The role of gastric microbiota in gastric cancer

Oliver A. Stewart, Fen Wu, and Yu Chen

ABSTRACT
Gastric cancer represents one of the leading causes of cancer deaths worldwide. Helicobacter pylori infection is the strongest risk factor associated with gastric cancer. Due to new molecular techniques allowing greater identification of stomach microbes, investigators are beginning to examine the role that bacteria other than H. pylori play in gastric cancer development. Recently, researchers have investigated how the composition of the gastric microbiota varies among individuals with various stages of gastric disease. Specific microbes residing in the stomach have been preferentially associated with gastric cancer patients compared to individuals with a healthy gastric mucosa. Studies conducted on the insulin-gastrin (INS-GAS) transgenic mouse model have provided additional insight into the association between the gastric microbiota and gastric cancer. The purpose of this article is to review the current state of literature on the relationship between the gastric microbiota and gastric cancer based on clinical studies performed to date.

ARTICLE HISTORY
Received 10 January 2020
Revised 16 April 2020
Accepted 23 April 2020

KEYWORDS
Microbiome; gastric cancer; stomach cancer; microbiome diversity; specific microbes

Introduction
Gastric cancer is the fifth most common cancer worldwide and over 1 million new cases were diagnosed in 2018. Infection with Helicobacter pylori is widely regarded as the strongest risk factor for the development of gastric cancer. Over 50% of people are infected with H. pylori globally. Almost all cases of gastric cancer can be related to H. pylori. The vast majority of gastric cancer cases are the intestinal type of non-cardia gastric cancer that occur via a predictable progression from atrophic gastritis (AG) to intestinal metaplasia (IM) to gastric cancer (GC), and it is known that H. pylori infection plays an initial role in this cascade (Table 1). H. pylori infection causes inflammation of the gastric mucosa and destruction of the hydrochloric acid-secreting glands of the stomach, ultimately leading to a condition known as atrophic gastritis. Atrophic gastritis is a chronic inflammatory and hypochloridic state that has the potential to progress to gastric cancer. Although H. pylori infection is known to precipitate this cascade, only approximately 1–3% of infected individuals will subsequently develop gastric cancer. Other risk factors include advanced age, male gender, certain ethnic backgrounds, and environmental factors such as smoking and consumption of high salt and smoked foods containing nitrates. Genetic polymorphisms within pro-inflammatory genes have been implicated in gastric cancer development as well. However, until recent years, the relationship between the microbiota and gastric cancer has remained relatively unexplored. It has been hypothesized that the hypochlorydia associated with atrophic gastritis allows the stomach to be colonized by oral and lower bowel microbes that are not ordinarily present under its normal, harshly acidic conditions. Initial research of the gastric microbiota was limited partially due to difficulty culturing commensal microorganisms residing in the stomach. As a result, researchers previously believed the number of microbes capable of surviving in the stomach was limited. However, due to advances in PCR techniques and metagenomics, it is now clear that the stomach does in fact contain a robust microbiota. As a result of these technological advances,
increased investigation into the relationship between the gastric microbiota and gastric cancer is beginning to occur. It is necessary to evaluate and analyze the research findings to date in order to determine potential next steps for research in this novel field.

**Gastric cancer development and diversity of the microbiota**

With the advent of newer techniques for analyzing the microbial content of the stomach, it became possible to better characterize the gastric microbiota. Perhaps more importantly, it also became possible to explore how the composition of the microbiota differs across the spectrum from a healthy gastric mucosa to GC (Table 1). Interestingly, the vast majority of the studies conducted on the gastric microbiota to date have focused on intestinal type adenocarcinoma as opposed to diffuse gastric cancer, possibly due to the fact that *H. pylori* is more associated with intestinal type gastric cancer as opposed to diffuse type gastric cancer. One of the landmark studies examining the gastric microbiota came in 2006 when Bik et al. sought to characterize the bacteria present in the stomach of 23 adults via gastric mucosal biopsies obtained during upper endoscopy. In this study, 64 were non-culturable by traditional culture. Despite the limitations of this study, which include a small sample size and lack of gender diversity (22 of 23 subjects were male), it was one of the first studies to highlight the fact that the stomach has a more robust microbiota than previously believed and gave a glimpse into the types of microbes that inhabit the stomach. It is also worth noting that this study identified the presence of *H. pylori* in the stomach of several patients who were deemed *H. pylori*-negative by conventional diagnostic testing. This finding suggests that the presence of *H. pylori* in patients with gastric disease may actually be underestimated by conventional diagnostic tests.

Although the aforementioned study demonstrated that the gastric microbiota was more robust than previously hypothesized, it focused on relatively healthy subjects receiving upper endoscopy for symptoms of dyspepsia. Several studies have attempted to characterize differences in gut microbiota diversity by the severity of phenotypes including a normal gastric mucosa, AG, IM, and GC (Table 2). Of these studies, 2 studies did not find a significant difference in diversity indices of bacterial phyla between gastric cancer patients and controls. One of the two studies had a very small sample size, including 10 patients with non-cardia gastric cancer and 5 controls with dyspepsia. It is possible that the small sample size of the study left it statistically underpowered and thus made it difficult to detect potential differences in microbiota diversity between groups. However, the other study conducted by Wang et al. on a total of 315 patients, including 212 cases of chronic gastritis and 103 cases of gastric cancer, also found no

### Table 1. *H. pylori*-dependency and potential role of commensal bacteria in promotion of gastric cancer.

| Disease progression | Normal stomach | Active Gastritis | Atrophic gastritis | Intestinal metaplasia | Dysplasia | Carcinoma |
|---------------------|----------------|-----------------|-------------------|----------------------|----------|-----------|
| Cell type           | Gastric mucosal | Gastric mucosal | Gastric parietal cells | Intestinal, premalignant | Intestinal, dysplastic | Intestinal, malignant |
| pH                  | 2              | 2–4             | 4–7               | 4–7                  | 4–7      | 4–7       |
| *H. pylori* in stomach* | 5–6.5%<sup>a</sup> | >90%<sup>a</sup> | 30–72%<sup>a</sup> | 30–35%<sup>a</sup> | 24.6%<sup>a</sup> |
| Commensal bacteria  | Uninhabitable<sup>a</sup> | ±<sup>a</sup> | ++                | ++                   | ++       |
| Phase               | Normal     | Initiation      | Promotion          | Progression          |
| Stage               | *H. pylori*-dependent stage (years to decades) | *H. pylori*-independent stage (decades) |

*a. In stomach with normal acid production, *H. pylori* can survive on the gastric mucosa by using urease to increase the pH in surrounding tissues. Commensal bacteria lack the capability of effective competition with *H. pylori* in an acid environment. b. In atrophic gastritis, *H. pylori* starts to disappear because commensal bacteria are not protected against the low gastric pH, whereas the near neutral pH in an atrophic stomach allows commensal bacteria to overgrow.*
Table 2. Summary of studies examining the relationship between gastric cancer and microbiota diversity.

| Reference | Study Design | Sample Size | Subject Country of Origin | Sample Type | Sequencing Methods | Outcomes | Major Findings |
|-----------|--------------|-------------|---------------------------|-------------|--------------------|----------|----------------|
| Dicksved19 | Case-control | 10 GC patients, 5 dyspeptic controls | Sweden | Gastric biopsies from antrum and corpus | T-RLFP, 16S rRNA gene sequencing | Microbial composition of GC patients compared to controls | No significant difference between microbial composition of gastric cancer patients compared to controls |
| Wang19    | Case-control | 103 GC patients, 212 chronic gastritis controls | China | Gastric biopsies from antrum or within 5 cm of cancerous lesion | 16S rRNA pyrosequencing | Bacterial load of gastric mucosa; Biodiversity, composition, structure of microbiota | ↑ Bacterial load in GC patients ↑ Lactobacillus, Escherichia-Shigella, Nitrospirae, Burkholderia fungorum in GC patients |
| Aviles-jimenez20 | Case-control | 5 GC patients, 5 IM patients, 5 nonatrophic gastritis patients | Mexico | Gastric biopsies from antrum in non-cancer patients, biopsies from lesion in GC patients | G3 PhylolChip 16S rRNA microarray | Gastric microbiota diversity; Taxa abundance differences between groups | No significant difference in diversity index between GC and chronic gastritis |
| Coker21    | Case-control | 54 GC, 81 chronic gastritis patients | Portugal | Gastric biopsies or surgical specimens (non-neoplastic tissue adjacent to tumor only) | 16S rRNA next generation sequencing via ION PGM | Gastric microbial diversity | No significant difference in microbial diversity of gastric cancer patients compared to controls |
| Ferreira22 | Case-control | 11 GC, 10 IM, 10 chronic gastritis patients | Korea | Gastric biopsies from antrum and corpus (non-neoplastic tissue adjacent to tumor only) | 16S rRNA gene sequencing using high throughput sequencing platform, 454 GS FLX Titanium | Diversity of gastric microbiota | No significant difference in microbial diversity of gastric cancer patients compared to controls |
| Eun23      | Case-control | 12 GC, 20 functional dyspepsia patients | Singapore and Malaysia | Gastric biopsies from antrum | 16S rRNA gene sequencing using Illumina MiSeq | Gastric microbiota richness and phylogenetic diversity | No significant difference in microbial diversity of gastric cancer patients compared to controls |
| Castaño-Rodriguez24 | Case-control | 6 GC, 5 chronic gastritis patients | China | Gastric wash samples | Shotgun metagenomic sequencing | Gastric microbiota richness and relative bacterial abundance | No significant difference in microbial richness and phylogenetic diversity of gastric cancer patients compared to controls |
| Hu25       | Case-control | 6 GC, 5 chronic gastritis patients | Korea | Gastric biopsies from antrum | 16S rRNA gene sequencing using Illumina MiSeq | Relative bacterial abundance | No significant difference in microbial richness and phylogenetic diversity of gastric cancer patients compared to controls |

GC, gastric cancer; IM, intestinal metaplasia; AG, atrophic gastritis.
difference in the Chao1 richness estimator and Shannon’s diversity index between the two groups following analysis of gastric biopsy samples. Both Chao1 richness estimator and Shannon’s diversity index are measures of alpha diversity or the mean diversity within a sample. Shannon’s diversity index accounts for both richness (the number of taxa observed) and evenness (the relative abundance of specific taxa), whereas the Chao1 richness estimator considers species richness alone for a given sample. Although both of these diversity measures were not statistically different between gastric cancer and chronic gastritis patients, Wang et al. did find that patients with gastric cancer had an increased bacterial load, as measured by copy numbers of 16S rRNA gene per microgram of DNA, compared to patients with chronic gastritis. This finding supports the idea that increased bacterial load induced by hypochlorhydria may play a role in the development of gastric cancer.

Despite the results of the two aforementioned studies, the majority of studies have found differences in the diversity of the microbiota in patients with gastric cancer compared to controls. However, there have been mixed findings regarding the relationship between disease progression and the diversity of the gastric microbiota. Generally, all studies addressing this issue analyzed the gastric microbiota of patients on the spectrum from a normal gastric mucosa to gastric cancer by performing gene sequencing on mucosal biopsy samples obtained from patients via upper endoscopy. Several studies also included patients with gastritis (atrophic or unspecified chronic gastritis) or intestinal metaplasia in addition to gastric cancer as well. The majority of studies had similar exclusion criteria that included no PPI, H2 blocker, anti-inflammatory, or antibiotic use in the prior 2–6 months before sample collection. A study conducted by Aviles-Jimenez et al. used tissue samples from five patients with superficial gastritis, five patients with IM, and five with GC to examine changes in the microbiota. Following analysis, they ultimately concluded that the data suggested that bacterial diversity decreased at the genus level as patients progressed from superficial gastritis to IM and GC. Similarly, a subsequent study conducted on a larger cohort of Chinese patients (21 patients with superficial gastritis, 23 with AG, 17 with IM, and 20 with GC) also concluded that IM and GC patients had significantly reduced microbial richness compared to patients with superficial gastritis. A study of 81 Portuguese patients also found that patients with gastric cancer had significantly lower microbial diversity compared to patients with chronic gastritis. In addition, a study conducted by Hu et al. on a small sample of 11 Chinese patients also found a lower level of microbial diversity in gastric cancer patients compared to patients with chronic gastritis. Notably, despite the small sample size, this was the only study that used metagenomic sequencing to examine the composition of the gastric microbiota. In addition, this study utilized gastric wash samples rather than tissue samples obtained from biopsy for the analysis.

However, there have also been studies suggesting that gastric cancer is associated with increased diversity and richness of the microbiota. One study examined the microbiota of 31 Korean patients with chronic gastritis, IM, or GC. While using similar exclusion criteria as comparable studies, they found that both evenness and diversity of gastric microbiota in the gastric cancer group was greater than in the two other groups. Similarly, a study of 32 Chinese subjects (12 patients with gastric cancer, 20 patients with functional dyspepsia) produced similar results and concluded that species richness and phylogenetic diversity were increased in gastric cancer compared to patients with functional dyspepsia.

All of the above studies compared the diversity of the gastric microbiota of patients with gastric cancer to patients at various stages of gastric disease progression. More recently, Liu et al. examined bacterial diversity and richness within specific gastric microhabitats in relation to gastric cancer development in 276 patients. Upon examining normal, peritumoral, and tumoral tissues from gastric cancer patients, the researchers noted decreased diversity and richness in peritumoral and tumoral microhabitats compared to normal gastric tissue. This finding suggests that the richness of gastric microbiota varies according to the gastric microhabitat within the same gastric cancer patient, not only as patients progress from atrophic gastritis to gastric cancer.
Currently, it is unclear whether there is a correlation between the diversity of the gastric microbiota and the progression from healthy gastric mucosa to gastric cancer. Although several studies used similar methods of data collection, exclusion criteria, molecular methods for analysis, and similar measures for diversity (via Shannon’s diversity index or Chao1 richness estimator), there is currently no consensus on the relationship between microbiota diversity and gastric cancer developmental stage. From a mechanistic standpoint, it is plausible that increased or decreased diversity of the microbiota can be associated with the development of gastric cancer. Although present in gastric tissue in >90% of patients with active acute gastritis,6,7 H. pylori is absent in gastric tissues in the large majority of patients with advanced AG, IM8 or gastric cancer9 even when serology is positive; this suggests the disappearance of active H. pylori infection during the later stages of gastric cancer development29 (Table 1). The loss of H. pylori and impairment of acid secretion in these lesions may facilitate the colonization of other bacteria in the stomach. It is possible that initial H. pylori infection leads to atrophic gastritis, higher pH levels in the stomach than usual, which would subsequently allow new microbes to colonize the stomach, increasing species diversity (Table 1). However, it is also plausible that the inflammation associated with H. pylori infection would produce a gastric environment that is inhospitable to most microorganisms, resulting in a restricted niche in which fewer microorganisms can reside. Another factor to consider in these studies is that the ethnicity of the patients examined was relatively homogenous within each study. The microbiota of Chinese, Swedish, Mexican, Portuguese, and Malaysian patients were examined across these studies (Table 2). However, only one ethnic population was examined within any one particular study. We know that dietary habits are one of the many factors that can influence the gastric microbiota, and it is unclear how ethnic factors shape the composition of a healthy individual’s gastric microbiota, let alone the impact on the microbiota in the progression to gastric cancer.10 A larger multicenter, multicultural study focusing on the changes in the microbiota in individuals progressing from normal, healthy gastric mucosa to gastric cancer is certainly warranted.

The potential role of specific microbes in gastric cancer development

The impaired acid-secretion associated with H. pylori infection may facilitate the colonization of other bacteria in the stomach (Table 1). Although there is not a consensus on the relationship between microbiota diversity and gastric cancer, several studies have shown associations between specific microbes and gastric cancer (Table 2). For example, the genus Lactobacillus has been found to be present in higher proportions in gastric cancer patients compared to controls in several studies20,23,24 (Table 2). Castaño-Rodriguez et al. reported higher levels of relative abundance in both Lactobacillus and Lactococcus genera comparing gastric cancer patients with individuals with functional dyspepsia.24 Although no causal role was demonstrated in the study, researchers do suggest a potential mechanism for the overrepresentation of these bacterial genera in gastric cancer patients. Both Lactococcus and Lactobacillus genera contain microbes that produce lactic acid and can theoretically aid tumor progression given that lactate can serve as an energy source for tumor growth and angiogenesis.30 Another study demonstrated that the Lachnospiraceae family was increased in gastric cancer patients compared to controls.20 Previous research suggests that microbes in the Lachnospiraceae family are often decreased in inflammatory processes, so it is possible that these microbes play a role in the regulation of inflammation that warrants further exploration.31 In addition to an increased proportion of Lachnospiraceae and Lactobacillus, Wang et al. reported that the phylum Nitrospirae was present in all patients with gastric cancer but completely absent in patients with chronic gastritis.19 Notably, several members of the Nitrospirae phylum are known to play a role in the metabolism of nitrates and nitrites.32 It is known that the consumption of nitrates is a significant risk factor for the development of gastric cancer, and it is plausible that the production of carcinogenic N-nitroso compounds can be increased by these bacteria.33 Several bacterial genera typically found in the oral
cavity including *Fusobacterium*, *Veillonella*, *Leptotrichia*, *Haemophilus*, and *Campylobacter* have also been found in higher relative abundances in gastric cancer patients. *Fusobacterium*, in particular, is a pro-inflammatory oral bacterial genus and has received attention in the past for a potential role in the development of colon and breast cancer. Interestingly, *Streptococcus bovis*, a bacterium which has a known association with colorectal cancer, has also been found in increased proportion in gastric cancer patients but this finding has not been replicated in other studies. In addition, *Propionibacterium acnes*, a well-known skin flora, has also been demonstrated to be over-abundant in gastric tumoral tissues, and it is hypothesized that its production of short-chain fatty acids may contribute to a lymphocytic gastritis.

One study demonstrated decreased abundance of *Sphingobium yanoikuyae* in patients with gastric cancer compared to patients with superficial gastritis. This species is capable of degrading aromatic hydrocarbons, which are a group of molecules that has potential carcinogenic effects. This study was the first study to suggest a negative association between *Sphingobium yanoikuyae* and gastric cancer. Park et al.’s 2019 study found an increased level of abundance of Rhizobiales in patients with intestinal metaplasia compared to patients with chronic superficial gastritis. In addition, they found an increased abundance of genes encoding type IV secretion system (T4SS) proteins in the metagenome of patients with intestinal metaplasia. T4SS is one type of secretion system used by microorganisms to transport macromolecules across the cell envelope. Many pathogenic bacteria use the T4SS in order to transfer proteins known as virulence factors that confer a bacterium with its pathogenicity. T4SS proteins consist of a smaller subset of proteins that allow injection of *H. pylori*’s proposed main virulence factor, CagA, from the bacterial cytoplasm to the cytoplasm of gastric epithelial cells. Although the researchers did not find direct evidence of horizontal genetic transfer between Rhizobiales and *H. pylori*, they hypothesized that it is possible that T4SS genetic transfer occurs between *H. pylori* and members of the microbiota, thus contributing to *H. pylori*’s carcinogenicity.

### A transgenic mouse model of gastric cancer

Several of the aforementioned studies suggest a relationship between alterations to the gastric microbiota composition and the development of gastric cancer (Table 2). There are even plausible explanations for the potential roles that certain microbes can play in gastric cancer development. Although no cause-and-effect relationship can be established in these studies of human subjects, recent studies performed on a transgenic mouse model have further elucidated the relationship between the microbiota and gastric cancer. The insulin-gastrin transgenic (INS-GAS) mouse model has high circulating gastrin levels and invariably goes on to develop atrophic gastritis with achlorhydria. In addition, when infected with *H. pylori*, 80% of these transgenic mice go on to develop gastrointestinal neoplasia by 6 month postinfection. This number represents a stark increase from the proportion of human patients who go on to develop neoplasia following *H. pylori* infection. Using the INS-GAS mouse model, several studies (Table 3) have attempted to establish a causal link between the microbiota and the development of gastric cancer.

The first transgenic mouse study that suggested an association between the microbiota and gastric cancer came in 2008. In Lee et al.’s study, after treating INS-GAS mice with triple therapy (metronidazole, omeprazole, clarithromycin), investigators found that eradication of *H. pylori* in these mice reduced the severity of gastric dysplasia in the mice several weeks postinfection. Interestingly, they also treated INS-GAS mice with no prior *H. pylori* infection with triple therapy and found that doing so reduced the severity of dysplasia in these mice as well. This data suggests that the antibiotic treatment was potentially exerting its effect on microorganisms other than *H. pylori*. Given that this study focused primarily on the role of *H. pylori* eradication on the development of gastric cancer, the investigators did not measure changes in microbial diversity or abundances of specific microbial taxa following triple therapy treatment. However, given that we know antibiotic treatment affects the composition of the microbiota in the colon and stomach, it is
possible that the effect of triple therapy on the composition of other bacterial taxa in uninfected mice is associated with reduced gastric dysplasia.

Other studies have also utilized the INS-GAS mouse model to explore the association between the gastric microbiota and gastric cancer. Particularly, studies have attempted to demonstrate how the complexity of the microbiota affects gastric cancer development in the INS-GAS mouse model. Through manipulating the microbiota of the transgenic mouse model, Lofgren et al. demonstrated that germ-free INS-GAS mice developed a reduced number of gastric lesions compared to INS-GAS with a more complex microbiota at 11 month postinfection with *H. pylori*. Lertpiriyapong et al. further expanded upon this knowledge by assessing if the presence of a diverse microbiota is a necessary requirement for the development of gastric lesions. In order to do this, investigators compared the postinfection gastric cancer risk in INS-GAS mice with three different microbiota compositions: complex, germ-free, and restricted (containing only *Lactobacillus, Clostridium,* and *Bacteroides* genera). In their study, they found that a restricted gastric microbiota was associated with a similar rate of gastric cancer development as a complex microbiota in the mice. However, compared to the germ-free mice, the INS-GAS mice with the restricted microbiota had significantly increased gastric pathology.

**Table 3. Summary of studies examining gastric cancer in the INS-GAS mouse model.**

| Reference | Sample Size | Primary Intervention | Outcomes | Major Findings |
|-----------|-------------|----------------------|----------|----------------|
| Wang      | 8 INS-GAS, 18 wild type mice | Monoinfection with *H. felis* | Parietal cell number and gastric histology at 20 months of age | ↑ gastric metaplasia, dysplasia, carcinoma in situ, and gastric cancer in INS-GAS mice; Accelerated rate of gastric carcinoma development in *H. felis* infected mice |
| Lofgren   | 86 germ free INS-GAS mice, 5 SPF mice | Monoinfection with *H. pylori* | Gastric lesion scores at 5, 7, 9, and 11 months postinfection | Delayed development of gastric lesions in germ free INS-GAS mice compared to SPF INS-GAS mice; ↓ Firmicutes, ↑ Bacteroidetes in *H. pylori*-infected SPF INS-GAS mice |
| Lee       | 54 specific pathogen free INS-GAS mice with *H. pylori* infection and controls without *H. pylori* infection | Eradication of *H. pylori* with omeprazole, metronidazole, and clarithromycin | Severity of gastric dysplasia at 8, 12, 22, and 28 weeks postinfection | *H. pylori* eradication at 8 weeks postinfection reduced GIN risk to that of *H. pylori* uninfected mice at 28 weeks postinfection; *H. pylori* eradication at 12 and 22 weeks postinfection prevented progression to high-grade GIN at 28 weeks postinfection |
| Lertpiriyapong | 32 germ free, 27 restricted, 19 complex microbiota INS-GAS mice; 12 mice monoinfected with *H. pylori*, 22 restricted flora with *H. pylori* infection, 24 complex flora with *H. pylori* infection | Monoinfection with *H. pylori* | Gastric pathology 7 months postinfection, mRNA expression of cancer-related genes 7 months postinfection | More severe gastric pathology seen in *H. pylori* infected mice with complex and restricted flora compared to germ free *H. pylori* infected mice at 7 months postinfection; ↓ expression of FoxP3+, regulatory T cells in INS-GAS mice coinfected with *H. polygyrus and H. pylori* compared to *H. pylori* monoinfected mice |
| Whary     | 12 *H. pylori* infected, 13 *H. polygyrus* infected, 10 *H. pylori + H. polygyrus* infected, 9 control INS-GAS mice | Coinfection with *H. pylori and H. polygyrus* | Gastric lesion scores 5 months postinfection, immunohistochemistry with T cell marker phenotyping, mRNA expression levels in gastric secretions | *Lactobacillus murinus, Clostridum and Bacteroides* in male restricted microbiota INS-GAS mice coinfected with *H. polygyrus and H. pylori* had reduced gastric atrophy, dysplasia and alterations in gastric flora compared to *H. pylori* monoinfected mice |

INS-GAS, insulin-gastrin transgenic mice; SPF, specific pathogen-free mice; GIN, gastric intraepithelial neoplasia.
gastric corpus inflammation, epithelial defects, oxyntic gland atrophy, epithelial hyperplasia, and dysplasia. Taken together, the data indicate that *H. pylori* can act synergistically with a community of bacteria to promote gastric neoplasia. These results suggest that the microbiota may play a role in the development of gastric cancer following *H. pylori* infection, but a diverse microbiota may not necessarily be a requirement for the development of gastric cancer. Previous researchers have hypothesized that the achlorhydria associated with gastric atrophy following *H. pylori* infection may allow overgrowth of commensal bacteria in addition to colonization from bacteria of the lower bowel. It is possible that this overgrowth of bacteria may play a role in gastric cancer development. However, in the INS-GAS mouse model, it appears that the presence of only a limited range of microbes was sufficient to produce gastric cancer at similar rates as the more complex microbiota.

In addition to suggesting that the gastric microbiota plays a role in the development of gastric cancer following *H. pylori* infection, studies of INS-GAS mice also have the potential to provide further insight regarding potential therapeutic interventions for individuals in gastric disease progression. For example, researchers have demonstrated that INS-GAS mice co-infected with *H. pylori* and a helminth, *H. polygyrus*, have lower rates of *H. pylori*-associated gastric atrophy, dysplasia, and are less susceptible to gastric colonization with lower bowel flora than INS-GAS mice infected with *H. pylori* alone. The researchers hypothesized that this finding is due to helminth-induced upregulation of a Th2-associated inflammatory response and increased regulatory T cell recruitment to the stomach where they preserve parietal cell function and promote maintenance of normal gastric pH. This study suggests that immunomodulation due to other pathogens may be a potential therapeutic modality in the prevention of progression from *H. pylori*-related gastritis to gastric cancer that warrants further investigation.

**Discussion**

Research on the human microbiome has greatly increased in recent years as investigators and clinicians have explored the role of the microbiome in various disease processes ranging from infectious diseases, various types of cancer, respiratory disease, metabolic disease, and autoimmune diseases. However, studying the gastric microbiota previously presented a significant challenge to researchers due to the harshly acidic conditions of the stomach and the limitations of previous culture techniques. However, with the advent of new PCR techniques and metagenomics analyses, gastric microbiota research has increased over the last decade.

The majority of research conducted on the gastric microbiota to date suggests that the microbiota is altered during the progression from a normal, healthy gastric mucosa to gastric cancer. However, although there seems to be a general consensus that the microbiota is altered in gastric cancer patients compared to controls, studies conducted to date have demonstrated mixed results regarding whether the microbiota of gastric cancer patients exhibits increased or decreased diversity compared to the microbiota of healthy individuals. Although the majority of studies used the same measures of species diversity in their analyses (typically Chao1 richness estimator or Shannon’s diversity index), studies did differ in their data collection techniques and utilized different sequencing software (Table 2). It is unclear whether this impacted genome construction and subsequent microbial species identification. These differences in sequencing technique could partially explain differences in the findings of species diversity between the studies. One major limitation of the studies on the gastric microbiota and gastric cancer development is the fact that all of the data in these studies is retrospective and correlational in nature. As the continuum of gastric cancer development takes decades, it was not feasible to follow the same individuals throughout the process. All of the studies used a cross-sectional study design, assessing diversity differences in patients with different phenotypes at one point in time. Thus, longitudinal and prospective studies are needed to assess the changes in gut microbiota over time. These studies are somewhat impractical given that only 3% of individuals who are infected with *H. pylori* go on to develop gastric cancer. However, as a result,
previous studies have compared the microbiota of patients with varying stages of gastric disease to each other without first establishing the baseline composition of the gastric microbiota in any individual. As a result, it is impossible to account for the impact of diet, previous illness, or ethnic differences on the gastric microbiota of the patients in these studies. Furthermore, any treatment or lifestyle changes subsequent to the disease could also influence the gastric microbiota. Therefore, it remains impossible to establish any sort of causative link between the microbiota and gastric cancer. Collection of data on treatments and diets may help control the confounding factors.

Although studies on the role of the gastric microbiota in gastric cancer do not demonstrate causality, mouse studies using the INS-GAS mouse model have helped to further elucidate the role of the microbiota in gastric cancer. Overall, the mouse studies conducted to date suggest that the presence of a microbiota following H. pylori infection hastens gastric cancer development but only a limited microbiota may be necessary to achieve these effects. Studies replicating the results of Lertpiriyapong et al.’s experiment should be performed to confirm that only a limited gastric microbiota is necessary to speed gastric cancer development in INS-GAS mice. In addition, further investigation regarding the role of immunomodulation in preventing gastric disease progression is certainly warranted, as INS-GAS mouse models have suggested that co-infection by other pathogens such as helminth may be protective through upregulation of regulatory T cell production. Metagenomic studies in particular may have the potential to identify the gene family of the microbiota involved in this process. In addition, although no individual bacterial member of the microbiota has been identified necessary for the development of gastric cancer following H. pylori infection, several bacteria have been found in increased proportions in gastric cancer patients across multiple studies. Of these, several bacterial genera have plausible mechanisms aiding tumor development, which range from inducing inflammation to supplying energy for tumor growth or the production of carcinogenic N-nitroso compounds. Other bacteria with plausible protective mechanisms of action have been found to have a decreased abundance in gastric cancer patients, such as Sphingobium yanoikuyae. Given that manipulation of the microbiota is more feasible in the INS-GAS mouse model compared to human patients, it is possible to conduct more targeted research on microbes with plausible mechanisms for causing gastric cancer.

Other future steps in research should include large, multicenter prospective studies conducted on human subjects with an emphasis on identifying the presence of specific bacterial species and underlying pathways as the microbiota changes in gastric cancer progression. As research on the gastric microbiota increases, it is possible that the presence of certain changes to the microbiota can be used as a surrogate for monitoring disease progression. In addition, it is also possible that manipulating the gastric microbiota, separate from the eradication of H. pylori, has the potential to represent a disease-modifying therapy that can affect the risk of developing gastric cancer.

Disclosure of potential conflicts of interest

The authors report no conflict of interest.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding

This work was supported by the National Institutes of Health [R01 CA204113, P42 ES010349, P30 ES000260, and P30 ES009089].

ORCID

Fen Wu http://orcid.org/0000-0003-0997-0890
Yu Chen http://orcid.org/0000-0002-1519-4894

References

1. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. Prz Gastroenterol. 2019;14(1):26–38. doi:10.5114/pg.2018.80001.
2. Wroblewski LE, Peek RM Jr., Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev. 2010;23(4):713–739. doi:10.1128/CMR.00011-10.
3. Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev. 2010;23(4):713–739. doi:10.1128/CMR.00011-10.
4. Chen Y, Segers S, Blaser MJ. Association between Helicobacter pylori and mortality in the NHANES III
study. Gut. 2013;62(9):1262–1269. doi:10.1136/gutjnl-2012-303018.
5. Park YH, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. J Cancer Prev. 2015;20(1):25–40. doi:10.15430/JCP.2015.20.1.25.
6. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1(8390):1311–1315. doi:10.1016/S0140-6736(84)91816-4.
7. Hirschl A, Potz R, Stanek G, Rotter M, Pötzi R, Gangl A, Holzner JH. Occurrence of campylobacter pyloridis in patients from Vienna with gastritis and peptic ulcers. Infection. 1986;14(6):275–278. doi:10.1007/BF01643961.
8. Kwak HW, Choi JJ, Cho SJ, Lee JY, Kim CG, Kook M-C, Ryu KW, Kim Y-W. Characteristics of gastric cancer according to Helicobacter pylori infection status. J Gastroenterol Hepatol. 2014;29(9):1671–1677. doi:10.1111/jgh.12605.
9. Galiatsatos P, Wyse J, Szilagyi A. Accuracy of biopsies for Helicobacter pylori in the presence of intestinal metaplasia of the stomach. Turk J Gastroenterol. 2014;25(1):19–23. doi:10.5152/tjg.2014.6476.
10. Naylor G, Axon A. Role of bacterial overgrowth in the stomach as an additional risk factor for gastritis. Can J Gastroenterol. 2003;17(Suppl B):13B–17B. doi:10.1155/2003/350347.
11. Bjorkholm B, Falk P, Engstrand L, Nyren O. Helicobacter pylori: resurrection of the cancer link. J Intern Med. 2003;253(2):102–119. doi:10.1046/j.1365-2796.2003.01119.x.
12. El-Omar EM, Carrington M, Chow W-H, McColl KEL, Bream JH, Young HA, Herrera J, Lissowska J, Yuan - C-C, Rothman N, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404(6776):398–402. doi:10.1038/35006081.
13. Sheh A, Fox JG. The role of the gastrointestinal microflora in Helicobacter pylori pathogenesis. Gut Microbes. 2013;4(6):505–531. doi:10.4161/gmic.26205.
14. Mattarelli P, Brandi G, Cabalrese C, Fornari F, Prati GM, Biavati B, Sgorbati B, et al. Occurrence of Bifidobacteriaceae in human hypochlorhydria stomach. Microb Ecol Health Dis. 2014;25:21379.
15. Monstein HJ, Tiveljung A, Kraft CH, Borch K, Jonasson J. Profiling of bacterial flora in gastric biopsies from patients with Helicobacter pylori-associated gastritis and histologically normal control individuals by temperature gradient gel electrophoresis and 16S rDNA sequence analysis. J Med Microbiol. 2000;49(9):817–822. doi:10.1099/0022-1317-49-9-817.
16. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59–65. doi:10.1038/nature08821.
17. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, Perez-Perez G, Blaser MJ, Relman DA. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A. 2006;103(3):732–737. doi:10.1073/pnas.0506655103.
18. Dicksevd J, Lindberg M, Rosenquist M, Enroth H, Jansson JK, Engstrand L. Molecular characterization of the stomach microbiota in patients with gastric cancer and in controls. J Med Microbiol. 2009;58(Pt 4):509–516. doi:10.1099/jmm.0.007302-0.
19. Wang L, Zhou J, Xin Y, Geng C, Tian Z, Yu X, Dong Q. Bacterial overgrowth and diversification of microbiota in gastric cancer. Eur J Gastroenterol Hepatol. 2016;28(3):261–266. doi:10.1097/MEG.0000000000000542.
20. Aviles-Jimenez F, Vazquez-Jimenez F, Medrano-Guzman R, Mantilla A, Torres J. Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. Sci Rep. 2014;4:4202. doi:10.1038/srep04202.
21. Coker OO, Dai Z, Nie Y, Zhao G, Cao L, Nakatsu G, Wu WK, Wong SH, Chen Z, Sung JYJ, et al. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut. 2018;67(6):1024–1032. doi:10.1136/gutjnl-2017-314281.
22. Ferreira RM, Pereira-Maques J, Pinto-Ribeiro I, Costa JL, Carneiro F, Machado JC, Figueiredo C. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. Gut. 2018;67(2):226–236. doi:10.1136/gutjnl-2017-314205.
23. Eun CS, Kim BK, Han DS, Kim SY, Kim KM, Choi BY, Song KS, Kim YS, Kim JF. Differences in gastric mucosal microbiota profiling in patients with chronic gastritis, intestinal metaplasia, and gastric cancer using pyrosequencing methods. Helicobacter. 2014;19(6):407–416. doi:10.1111/hel.12145.
24. Castano-Rodriguez N, Goh KL, Fock KM, Mitchell HM, Kaakoush NO. Dysbiosis of the microbiome in gastric carcinogenesis. Sci Rep. 2017;7(1):15957. doi:10.1038/s41598-017-16289-2.
25. Hu YL, Pang W, Huang Y, Zhang Y, Zhang CJ. The gastric microbiome is perturbed in advanced gastric adenocarcinoma identified through shotgun metagenomics. Front Cell Infect Microbiol. 2018;8:433. doi:10.3389/fcimb.2018.00433.
26. Park CH, Lee AR, Lee YR, Eun CS, Lee SK, Han DS. Evaluation of gastric microbiome and metagenomic function in patients with intestinal metaplasia using 16S rRNA gene sequencing. Helicobacter. 2019;24(1):e12547. doi:10.1111/hel.12547.
27. Kim BR, Shin J, Guevarra R, Lee JH, Kim DW, Seol K-H, Lee J-H, Kim HB, Isaacson RE. Deciphering diversity indices for a better understanding of microbial communities. J Microbiol Biotechnol. 2017;27(12):2089–2093. doi:10.4045/jm.1709.09027.
28. Liu X, Shao L, Liu X, Ji F, Mei Y, Cheng Y, Liu F, Yan C, Li L, Ling Z, et al. Alterations of gastric mucosal microbiota across different stomach microhabitats in a cohort of 276 patients with gastric cancer. EBioMedicine. 2019;40:336–348. doi:10.1016/j.ebiom.2018.12.034.
29. Karnes WE Jr., Samloff IM, Siurala M, Kekki M, Sipponen P, Kim SWR, Walsh JH. Positive serum anti-body and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis. Gastroenterology. 1991;101(1):167–174. doi:10.1016/0016-5085(91)90474-Y.

30. Sonveaux P, Copetti T, De Saedeleer CJ, Vegran F, Verrax J, Kennedy KM, Moon EJ, Dhup S, Danhier P, Frerart F, et al. Targeting the lactate transporter MCT1 in endothelial cells inhibits lactate-induced HIF-1 activation and tumor angiogenesis. PLoS One. 2012;7(3):e33418. doi:10.1371/journal.pone.0033418.

31. Berry D, Reinisch W. Intestinal microbiota: a source of novel biomarkers in inflammatory bowel diseases? Best Pract Res Clin Gastroenterol. 2013;27(1):47–58. doi:10.1016/j.bpg.2013.03.005.

32. Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, Laughlin RC, Gomez G, Wu J, Lawhon SD, et al. Host-derived nitrate boosts growth of E. coli in the inflamed gut. Science. 2013;339(6120):708–711.

33. Chen Y, Peng Y, Yu J, Chen T, Wu Y, Shi L, Li Q, Wu J, Fu X. Invasive Fusobacterium nucleatum activates beta-catenin signaling in colorectal cancer via a TLR4/ P-PAK1 cascade. Oncotarget. 2017;8(19):31802–31814. doi:10.18632/oncotarget.15992.

34. Montalban-Arques A, Wurm P, Trajanoski S, Schauer S, Kienesberger S, Halwachs B, Gorkiewicz G, et al. Propionibacterium acnes overabundance and natural killer group 2 member D system activation in corpus-dominant lymphocytic gastritis. J Pathol. 2016;240(4):425–436. doi:10.1002/path.4782.

35. Rego AT, Chandran V, Waksman G. Two-step and one-step secretion mechanisms in Gram-negative bacteria: contrasting the type IV secretion system and the chaperone-usher pathway of pilus biogenesis. Biochem J. 2010;425(3):475–488. doi:10.1042/BJ20091518.

36. Walden K, Rivera-Calzada A, Waksman G. Type IV secretion systems: versatility and diversity in function. Cell Microbiol. 2010;12(9):1203–1212. doi:10.1111/j.1462-5822.2010.01499.x.

37. Backert S, Selbach M. Role of type IV secretion in Helicobacter pylori pathogenesis. Cell Microbiol. 2008;10(8):1573–1581. doi:10.1111/j.1462-5822.2008.01156.x.

38. Wang TC, Dangler CA, Chen D, Goldenring JR, Koh T, Raychowdhury R, Coffey RJ, Ito S, Varro A, Dockray GJ, et al. Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. Gastroenterology. 2000;118(1):36–47. doi:10.1016/S0016-5085(00)70412-4.

39. Lofgren JL, Whary MT, Ge Z, Muthupalani S, Taylor NS, Mobley M, Potter A, Varro A, Eibach D, Suerbaum S, et al. Lack of commensal flora in Helicobacter pylori-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. Gastroenterology. 2011;140(1):210–220. doi:10.1053/j.gastro.2010.09.048.

40. Lee CW, Rickman B, Rogers AB, Ge Z, Wang TC, Fox JG. Helicobacter pylori eradication prevents progression of gastric cancer in hypergastrinemic INS-GAS mice. Cancer Res. 2008;68(9):3540–3548. doi:10.1158/0008-5472.CAN-07-6786.

41. Lertpiriyapong K, Whary MT, Muthupalani S, Lofgren JL, Gamazon ER, Feng Y, Ge Z, Wang TC, Fox JG. Gastric colonisation with a restricted commensal microbiota replicates the promotion of neoplastic lesions by diverse intestinal microbiota in the Helicobacter pylori INS-GAS mouse model of gastric carcinogenesis. Gut. 2014;63(1):54–63. doi:10.1136/gutjnl-2013-305178.

42. Whary MT, Muthupalani S, Ge Z, Feng Y, Lofgren J, Shi HN, Taylor NS, Correa P, Versalovic J, Wang TC, et al. Helminth co-infection in Helicobacter pylori infected INS-GAS mice attenuates gastric premalignant lesions of epithelial dysplasia and glandular atrophy and preserves colonization resistance of the stomach to lower bowel microbiota. Microbes Infect. 2014;16(4):345–355. doi:10.1016/j.micinf.2014.01.005.

43. Wang BH, Yao MF, Lv LX, Ling ZX, Li LJ. The human microbiota in health and disease. Engineering. 2017;3(1):71–82. doi:10.1016/J.ENG.2017.01.008.