dysplasia and gastric adenocarcinoma occurred significantly more frequently in *H. pylori*-infected iron-depleted gerbils compared with iron-replete gerbils.[129] A low iron diet did not produce these effects in animals infected with an isogenic *cagA*-mutant strain. These data demonstrate that dietary iron depletion significantly increases the severity of gastric inflammation and accelerates the development of *H. pylori*-induced premalignant and malignant lesions in a rodent model of gastric cancer.

Cholesterol

H. pylori is auxotrophic for cholesterol,[130] and therefore, the dietary content of cholesterol could potentially have an impact on *H. pylori* colonization of the stomach or *H. pylori*-induced gastric disease. In vitro experiments showed that *H. pylori* follows a cholesterol gradient and extracts lipid from plasma membranes of epithelial cells for subsequent glucosylation. Excessive cholesterol promoted phagocytosis of *H. pylori* by antigen-presenting cells, such as macrophages and dendritic cells, and enhances antigen-specific T cell responses.[130] A cholesterol-rich diet led to T cell-dependent reduction of the *H. pylori* burden in the stomach. Thus, a diet with high cholesterol content may reduce the risk of subsequent inflammation and injury induced by *H. pylori*.

Possible Mechanisms by which Diet May Influence Gastric Cancer Risk

There are several potential mechanisms by which dietary constituents may protect against or augment the development of gastric cancer. One possibility is that dietary factors may exert direct effects on the host. For example, direct effects of dietary constituents on the gastric epithelium might either raise or lower the threshold for malignant transformation, or dietary components might damage the gastric mucosa, thereby allowing increased entry of carcinogens into gastric tissue. Certain dietary components are known to interact with intestinal immune receptors and thereby regulate intestinal immunity,[131] and potentially similar

phenomena might occur in the stomach. The composition of the diet might influence the composition of the gastric microbiota, or may favor the proliferation of *H. pylori* variants with adaptive properties. Dietary factors may also influence epigenetic alterations, as documented in a recent study which reported that dietary folic acid supplementation protected against loss of global DNA methylation and markedly reduced the development of gastric dysplasia and inflammation in *Helicobacter*-infected INS-GAS mice.[132] Finally, dietary constituents might modulate gene expression in *H. pylori*, resulting in increased or decreased production of bacterial virulence factors. These potential mechanisms by which diet can modulate gastric cancer risk are not mutually exclusive, and it seems possible that several may be operative. Several recent studies have focused in particular on the concept that diet may directly influence the pathogenic potential of *H. pylori* by augmenting the expression and function of cancer-associated microbial virulence determinants. In the following sections, we review these recent findings.

Effect of Salt on *H. pylori* Virulence

Gene expression in several bacterial pathogens, including *Vibrio cholerae, Salmonella enterica*, *Listeria monocytogenes* and *Campylobacter jejuni* is regulated in response to salt concentration or osmotic stress.[133] Similarly, *H. pylori* gene expression is altered in response to changes in the concentrations of sodium chloride present in the bacterial culture medium.[134-136] In response to high sodium chloride concentrations, *H. pylori* cells change from a typical spiral shape to a more elongated shape and form chains.[135] The sodium chloride concentrations tested in these in vitro studies ranged from 0.25% (43 mmol/L) to 2.0% (342 mmol/L), which it hypothesized to be similar to the gastric luminal salt concentrations that are attained in some populations.[134] However, salt concentrations in the gastric mucus layer (the natural niche for *H. pylori*) are likely to be lower than in the gastric lumen.

Both transcriptional studies and proteomic studies have revealed increased expression of *cagA* in response to high salt conditions.[134,136] Concordant with the in vitro results, increased *cagA* transcription was detected in vivo in *H. pylori*-infected gerbils that were fed a high-salt diet compared with those on a regular diet.[42] Analysis of 36 *H. pylori* strains isolated from unrelated persons revealed marked differences among strains in salt-responsive CagA expression.[137] Sequence analysis of the *cagA* promoter region in these strains revealed a DNA motif (TAATGA) that was present in either one or two copies. Salt-induced upregulation of CagA expression was detected more commonly in strains containing two copies of the TAATGA motif than in strains containing one copy.[137] Mutagenesis experiments confirmed that two copies of the TAATGA motif are required for salt-induced upregulation of CagA expression.

Effects of Iron on *H. pylori* Gene Expression and Virulence

Iron is an essential micronutrient for virtually all microorganisms, including *H. pylori*. Due to limited availability of free iron in the host, *H. pylori* has evolved multiple mechanisms for iron acquisition in vivo.[138-140] In contrast to many mucosal pathogens, *H. pylori* does not synthesize siderophores,[140] but can utilize several iron-containing host factors, including lactoferrin, transferrin and hemoglobin, as iron sources. Transferrin and lactoferrin in the iron-bound state can support growth of *H. pylori* as the sole iron source. *H. pylori* can utilize both Fe^{2+} (ferrous) iron, which is relatively soluble and readily available under anaerobic and acidic conditions, as well as Fe^{3+} (ferric) iron, which is typically complexed with binding molecules such as lactoferrin, transferrin, or heme.[141] Several *H. pylori* proteins are known or predicted to have important roles in iron acquisition. These include three putative FecA homologs (which are predicted to recognize ferric iron), FrpB (which binds heme), TonB/ExbB/ExbD proteins (which aid in iron transport) and FeoNB (which is predicted to function as a ferrous iron transport).[138-141] Iron storage proteins are produced by *H. pylori* to ensure that oxidative stress is not induced by the presence of free iron within the bacterial cell. These include NapA and Pfr, ferritin-like proteins that function to store iron in a non-reactive state.[140,141]

Experiments using *H. pylori* DNA microarrays have detected numerous growth phase-dependent gene expression changes that occur in response to iron starvation conditions.[142] Several virulence genes were found to be differentially expressed, including *cagA* and *vacA*.[142] One mechanism by which *H. pylori* regulates gene expression in response to environmental iron concentrations involves the global ferric uptake regulator (Fur). In comparison to Fur proteins in most other bacteria, *H. pylori* Fur appears to be unique in that it can function as either a transcriptional repressor or a transcriptional activator under iron-limiting or iron-replete conditions.[140,141] This is due to the ability of Fur to bind to cognate promoters in either an iron-bound or an iron-free (apo) form. Iron-bound Fur can repress *fur* transcription, and thus, *fur* expression is governed by an autoregulatory mechanism.[140,141] Global analyses have been conducted using DNA microarrays and proteomics techniques to identify members of the *H. pylori* Fur regulon; these include genes involved in iron storage and transport, as well as genes involved in motility, energy metabolism and respiration.[143-145] The consensus sequence recognized by Fur is a 7-1-7 motif with dyad symmetry, and a recent study used this sequence as a probe to identify multiple additional targets within the *H. pylori* genome that are regulated by Fur.[146] Fur-regulated genes not only included those encoding previously identified iron acquisition systems but also genes encoding virulence proteins, such as CagA and gamma-glutamyltransferase (GGT).[146]

To evaluate the relationship between iron availability and virulence gene expression in the context of host cells, a recent study utilized a Transwell system containing polarized MDCK epithelial cells to demonstrate that wild-type *H. pylori* could replicate and form microcolonies on the apical surface of epithelial cell monolayers, whereas *cagA*$^-$ isogenic mutants did not.[147] In contrast, the *cagA*$^-$ mutant strain was able to form microcolonies if ferric iron was added to the apical chamber.[148] More in-depth mechanistic studies revealed that CagA, as well as VacA, coordinate an orchestrated mislocalization of transferrin and transferrin receptors from the basolateral surface to apical surface microniches where *H. pylori* were attached[148] (**Fig. 2**). This action

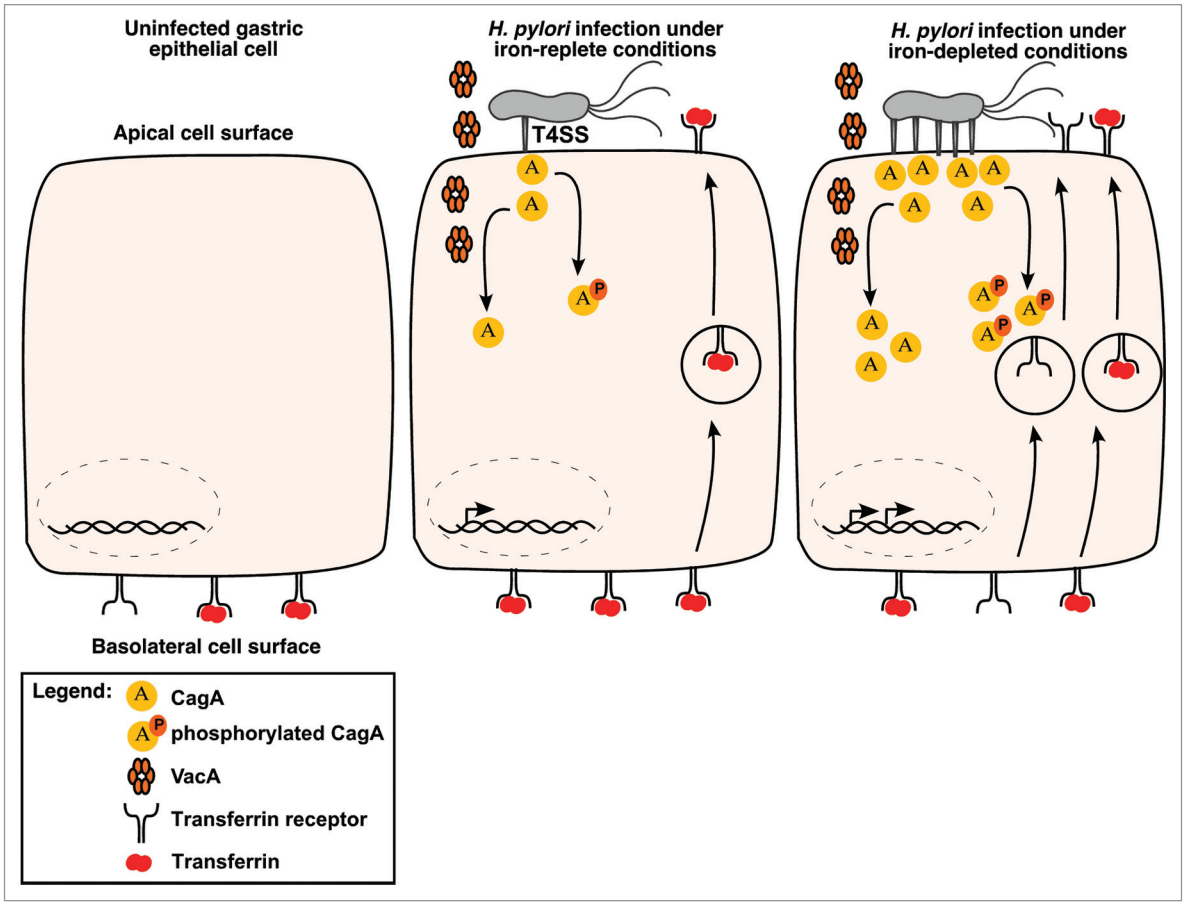

Figure 2. Effect of low-iron conditions on interactions between *H. pylori* and host cells. CagA and VacA each induce mislocalization of transferrin receptors from the basolateral cell surface to the apical surface where *H. pylori* are localized. Under iron-deplete conditions, CagA production is increased, the formation of pili associated with the *cag* type IV secretion system is increased and translocation of CagA into host cells occurs at increased levels. This potentially leads to a more robust mislocalization of transferrin receptors to the apical membrane.

of CagA was dependent on the presence of EPIYA phosphorylation motifs. The role of CagA in promoting bacterial growth in co-culture systems mimics results observed in vivo, as *H. pylori cagA*$^-$ mutant strains exhibited a significant decrease in colonization density in Mongolian gerbils compared with wild-type *H. pylori*.[148]

To further investigate relationships between iron concentration and *H. pylori*-host cell interactions, *H. pylori* has been cultured in vitro under iron-replete or iron-restricted conditions induced by iron chelation with dipyridyl and then co-cultured with gastric epithelial cells. Cells co-cultured with *H. pylori* grown under iron-restricted conditions contained significantly higher levels of phosphorylated CagA and produced significantly higher levels of IL-8 than did cells that were co-cultured with bacteria grown under iron-replete conditions.[129] Scanning EM studies to analyze the formation of *cag* T4SS-associated pili at the bacteria-host cell interface revealed that the number of pili formed by *H. pylori* grown under iron-restricted conditions was significantly higher than the number formed by *H. pylori* grown under iron-replete conditions.[129] The enhanced production of pili stimulated by dipyridyl was abrogated following the addition of exogenous iron. These experiments indicate that exposure of *H. pylori* to low iron conditions enhances the ability of the bacteria to cause alterations in gastric epithelial cells.

An important goal is to differentiate the specific effects of iron deficiency on *H. pylori* virulence vs. the effects of iron deficiency on the host. This topic has been addressed by examining proinflammatory cytokine levels in gastric tissue from gerbils. Infection of gerbils with *H. pylori* resulted in significantly increased gastric levels of *IL-1*β, *IFN*γ and *TNF*α, compared with levels in uninfected control animals; however, there were no differences in the levels of these cytokines between *H. pylori*-infected gerbils under iron-replete vs. iron-depleted conditions.[129] Although these results do not completely exclude effects exerted by iron deficiency on the host, these data, when viewed within the context of in vitro data, suggest that the direct effects of iron deficiency on *H. pylori*, particularly enhanced function of the *cag* T4SS, likely contribute to the increased severity and incidence of gastric disease observed in this model.

Selection of *H. pylori* Variants with Adaptive Properties in Response to Dietary Conditions

To test the hypothesis that *H. pylori* adapts to particular environmental conditions associated with changes in diet, *H. pylori* strains that were cultured from gerbils fed a low iron diet or a regular diet have been compared using two-dimensional (2D) DIGE/mass spectrometry. Multiple proteins differed in abundance when comparing *H. pylori* strains isolated from iron-deplete vs. iron-replete gerbils, including proteins that mediate survival, microbial adherence and function of the *cag* T4SS.[129] For example, *H. pylori* FlaA and FlaB, the major flagellin subunits, were significantly upregulated in bacteria cultured from the iron-deplete animals. *H. pylori* strains isolated from iron-depleted gerbils also expressed significantly higher levels of CagA.[129] To further investigate differences between these two types of *H. pylori* strains, gastric epithelial cells were co-cultured with *H. pylori* strains isolated from iron-depleted gerbils or iron-replete gerbils. The former cells contained significantly higher levels of phosphorylated CagA and produced significantly higher levels of IL-8 when compared with cells co-cultured with strains isolated from iron-replete gerbils.[129] When co-cultured with gastric epithelial cells, the number of *cag* T4SS pili was significantly increased for strains isolated from iron-depleted vs. iron replete gerbils.[129]

As another approach for analyzing relationships between properties of the bacteria and the iron state of the host, the properties of *H. pylori* strains isolated from patients in a geographic region with a high risk of gastric cancer have been correlated with serum ferritin levels of the corresponding patients. Strains from patients with low serum ferritin levels induced more robust inflammatory responses compared with strains isolated from patients with high ferritin levels.[129] Collectively, these results suggest that prolonged exposure of *H. pylori* to low iron conditions in vivo leads to stable changes in the bacteria, including increased activity of *cag* PAI-mediated phenotypes.

Conclusions

Gastric cancer risk is determined by many factors, including properties of the *H. pylori* strain, the host genotype and environmental exposures such as diet, each affecting the level of long-term interactions between *H. pylori* and humans. Among the approximately one-half of the world's population that is infected with *H. pylori*, fewer than 5% will ever develop gastric cancer. Side effects associated with *H. pylori* treatment, including the development of antibiotic resistance in *H. pylori* and other commensal flora, preclude the use of *H. pylori* eradication campaigns on a population-wide basis. Thus, there is great interest in developing techniques to identify sub-populations at high risk for disease. Such targeted therapy requires the use of biological markers other than simply detection of *H. pylori per se*. For example, iron deficient persons with high-expression *IL-1*β polymorphisms who are colonized by *cag*$^+$ strains may represent a population that is most likely to derive benefit from *H. pylori* eradication, and treatment of such persons could result in a substantially reduced cancer risk. In future studies, it will be important to gain deeper insight into the pathogenesis of *H. pylori*-induced gastric adenocarcinoma, not only to develop more effective means for preventing and treating this cancer, but also because it might serve as a paradigm for understanding the role of chronic inflammation in the pathogenesis of other malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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