Risk factors associated with gastric malignancy during chronic *Helicobacter pylori* Infection

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Abstract
Chronic *Helicobacter pylori* (*Hp*) infection is considered to be the single most important risk factor for the development of gastric adenocarcinoma in humans, which is a leading cause of cancer-related death worldwide. Nonetheless, *Hp* infection does not always progress to malignancy, and, gastric adenocarcinoma can occur in the absence of detectable *Hp* carriage, highlighting the complex and multifactorial nature of gastric cancer. Here we review known contributors to gastric malignancy, including *Hp* virulence factors, featuring the vacuolating cytotoxin (VacA), the cytotoxin-associated gene A (CagA), and other bacterial components that promote chemotaxis, colonization, and the establishment of chronic inflammation. In addition, we discuss host factors including sex, age, and genetic polymorphisms associated with host inflammation. Moreover, we consider environmental variables that influence cancer risk, such as nutritional status, socio-economic status, and smoking. In addition to these relatively well-studied contributors to gastric cancer risk, the resident gastric microflora in humans have more recently been proposed as an additional risk factor for disease progression in *Hp*-infected individuals. Molecular approaches for microbe identification have revealed differences in the gastric microbiota composition between cancer and non-cancerous patients, as well as infected and uninfected individuals. Although the reasons underlying differences in microbial community structures are not entirely understood, gastric atrophy and hypochlorhydria that accompany chronic *Hp* infection may be a critical driver of gastric dysbiosis that promote colonization of microbes that contribute to increased risk of malignancy. However, definitive evidence that the gastric microbiota influences the emergence of gastric cancer does not exist. In summary, while controversial and unresolved, the importance of the gastric microbiota as a risk factor for gastric malignancy is a vital area of current research.

Keywords: *Helicobacter pylori*, gastric cancer, adenocarcinoma, risk factors, virulence factors, vacuolating cytotoxin, VacA, CagA, gastric microbiota, microbiome, host factors, polymorphism, co-infection, hypochlorhydria, inflammation, nutritional factors.
1. Introduction

Gastric adenocarcinoma is the fifth most common malignancy in the human population, and worldwide, is one of the leading causes of cancer-related deaths\(^1\). For nearly 40 years, the single most important risk factor for the elaboration of gastric adenocarcinoma has been recognized to be chronic infection with the gastric bacterium, Helicobacter pylori (Hp)\(^2\)-\(^4\). The rate of gastric cancer is significantly lower in individuals that have undergone Hp eradication therapy\(^5\)-\(^9\). However, the question of whether Hp infection should be universally eradicated regardless of life stage, or the severity of damage within the gastric mucosa\(^10\)-\(^13\), is particularly interesting, considering increasing evidence that Hp carriage may convey advantages to children at younger ages\(^14,15\). Ultimately, any policy regarding comprehensive eradication of Hp must be based on evidence-based analysis of risk factors promoting the advancement of chronic infection to malignant disease. In this review, we consider the contributions of both well-studied and emerging-risk factors for the progression of chronic Hp infection to gastric cancer.

2. Known risk factors promoting the advancement of chronic Hp infection to gastric cancer in humans

Worldwide, over 4.4 billion individuals were infected with Hp in 2015, with the infection rates varying among regions\(^16\). Latin America, the Caribbean, and Africa had the highest prevalence of Hp infection, whereas the lowest prevalence of Hp infection was identified in North America\(^17\). As Hp infection is regarded as the major contributor to the development of gastric cancer, the prevalence of Hp infection should geographically correspond to the regions with highest rate of gastric cancer. While this logic certainly holds true for explaining the relatively low gastric cancer cases in the Western countries\(^17\), discrepancies emerge when this reasoning is applied to cases in the Eastern countries. Within Asia, the prevalence of Hp infection is reported to be higher in the Indian subcontinent (including India, Pakistan, and Bangladesh) compared to that of the East Asian countries (including South Korea, Japan, China)\(^17\). When compared to India, however, Japan experiences about 100-fold higher incidences of gastric cancer\(^18\). South Korea, Mongolia, and Japan have the top three rates in the world\(^19,20\), suggesting that the region-specific factors can contribute to disease emergence. Within Hp-infected individuals, complex interactions between bacterial virulence factors, host susceptibility genes, and environmental factors, are recognized as risk determinants for progression of chronic infection to gastric cancer\(^21,22\).

2.A. Virulence factors and Hp-dependent disease

Only recently have animal studies revealed that subsequent to transmission to the stomach, Hp can colonize deep within the glands of the gastric mucosa\(^23\)-\(^25\). Colonization is accompanied by the generation of a sustained inflammatory state, as well as other changes to gastric tissue, which is likely to involve locally-activated myeloid cells and shifts in the portfolio of circulating effector T cells. The shaping of the chronic Hp infection microenvironment is influenced by a repertoire of virulence factors, whose elaboration are sometimes strain-specific. Among the most highly studied virulence factors associated with disease-causing strains of Hp, are the vacuolating cytotoxin (VacA) and the
cytotoxin-associated gene A (CagA). However, the portfolio of Hp factors that contribute to colonization, long-term infection, and disease progression, extend well beyond VacA and CagA.

2.A.1. VacA

During infection of the gastric epithelium, Hp secrete VacA, which is a pore-forming intracellular-acting protein exotoxin, that is best known for the biogenesis of large intracellular vacuoles in many epithelial-derived cell lines. While almost all Hp strains possess the gene encoding VacA, substantial DNA sequence variation occurs within three distinct regions of vacA, called the s, m, and i regions. Epidemiological studies of human populations have revealed distinct alleles within each of these regions associated with increased risk for Hp-mediated gastric pathophysiology and disease. Hp strains that harbor s1/m1/i1 vacA express a form of toxin associated with greater cytotoxicity and are closely associated with the development of gastric cancer.

Despite intensive study over the past 30 years, the in vivo role of VacA as an important determinant of chronic Hp infection and disease progression remains poorly understood. Animal studies support the idea that VacA is important for colonization and pathogenesis of Hp. Direct administration of VacA-containing Hp bacterial extracts to the stomachs of mice by oral gavage revealed substantial damage to the gastric epithelium, leading to the widely-held belief that the central role for VacA during infection is to damage the gastric epithelium. Consistent with such a model, VacA has been demonstrated to induce the death of both primary and immortalized cells by several different mechanisms. However, cellular modulatory activities of VacA, not associated with toxin-mediated killing of host cells, have also been identified at lower concentrations of VacA. These activities include intracellular vacuole biogenesis (vacuolation), targeting of mitochondria to disrupt metabolic homeostasis, and the modulation of cellular tight junctions. The presence of VacA also induces autophagy. Within the context of in vivo Hp infection, VacA may also contribute to decreased acid production and mucus secretion, alterations that have been previously associated with gastric Hp infection.

In addition to the modulatory effects of VacA associated with gastric epithelial cells, multiple studies have indicated that VacA also targets immune cells, including myeloid cells, in a manner that modulates regulatory T-cell proliferation.

2.A.2. CagA

CagA is an important Hp virulence factor, encoded by a gene within a pathogenicity island, contributes to the risk of developing gastric cancer, and is classified as the only bacterial oncoprotein known. Patients infected with CagA+ strains of Hp are at a 5.8-fold higher risk to develop gastric cancer than strains not producing CagA. In contrast to VacA, which is secreted into the extracellular milieu, delivery of CagA to host cells requires a direct contact via the Type IV Secretion System pilus. Upon entry into gastric epithelial cells, CagA is phosphorylated by host oncoproteins, such as c-Abl and c-Src tyrosine kinases, which triggers downstream signaling believed to facilitate Hp persistence. Apoptosis, inflammation, cell adhesion, and other core cellular functions have been found to be altered due to CagA effects on signaling, which may contribute to the development of gastric malignancy.
example, CagA promotes an anti-apoptotic state through the inactivation of pro-apoptotic factors, while at the same increases expression of anti-apoptotic factors. CagA-mediated signaling also activates β-catenin, a transcription factor that regulates cancer related genes. In addition, the cag-pathogenicity island promotes the inflammatory microenvironment that causes cumulative gastric damage, a potential contributor to the development of gastric cancer.

2.A.3. Additional virulence factors

While VacA and CagA are perhaps the most well-studied Hp virulence factors, additional critical virulence strategies have been identified to facilitate chronic infection.

2.A.3.a Motility

Subsequent to transmission into the stomach lumen, Hp colonize the gastric tissue, in part by flagellar-mediated motility deep into the gastric glands associated with the mucosa. Hp possess several chemotaxis systems that have been demonstrated to be critical for Hp colonization and persistence. Chemotaxis allows Hp to move out of the acidic environment and into the gastric glands during the initial colonization.

2.A.3.b Adherence

Hp generate many outer membrane proteins, some of which mediate bacterial adhesion to host cell surfaces. BabA is an adhesin required for establishing chronic infection, by binding to fucosylated blood group antigens expressed on gastric epithelial cells and gastric mucus. SabA, another outer membrane protein (OMP) expressed by Hp, binds to sialylated glycans on the host cell surface for attachment. Hp-infected individuals have elevated levels of sialylated glycoconjugates, that decrease upon infection eradication.

2.A.3.c. Establishment of the inflammatory microenvironment

One of the most-studied gastric alterations accompanying Hp infection is the establishment of a persistent inflammatory microenvironment, which includes increases in tissue and circulatory levels of pro-inflammatory cytokines. Sustained inflammation, not only results in persistent tissue damage, but also can result in the enrichment of cancer stem cells (CSCs), and ultimately increase the risk of developing gastric cancer. Outer Inflammatory Protein A (OipA) is expressed by clinical Hp strains, and is associated with increased expression of the pro-inflammatory cytokine IL-8. OipA phosphorylates focal adhesion kinase, which activates a signaling cascade that results in an increased IL-8 expression. Another virulence factor that contributes to inflammation is neutrophil activating protein A (NapA), which interacts with Toll-like receptors (TLRs) during infection. TLR signaling results in neutrophil trans-endothelial migration, which contributes to Hp persistence by promoting the sustained inflammatory environment through the production of reactive oxygen species, resulting in sustained injury to the gastric tissue and disruption of epithelial barrier.

2.B. Host factors and Hp-dependent disease

While genotypic variance of infecting Hp strains can partially account for infection outcome and disease risk, additional factors have also been implicated to contribute to progression of chronic infection to serious gastric pathophysiology and malignancy. Predisposing elements for
the acquisition of \textit{Hp}, as well as \textit{Hp}-associated diseases, involve both host genetic differences and environmental influences.

2.B.1. Age.

The acquisition of \textit{Hp} typically occurs during early childhood, before the age of 3 years, and the risk declines drastically after the age of 5 years\textsuperscript{69}. \textit{Hp} transmission rates to young children are very high within families with \textit{Hp}-infected members\textsuperscript{70}.

2.B.2. Host genetic polymorphisms associated with increased inflammation

During \textit{Hp} infection, the immune response is controlled, in part, by the level of pro- and anti-inflammatory cytokines secreted by both epithelial cells, myeloid cells, and T lymphocytes and immune cells, including Th1, Th2, Th17, and Treg cells\textsuperscript{71}. Host polymorphisms in the genes encoding specific cytokines can increase the degree of inflammation, making some \textit{Hp}-infected individuals more susceptible to the development of gastric cancer.

2.B.2.a. IL-1\textbeta

IL-1\textbeta an important mediator of the inflammatory response, and elaborates function associated with multiple cellular processes, including programmed cell death, and cell differentiation and proliferation. As a pro-inflammatory cytokine, the overproduction of IL-1\textbeta by macrophages influences the pathophysiology associated with \textit{Hp}-infected individuals\textsuperscript{72}. IL-1 genetic polymorphisms linked to increased IL-1\textbeta expression in humans are described to be associated with the development of \textit{Hp}-related gastric diseases, including intestinal metaplasia, atrophic gastritis, and gastric cancer\textsuperscript{73,74}.

2.B.2.b. TNF-\alpha

TNF-\alpha plays an important role in maintaining homeostasis of the immune system and host defense, and is overexpressed in the gastric mucosa of \textit{Hp}-infected patients\textsuperscript{75,76}. In particular, TNF-\alpha-307A (historically mislabeled as position 308) is described to increase its expression\textsuperscript{77} and is associated with increased risk of developing gastric cancer\textsuperscript{78-80}. While additional polymorphisms have been detected at TNF-\alpha-238 (only in Eastern populations), -857, -863, and -1031, not all identified single nucleotide polymorphisms (SNP) have been associated with gastric malignancy\textsuperscript{80}. Notably, there is not complete consensus as to the biological relevance of the TNF-\alpha-307A SNP, as another study reported that mucosal expression of TNF-\alpha or inflammation, in response to \textit{Hp} infection between populations harboring the parental sequence and populations harboring the TNF-\alpha-307A SNP, were not statistically different\textsuperscript{73}.

2.B.2.c. IL-8

IL-8 is secreted by phagocytes and mesenchymal cells, and functions as a chemoattractant for neutrophils\textsuperscript{81}. Increased expression of IL-8 in the gastric mucosa is highly associated with gastric cancer, as it potentiates the epithelial-mesenchymal transition, increases angiogenesis, and promotes extracellular matrix remodeling\textsuperscript{82,83}. In Japan, a positive association was discovered between \textit{Hp}-positive gastric cancer patients with a genetic polymorphism of pro-inflammatory IL-8\textsuperscript{84}. Carriers of IL-8-251A displayed more severe gastric atrophy than in individuals carrying IL-8-251T/T\textsuperscript{84,85}.

2.B.2.d. IL-10
IL-10 is an anti-inflammatory cytokine, generated by monocytes and Th2 cells to curb and control the degree of inflammation perpetuated by the pro-inflammatory cytokines released during infection. Polymorphisms of anti-inflammatory IL-10 are also reported to be associated with gastric atrophy, intestinal metaplasia, and gastric cancer. A study of 207 Hp-infected individuals with chronic gastritis revealed that the carriers of the IL-10−1082G/−819C/−592C genotypes had increased gene transcript expression and were associated with colonization by more virulent Hp strains (cagA+, vacAs1+, babA2+)73.

2.C. Environmental factors and Hp-dependent disease
2.C.1. Socioeconomic status

Higher prevalence of Hp infection is generally associated with low socioeconomic status, where individuals residing in overcrowded areas are at high risk of infection. As high as 90% of individuals born before 1950s in Japan were Hp-positive, which is a stark contrast to less than 2% infection rate in individuals born after 2000s. As the acquisition of Hp is more likely during childhood, the improvements in the quality of life and hygiene practices during the early years are attributed to the drastic change in epidemiology.

2.C.2. Hp Co-infection with other pathogens

Altered gastric function during Hp infection, particularly decreased acid and mucus production, are thought to create an environmental favorable for parasite co-colonization. A strong correlation between the presence of Hp and various gastrointestinal parasites is reported, but the incidence of gastric disease in co-infected individuals depends on the identity of the parasite. On one hand, several protozoan parasites, including Giardia lamblia and Entamoeba histolytica, which are single-cell eukaryotes, co-infect in Hp-positive individuals. The Th1 adaptive immune response to G. lamblia and E. histolytica infections, along with Hp co-infection, is linked to higher inflammation and exacerbated gastric mucosal damage than in patients with Hp infection alone.

On the other hand, the presence of intestinal helminths is associated primarily with a Th2-dominant immune response, which can alleviate the effects of the proinflammatory Th1 response driven by Hp. Higher or lower gastric cancer rates, in two different regions of Columbia with similar Hp incidence, were attributed to the absence or presence of intestinal helminths, respectively. In these same populations, a strong association exists between seropositivity for the helminth Ascaris lumbricoides and lower inflammatory response.

2.C.2. Dietary factors and nutritional status

Among the most studied dietary factors that affect disease risk in Hp infected individuals is excessive salt intake. Infection studies using animal models revealed a strong correlation between Hp colonization, Hp-induced adenocarcinoma, and high-salt diet. Given that Hp can sense and preferentially colonize injured regions of the stomach, the link between excessive salt intake and damage to the gastric mucosal barrier may contribute to the development of gastric cancer.

Iron-deficiency is commonly associated with Hp infection, which is demonstrated to be linked to increased risk of gastric cancer. Hp-infected Mongolian gerbils on
a low-iron diet developed cancerous lesions in the gastric lining after 12 weeks post infection\(^9_6\). Additionally, \textit{Hp} strains obtained from anemic individuals were more virulent and yielded heightened pro-inflammatory response compared to strains isolated from individuals with high iron level\(^9_6\).

The consumption of N-nitroso compounds within prepared foods, especially cured meats and fish\(^9_7\), is also thought to be a major contributor to carcinogenesis. \textit{In vivo} nitrosation occurs in the stomach, facilitated by low acidity and catalyzed by some bacterial species\(^9_8\), as further discussed below. The gastric nitrite levels are elevated in individuals residing in areas with higher risk for gastric cancer relative to low-risk areas\(^9_9,10_0\). As a scavenger of nitrites, ascorbic acid is essential in reducing the level of N-nitroso compounds\(^9_8\). The level of ascorbic acid in the gastric environment is decreased in \textit{Hp}-infected individuals, and recovers upon \textit{Hp} clearance in individuals that experienced reduction of gastric acidity\(^10_1\). Low ascorbic acid is attributed to \textit{Hp} promoting the oxidation of ascorbic acid and dampening its secretion\(^10_1\).

### 2.C.3. Smoking

A study based on a questionnaire survey and blood sample collection of non-cardia gastric cancer and control individuals suggested that smoking increases gastric cancer risk in \textit{Hp}-infected individuals\(^10_2\), due in part to the presence of carcinogenic nitrosamines found in tobacco smoke\(^10_3\) and depletion of plasma antioxidants, including ascorbic acid, which acts as a scavenger of nitrites\(^10_1,10_4\).

#### 3. The gastric microbiome and disease progression during \textit{Hp} infection

As described above, compelling evidence exists for the impact of \textit{Hp} virulence factors, human genetic variation, and environmental variables on gastric cancer risk in chronically infected humans. However, the results of many of these previous studies have, to some extent, been historically interpreted in the context of the stomach as a mono-culture environment dominated by \textit{Hp}, without consideration of the impact and influence of gastric microbiota. Here we review the emergence of the gastric microbiota as a potential risk factor of gastric cancer.

### 3.A. The evolving landscape of the stomach as a microbial niche

Forty years ago, the widely-held view of the inability of the sterile gastric environment to support resident microbiota, was turned upside-down with the discovery of \textit{Hp} in the human stomach. However, the existence and potential role of resident gastric microbiota (beyond \textit{Hp}) in the progression of chronic \textit{Hp} infection to malignancy has remained murky, due in part to the inability to reliably culture microbes from stomach lumen or tissue. More recently, the emergence of culture-independent, molecular techniques has begun to lift the veil over the richness and diversity of microbial communities that are resident within the gastric environment. Today, our view of a highly diverse gastric microbiota in humans is coming increasingly into focus\(^10_5\)\(^-\)\(^10_7\). Indeed, recent studies have revealed differences in both composition and community structures of gastric microbiota between \textit{Hp}-infected and uninfected individuals, as well patients with gastritis versus patients with gastric cancer.

The microbial community structure of the stomach in *Hp*-uninfected humans has been interrogated using several different approaches, revealing a complex and diverse composition of microbes. While person-to-person variation exists, the gastric microbiota in uninfected subjects has been reported to be dominated by several microbial phylotypes. In particular, the Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Fusobacteria have been reported as the dominant phyla, as determined using small subunit 16S rDNA clone library approaches\(^{108}\), 16S rRNA gene amplicon-based analyses\(^{109}\), and culture-based analyses\(^{106}\).

There has not been universal agreement on the gastric microbiota in *Hp*-infected individuals. Using a small subunit 16S rDNA clone library approach, one study reported that the composition of the gastric community was not affected by the presence of *Hp*, and *Hp* did not have a negative effect on the diversity and evenness of the other members of the gastric community\(^{108}\). In comparison, gastric samples from *Hp*-infected subjects individuals were reported to be highly enriched in Proteobacteria\(^{109}\), relative to samples from uninfected subjects. Class level phylogenetic analysis revealed that Proteobacteria of uninfected subjects included β-Proteobacteria and γ-Proteobacteria in equal ratios whereas ε-Proteobacteria dominated the gastric microbiota of *Hp*-infected individuals\(^{107,109}\). Using culture-based identification, *Hp*-positive individuals were characterized by increased relative abundance of non-*Helicobacter* species from Proteobacteria, Spirochetes, and Acidobacteria, and with decreased abundance of Actinobacteria, Bacteroidetes and Firmicutes\(^{106}\). In one of the first pediatric studies of the gastric microbiota\(^{10}\), 16S ribosomal RNA gene high-throughput sequencing revealed that relative to *Hp*-infected subjects, the microbial community structures of *Hp*-uninfected individuals are characterized by enhanced richness and diversity, and more specifically, higher abundance of β-Proteobacteria and γ-Proteobacteria, Bacteroidia and Clostridia classes. Despite emerging patterns in microbial community structures, it is also clear that the diversity of the gastric microbiota extends well beyond the dominant phyla described in multiple studies, as deep sequencing of 16sRNA has revealed more than 600 bacterial phylotypes\(^{111}\).


Several lines of evidence have provided hints that the gastric microbiota may have an important role in promoting gastric cancer. A retrospective study revealed that, relative to 81 individuals with chronic gastritis, the gastric microbiomes in gastric cancer patients were relatively depleted of *Helicobacter*\(^{112}\). The gastric microbiota of patients with gastric cancer were less diverse, although enriched in some bacterial genera, including intestinal microbial flora with nitrosation activity, thereby supporting the presence of a dysbiotic microbiota predicted to have genotoxic potential\(^{112}\).

In contrast, another study, revealed more diverse community structures in tissue from cancer patients, whose stomachs were characterized by bacterial overgrowth\(^{113}\). Strikingly, microbial communities from the cancer patients were enriched with genera of bacteria with potential cancer-causing
activities. Specifically, some species of bacteria can functionally catalyze the nitroso-conjugation of bile acids within the intestine, resulting in N-nitrosoglycocholic acid and N-nitrosotaurocholic acid, which possess mutagenic properties, which, as described immediately below, can contribute to the development of gastric cancer.

3.C. Gastric microbiota and cancer risk.

The underlying changes in the stomach environment that promote enrichment of microbes with nitroso-conjugating activities are not entirely understood. However, a likely contributor is the well-documented reduction in gastric acid production (hypochlorhydria) that accompanies chronic Hp infection, and is thought to be a major component of the gastric atrophy that precedes neoplasia.

Gastric alterations in Hp infected patients, induced by administration of proton-pump inhibiting agents (PPIs) such as omeprazole, resemble those changes observed in patients with Hp-induced atrophic gastritis which is a major condition predisposing to gastric cancer. Several studies support an association between higher stomach pH and increased colonization of non-Hp bacterial species in the stomachs of individuals administered PPI-treatments promote the overgrowth of pathobionts, that can promote inflammatory responses in the stomach, which is a potential contributor to the development of gastric cancer. Under conditions of reduced acidity, bacteria were cultured out of the stomach that reduce dietary nitrate to nitrite and also facilitate intra-gastric formation of N-nitroso compounds, suggesting the presence of strains with oncogenic potential.

Other studies have employed human gastrin-overexpressing transgenic mice (INS-GAS mice). These studies revealed that germ-free INS-GAS animals were cancer-free when infected for 7 months with Hp. In contrast, gastric cancer was evident in INS-GAS mice infected with Hp under specific pathogen-free (SPF) conditions, indicating that in least this infection model, Hp infection was not sufficient to induce malignancy.

In support of the interpretation that additional microbes were required for the development of gastric cancer within Hp-infected animals under SPF conditions, neoplastic lesions were detected in Hp-infected animals that had been co-infected with ASF356 Clostridium species, ASF361 Lactobacillus murinus, and ASF519 Bacteroides species, three intestinal bacteria, analogous to Hp-infected INS-GAS mice under SPF conditions. While these results suggest associations between the development of gastric cancer within Hp-infected subjects and other gastric bacteria, definitive evidence that the gastric microbiota influences the emergence of gastric cancer does not exist.

4. Summary and Outlook

The discovery of chronic Hp infection as the single most important risk factor for gastric adenocarcinoma in humans impacted the biomedical community by challenging long-held dogma about the etiology of cancerous diseases. Nonetheless, Hp infection does not always progress to malignancy, and, gastric adenocarcinoma can occur in the absence of detectable Hp carriage, highlighting the complex and multifactorial nature of this disease. Here, we have highlighted several known classes of risk-factors for gastric cancer, including Hp virulence, host genetics, and several assorted external influences. Increased appreciation for the gastric microbiota has
sparked recent studies into how other microbes might impact the outcome of chronic *Hp* infection. While research in this area is still at an early stage, evidence has emerged that the gastric microbiota is influenced by the presence or absence of *Hp*. Moreover, the microbial community structures differ in *Hp* gastric individuals with gastritis and patients with gastric cancer. Hypochlorhydria is associated with the gastric atrophy that precedes neoplasia in *Hp*-infected individuals, and there is some evidence that increased pH in the gastric environment is responsible for dysbiosis and enrichment of microbes with metabolic potential linked with carcinogenesis. However, there is currently a dearth of data to establish causal linkage between gastric dysbiosis and gastric cancer.

Early forays into the stomach have provided a glimpse into the gastric microbial landscape of humans. Although these studies suggest that the stomach microbiota are more complex than originally thought, a challenge in the near future will be to discriminate between microbes that stably inhabit the human stomach, from those that are only transiently associated with gastric tissue. Ultimately, discerning both the role of individual microbes, as well as the collective microbes and metagenomic potential within gastric microbial communities, in promoting gastric cancer in humans will be a difficult and challenging goal that will require new and innovative ways of assessing these communities in vivo. In the near future, objectives will include identifying the diversity of microbial communities that occupy distinct gastric niches, and, defining the dynamics of microbial communities structures as a function of disease state progression in *Hp*-infected individuals. In summary, while controversial and unresolved, the importance of the gastric microbiota as a risk factor for gastric malignancy is a vital area of current research.

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