The Gastric Microbiome and Its Influence on Gastric Carcinogenesis
Current Knowledge and Ongoing Research

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INTRODUCTION

The human body is thought to house more than 100 trillion microbes. These microbial communities have a significant impact on their human hosts.\textsuperscript{1} They influence everything from pathogen defense to digestion to immune system maturation. The microbiome is also linked to the development of several autoimmune diseases and cancers, including colorectal, pancreatic, and gastric cancers.\textsuperscript{2} A major risk factor for the development of gastric cancer (GC) is infection by the bacteria Helicobacter pylori.

KEYWORDS

- Gastric microbiome
- Helicobacter pylori
- EBV
- Gastric cancer
- Microbial identification

KEY POINTS

- Gastric cancer is the fourth leading cause of cancer-related deaths worldwide, most commonly caused by chronic infection with intracellular bacterium Helicobacter pylori.
- H pylori is the most common cause of peptic ulcer disease. The drivers that determine malignant transformation over self-limiting ulcer and chronic gastritis remain unknown.
- In addition to H pylori, the stomach hosts a diverse and active microbial community whose role in host response and pathogenesis has yet to be fully delineated.
- The gastric microbiome is likely a marker of host health and influences the inflammatory response within upper gastrointestinal cancers.

INTRODUCTION

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pylori, a common inhabitant (although not always pathogen) of the human stomach. GC is the fourth most common cancer worldwide. The study of H pylori and its associated gastric microbiome is of increasing importance in the study of gastrointestinal (GI) diseases and chronic immune response. Understanding the impact of microbial community structure and function on tumorigenesis and immune response will broaden the understanding of GC tumorigenesis and may impact development of therapeutic and preventative approaches.

MICROBIOME DIVERSITY ALONG THE GASTROINTESTINAL TRACT

Although parts of the GI tract are arguably primed for microbial life, the stomach is not intuitively such an environment. The human stomach is particularly unique in that its inhabitants are challenged by several antimicrobial chemicals, enzymes, structural barriers, and highly acidic conditions that are not collectively present in any other part of the human body. Although transient microbes may temporarily survive such an environment through spore formation, thickened cell walls, and acid resistance, those colonizing this environment must adapt to a highly variable and ever-changing landscape.

A benefit of the harsh gastric environment is that it enables segregation of digestive absorption of food from most of the microbial biomass, thereby prioritizing nutrient absorption. The production of salivary enzymes such as lipase and amylase as well as the conversion of nitrates into the antimicrobial compound NO_{2} by Lactobacilli in the mouth begins the process of dramatically reducing the microbial biomass before microbial entry into the stomach. Microbes entering the stomach are then exposed to hydrochloric acid secreted by parietal cells, which then enables the conversion of pepsinogen into pepsin, a potent enzyme that denatures proteins and inhibits microbial survival and growth.

In addition to antimicrobial enzymes, there are several other mechanisms used by the host to prevent microbial proliferation within the stomach. Antimicrobial mechanisms include the expression of immunoglobulin A (IgA), which is thought to limit mucosal penetration and potentially shape diversity of normal gut flora. In addition, the constitutive production of defensins and cathelicidins in epithelial cells as well as triggered expression of C-type lectins help to protect host mucosa from over colonization.

These challenges largely explain the discordance in the bacterial density in the stomach as compared with the colon. By some estimates, microbial cell numbers increase from 10^{1} to 10^{3} colony-forming units (CFU) mL^{-1} in the stomach to 10^{11}–10^{12} cfu mL^{-1} in the large intestine. Notably, the colonic bacterial density exceeds that found in any other known ecosystem.

Gastric Bacteria

Bacteria that are able to reside in the stomach demonstrate several mechanisms that enable colonization within the harsh gastric environment. Acid resistance is a significant factor for bacterial survival and proliferation. Bacteria such as Escherichia coli and H pylori exhibit increased membrane protein production and buffering capacity, allowing these bacteria to resist acid degradation upon entry to the stomach. Subsequent structural, enzymatic, and adhesive adaptive advantages further enable these bacteria and others to colonize the environment.

Multiple studies have attempted to characterize the microbial components of the stomach, either directly from biopsy samples or from gastric juices. There is increasing evidence that the microbiome profiles of the human stomach vary widely between...
individuals with some consistency as to common phyla, although not necessarily species. The most dominant bacterial phyla found across multiple studies are Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes9–14 (Table 1). Several studies also mention frequency of the phyla Fusobacteria.10,15–17 Numerous other phyla have also been detected in lower abundance across gastric samples.

Prevalent species consistent across multiple studies included Streptococcus sp, Lactobacillus sp, Veillonella sp, Prevotella sp, Rothia sp, and Neisseria sp (see Table 1). More than 65% of the phylotypes of bacteria found in the stomach are also those known to inhabit the human mouth3 (Fig. 1).

More than 260 microbial phylotypes have been isolated from as few as 3 separate gastric samples, with only 33 phyla shared between them.15 Gall and colleagues19 demonstrated that bacterial communities in the upper GI tract displayed higher inter-individual variation but overlapping community membership between anatomic sites, indicating that a single set of microbiome profiles of the gastric environment is not generalizable nor directly applicable to any one individual (see Fig. 1). This study also found that samples from the same individual were more likely to be phylogenetically similar to each other than to samples from the same anatomic site in other individuals.19

Bacterial composition and diversity seem to also depend on other species co-inhabiting the gut. Ecologically, many bacteria rely on commensal, syntrophic, and symbiotic relationships, and this is often reflected in community structure and diversity. For example, H pylori is known to reduce the acidity within the stomach, thus enabling other bacteria to colonize an environment where they might not otherwise survive. Therefore, the representation of dominant phyla in the stomach between the H pylori–positive and –negative profiles is notably different. In one study, up to 28% of total variance in gastric microbiota between subjects was explained by H pylori infection status.12

**Esophageal Bacteria**

Biopsies from the normal human esophagus commonly contain bacterial members of the phyla Firmicutes, Bacteroides, Actinobacteria, Proteobacteria, and Fusobacteria.20 Although the predominant phyla of the esophagus fit the profile of those found within the gastric environment, the species composition is fundamentally different.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Gram +/−</th>
<th>Phylum</th>
<th>Relative Abundance, %</th>
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<tbody>
<tr>
<td>Bifidobacteria sp</td>
<td>POS</td>
<td>Actinobacteria</td>
<td>10.7–46.8</td>
</tr>
<tr>
<td>Rothia sp</td>
<td>POS</td>
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<tr>
<td>Prevotella sp</td>
<td>NEG</td>
<td>Bacteroidetes</td>
<td>11.1–26</td>
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<tr>
<td>Enterococcus sp</td>
<td>POS</td>
<td>Firmicutes</td>
<td>29.6–51</td>
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<td>Gemella sp</td>
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<tr>
<td>Lactobacillus sp</td>
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<tr>
<td>Streptococcus sp</td>
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<tr>
<td>Staphylococcus sp</td>
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<tr>
<td>Veillonella sp</td>
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<tr>
<td>Fusobacterium sp</td>
<td>NEG</td>
<td>Fusobacteria</td>
<td>&lt;1.1</td>
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<tr>
<td>Campylobacter sp</td>
<td>NEG</td>
<td>Proteobacteria</td>
<td>6.9–10.8</td>
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<tr>
<td>Haemophilus sp</td>
<td>NEG</td>
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<td>Neisseria sp</td>
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Streptococcus sp comprised almost 40% of bacterial composition, whereas Prevotella sp and Veillonella sp were 17% and 14%, respectively. In one study, up to 87% of bacteria were shared between normal tissues from patients, indicating that the esophagus may house a less diverse population of bacteria between individuals than the stomach.20

A more detailed set of studies investigating esophageal microbiota demonstrated at least 2 distinct microbiome profiles distinguish healthy normal esophageal tissue from gastroesophageal reflux disease (GERD) patients. The profile associated with normal tissue was dominated by Streptococcus sp and en mass contained predominantly gram-positive bacteria belonging to the Firmicutes phyla. The profile most associated with esophagitis and GERD contained primarily gram-negative, anaerobic, or microaerophilic bacteria and a dominance of Bacteroidetes, Proteobacteria, Spirochaetes, and Fusobacteria phyla, respectively. Changes in relative abundance of these taxa appear to play a more significant role than absolute bacterial loads.21

Viruses

Viruses as autochthonous, nonpathogenic inhabitants of the human gastric environment have not been widely studied. To date, there are few known benign viral inhabitants of the stomach. Those known to cause severe gastritis, such as Norovirus, Rotavirus, Astroviridae, and Adenoviridae, are temporary infections generally cleared by the host immune system after a short infectious course. A significant viral pathogen associated with the development of a genetically distinct GC subtype is Epstein-Barr virus (EBV),22 described in detail later in this review. Human papilloma virus has been strongly correlated with esophageal squamous carcinoma and may play a
mechanistic role for the development of other cancers along the GI tract. Other oncogenic viruses infecting other regions of the body that may yet be found to influence the gastric environment include human cytomegalovirus, Merkel cell polyomavirus, and hepatitis B and C viruses. When combined statistically to those cancers caused by \textit{H pylori} bacterium, these microbial pathogens may account for more than 15% of the cancer burden worldwide.

\textbf{Archaea}

Archaea are single-celled prokaryotic organisms that are members of their own genetically distinct domain. They are notorious extremophiles. Found in geothermal vents, miles beneath the surface of the ocean, and even in volcanoes, this group consists of many organisms primed for otherwise inhospitable conditions.

The human gut is host to several archaea, notably methane-producing species known as methanogens. Two main species frequently inhabit the gut: \textit{Methanobrevibacter smithii} and \textit{Methanosphaera stadtmanae} (MSS), likely metabolically supporting surrounding bacterial communities. Although few studies to date have attempted to elucidate archaea from the stomach specifically, it is highly likely given their ecology that these microbes are found in abundance throughout the GI tract.

\textit{Methanoarchaea} are frequently able to form syntrophic interactions with a wide range of bacterial constituents, and in some cases, are necessary for their survival, metabolic function, and growth. For some beneficial bacteria, they are necessary cohabitants of the environment. They possess metabolic processes that convert common bacterial fermentation byproducts such as hydrogen and carbon dioxide and enable interspecies hydrogen transfer. Studies of archaea composition within patients with cancer suffering from chemotherapy-induced diarrhea demonstrated a decrease of methanogenic archaea in parallel with the loss of beneficial bacteria.

Despite ubiquitous distribution and high numbers within the human gut, archaea have not generally been recognized as significant components of the microbiota and the immune system. Associations between \textit{Methanobrevibacter oralis} and inflammatory periodontal disease have, however, led to further interest in the potential pathogenic potential of some archaea.

Blais Lecours and colleagues found increased prevalence of MSS in patients with inflammatory bowel disease (IBD). Only IBD patients developed a significant anti-MSS IgG response. Additional evidence suggests methane production by archaea is related to pathogenesis of constipation, irritable bowel syndrome (IBS), and obesity. Many patients with IBS and constipation excrete methane, suggesting an overabundance of methanogenic archaea in their gut. In a more recent study, differences in archaea and microbial composition were observed in colorectal tubular adenoma and adenocarcinoma cases between both healthy and diseased mucosa. It is apparent from such studies that archaea have the potential to influence both community structure within the microbiome and immune response. As such, they should not be overlooked.

\textbf{Fungi}

Fungi are a ubiquitous but often ignored component of the gastric microbiome. Surveys of the gastric environment identified hundreds of diverse fungal strains, including representatives from \textit{Candida albicans}, \textit{Candida tropicalis}, and \textit{Candida lusitanae} within both the healthy gut and those with GI ulcers. Fungi survive much higher acidities than many bacteria due to structural and biological adaptations. \textit{C albicans} are able to proliferate at a pH of 1.4 and upwards. Comparative studies of fungi colonizing gastric ulcers found 54.2% of cases had fungal infections compared with 10.3% of
those with chronic gastritis and 4.3% of controls. Measurements of antifungal antibodies and the presence of fungal antigens within these study subjects’ serum indicated that fungal colonizations were likely secondary features of other infections; however, they are likely still important in the context of the gastric microbial milieu.\textsuperscript{37}

### IDENTIFICATION METHODOLOGIES FROM CLINICAL SAMPLES

#### Experimental Approaches

Studying microbes within the gastric environment is particularly challenging given the low abundance and the lack of ability to culture these organisms. More than 80% of microbes are uncultivable.\textsuperscript{3}

Even with well-established culture independent methods such as the isolation and amplification of 16S ribosomal RNA (rRNA) genes by polymerase chain reaction (PCR) and quantitative PCR, microbial DNA isolation from human tissue is notoriously subject to bias. The bias of 16S-based methods often results from environmental conditions and the ability of extraction techniques to account for natural differences in microbial cell wall structure as well as susceptibility of certain microbial species to lysis. Bacteria in particular may exhibit varying levels of resistance to release their nucleic acids using chaotropic agents, enzymes, homogenization, or bead-beating.\textsuperscript{38} As such, there is not a single “gold standard” by which to determine environmental composition of human samples. Several methods are described in the literature to try to address this disparity with varying results in terms of diversity, species selection, and abundance.\textsuperscript{39,40} For any extractions intended for downstream sequencing, it is therefore important to be aware that extraction methodologies may not capture the full spectrum of bacterial composition of the samples.

Some other culture-independent methods used to obtain an accurate profile of these microbes include fluorescent in situ hybridization, dot-blot hybridization with rRNA-targeted probes, denaturing gradient gel electrophoresis, cloning, and more recently, whole genome shotgun (WGS) sequencing.\textsuperscript{38} Although no method is immune to bias, analyses like whole genome sequencing enable a much more sensitive means to obtain microbial sequences and data. Using additional computational pipelines, it is possible to achieve another lens with which to view the microbial constituents of the human microbiome.

#### Computational Identification of Microbiome

The computational approaches to identify the microbiome have been specifically designed to process 16S rRNA gene or WGS sequencing, respectively. The bacterial and archaeal 16S rRNA gene has been widely used as the phylogenetic marker for microbial characterization due to its universal presence in prokaryotes. Several different taxonomic schemes have been proposed by independent curators based on structural and functional attributes of microbes, and 16S rRNA sequences, for example, Berpey’s,\textsuperscript{41} National Center for Biotechnology Information. All major rRNA gene sequence databases, such as RDP,\textsuperscript{42} Greengenes,\textsuperscript{43} and SILVA,\textsuperscript{44} were designed based on different taxonomic schemes and accumulated vast reference sequences for phylogenetic analysis. Generally, algorithms for microbial identification from 16S rRNA data can be divided into 2 major categories: homology based and composition based.\textsuperscript{45} Homology-based approaches use traditional sequence alignment algorithms to compare similarity between sequencing data and reference 16S rRNA in the database. Composition-based methods build models based on the different features extracted from sequences, for example, GC content, codon usage, and frequencies of motifs. To date, most microbiome studies rely on 16S rRNA-based
method, because it is cost-effective, and data processing is easier than WGS data. The limitation of this method is that the taxonomic annotation is based on putative association of one single gene. In practice, only 1 of 2 variable regions of 16S rRNA is amplified. Because of its highly conserved nature, typically it only analyzes at the phyla or genus level, and identification at the species level could be less accurate. For example, different species within the same genus, such as *Bacillus cereus*, *Bacillus thuringiensis*, and *Bacillus anthracis*, have only a few base differences in their 16S rRNA gene sequences.

As the alternative approach, WGS appears to be more accurate to profile the microbiome to species level, even to subspecies level, but it is more expensive and requires extensive data analysis. Computational methods to identify microbes from WGS can generally be classified, similarly to 16S, as homology based and composition based. A potential limitation of WGS is that most algorithms require complete genomes to build databases in order to achieve precision. Although a recent publication partially addressed this problem by constructing the database with detected clade-specific markers, it sacrifices the identification accuracy to reduce the computational complexity. Unlike 16S rRNA genes that are specific to prokaryotes, WGS sequencing approach captures host DNA as well. The relative abundance of host contamination is as high as 99.9% in human clinical biopsies. Although a pipeline with extensive filtering steps that maps sequencing reads to different human sequence databases could remove most host content, the remaining reads may still lead to false identification within traditional microbiome profiling software, which is designed to process microbial DNA-enriched samples. Thus, additional steps are necessary to accurately characterize microbial species for host DNA-enriched samples.

A myriad of factors can affect microbial detection, including different experiment protocols, different reference databases, different species markers, and different mapping algorithms. Although it is unlikely that different identification approaches will agree in bacterial identification, the most abundant species should always be detected by all methods.

**EPIDEMIOLOGIC ASSOCIATIONS WITH DISEASES**

*Helicobacter pylori*

GC is a heterogeneous disease with several established risk factors, one of the most significant being chronic gastric inflammation due to infection by the gram-negative, facultative intracellular bacillus *H pylori*. Unlike other common factors causing acute gastritis, *H pylori*–associated acute gastritis is usually asymptomatic; thus, it could be easily ignored and delayed. Several studies show strong correlations between *H pylori* and acute gastritis. Harford and colleagues performed a long-term follow-up study on 35 cases of acute gastritis with hypochlorhydria. Twenty-eight of 35 participants either had a positive *H pylori* infection history or had a new infection within 12 months. Cheung and colleagues completed gastroscopies for 194 participants out of a population of 600 from Aklavik, Canada. Among 128 participants who were histologically *H pylori* positive, prevalence was 94% for acute gastritis and 100% for chronic gastritis.

After persistent colonization within the gastric mucosa, *H pylori* has been significantly associated with the development of different stages of gastric diseases, from chronic gastritis, gastric ulcers, atrophic gastritis, intestinal metaplasia, and finally, to GC. Several studies and meta-analyses support these findings.

Besides gastritis and GC, *H pylori* has also been identified as a risk factor for other upper GI diseases. The study by Eidt and colleagues found that nearly all patients...
with gastric mucosa-associated lymphoid tissue lymphoma are \textit{H pylori} positive. Although there are multiple factors that may cause functional dyspepsia, \textit{H pylori} is still considered a likely culprit.\textsuperscript{65,66} A recent retrospective study of 5156 patients revealed that \textit{H pylori} infection was significantly more common among those with GERD (odds ratio = 1.17).\textsuperscript{67} Interestingly, \textit{H pylori} also has been reported with inverse correlations to some diseases. Multiple meta-analyses revealed that decline of \textit{H pylori} colonization in the past few decades may be responsible for an increase of esophageal cancer incidence in Western countries.\textsuperscript{68,69} Although 95\% duodenal ulcer occurred in the presence of \textit{H pylori} infection,\textsuperscript{56} in the cohort of patients with duodenal ulcers, the incidence of GC was significantly lower than expected.\textsuperscript{70}

\textbf{Epstein-Barr Virus}

As another identified risk factor for developing GC, EBV\textsuperscript{71} is responsible for 5\% to 20\% of GC worldwide. Global infection prevalence of EBV is approximately 9\%, which is much lower than that of \textit{H pylori}. Only a small proportion of GC is associated with EBV infection; therefore, most epidemiologic studies cannot find strong correlations due to the limited data.\textsuperscript{72} Although EBV has been suspected in several other upper GI diseases, most studies to date are only case reports,\textsuperscript{73,74} and a lack of large-scale studies cannot currently support the causal relationships between EBV and these diseases.

\textbf{Other}

Because of a lack of understanding of the gastric microbiome as a whole, most epidemiologic studies are limited to \textit{H pylori} and EBV.\textsuperscript{75} Only a few studies have looked at the community of bacteria and their overall relationship to GC. Aviles-Jimenez and colleagues\textsuperscript{76} found a significant microbial difference between nonatrophic gastritis (NAG) and intestinal-type GC. \textit{Porphyromonas}, \textit{Neisseria}, TM7 group, and \textit{Streptococcus sinensis} decrease, whereas \textit{Lactobacillus coleohominis} and \textit{Lachnospiraceae} increase from NAG to GC. Seo and colleagues\textsuperscript{77} evaluated cancer tissue and matching normal gastric mucosa from 16 patients. According to the genus level comparison, they found that \textit{Propionibacterium} spp, \textit{Staphylococcus} spp, and \textit{Corynebacterium} spp had significantly reduced populations in cancer tissue, whereas \textit{Clostridium} spp and \textit{Prevotella} spp had significantly increased populations. Eun and colleagues\textsuperscript{78} used 454 sequencing to sequence the V5 region of 16S rDNA from 31 patients. They found that compared with chronic gastritis and intestinal metaplasia groups, the relative abundance of Bacilli class and Streptococcaceae families increased, and the Helicobacteraceae family was significantly lower in GC group.

\textbf{MOLECULAR ASSOCIATIONS AND MECHANISMS OF TUMORIGENESIS}

Bacteria within the human body produce low molecular weight substances that, although difficult to isolate, are attributable to epigenetic changes. The epigenetic changes include chromatin remodeling and signaling molecule changes, which regulate cellular differentiation and apoptosis as well as inflammation.\textsuperscript{79} These changes may also lead to tumorigenesis. The mechanisms behind these relationships are complex and may have larger implications for the study of oncogenic potential among several bacteria found within the human microbiome.

\textbf{Mechanisms of Helicobacter pylori Tumorigenicity}

\textit{H pylori} invades and damages the gastric mucosa using a series of competitive mechanisms specifically adapted to colonize the gastric environment. The major
mechanisms include structural and adhesive advantages such as flagella that enable motility and penetration of the gastric mucin layer. \textit{H pylori} binds to mucin and targets gastric epithelial cells. Its flagella are protected by a lipopolysaccharides-containing sheath and protein, which generate an immune response mediating mucus synthesis and secretion by epithelial cells.\textsuperscript{80,81} Another colonizing advantage of \textit{H pylori} is the production of urease enzyme. Urease produces ammonia from urea, thereby neutralizing nitric acid within the stomach and allowing access to the mucus layer. \textit{H pylori} is then able to colonize the epithelium and elicit an intricate immune response of inflammatory cytokines leading to peptic ulcers or chronic gastritis.\textsuperscript{3}

Highly carcinogenic strains of \textit{H pylori} carry the cytotoxin-associated gene A (cagA). This gene encodes for CagA, a known regulator of malignancy. Bacterial type IV secretions deliver CagA into gastric epithelial cells.\textsuperscript{82} CagA disrupts host signaling pathways by acting as a hub or extrinsic scaffold protein, in turn potentiating genomic instability or malignant transformation.\textsuperscript{83,84} Transgenic mice who systemically express wild-type CagA have been known to spontaneously develop GI carcinomas, whereas phosphorylation-resistant CagA fails to induce such malignancy.\textsuperscript{85} CagA is stabilized through phosphorylation, which in turn enables binding to SHP2 and subsequent activation of the Ras-Erk mitogenic pathway.\textsuperscript{86} Genes often overexpressed in GC are involved in this signaling pathway. The Ras-Erk mitogenic pathway include FGFR2, KRAS, EGFR, ERBB2, and MET, which are frequently investigated targets for drug treatment.\textsuperscript{87–89} Overexpression of above genes leads to unique DNA damage in transcribed regions and those proximal to telomeres in gastric cell lines and primary gastric epithelial cells.\textsuperscript{90}

In addition to CagA, several other bacterial virulence factors play a role in both the pathogenesis and the inflammatory potential of \textit{H pylori}. These virulence factors include Vacuolating cytotoxin A and its associated polymorphisms as well as outer membrane proteins BabA, HomA, and HomB.\textsuperscript{91} HomB in Western-type CagA strains have been directly associated with the development of GC.\textsuperscript{92}

The interactions between \textit{H pylori} and other microbiota within the gastric environment are complex and are mediated in part by host response. \textit{H pylori} uses BabA and Cag plasminogen activator inhibitor proteins to adhere to epithelial cells, which promotes expression of inflammatory cytokines, including IL-1β, IL-6, IL-8, and IL-10, as well as tumor necrosis factor-α, exacerbating immune responses.\textsuperscript{93–95} Immune exacerbation in turn likely impacts the success of other bacterial species. \textit{H pylori} also affects the hormones and density-dependent humoral and cellular immune responses within the gastric environment.\textsuperscript{96,97} Hormones that modulate immunity and gastric acid secretion promote a Th1 response.\textsuperscript{98,99} More research is needed to characterize the physiologic differences related to \textit{H pylori} status, including variation in the gastric microbiota, as well as its clinical implications.\textsuperscript{12}

**EVIDENCE FOR PROLONGED MICROBIOME SHIFTS ASSOCIATED WITH PRIOR INFECTION WITH HELICOBACTER PYLORI**

Another unique feature of \textit{H pylori} infection within the gut is its potential for long-term disturbances of the microbiota even after individuals clear infection. A recent study examining the stool of patients previously infected with \textit{H pylori} found that up to 18 months after eradication there is still a disruption in the phyla and genera of microbes seen within the lower GI tract. Yap and colleagues\textsuperscript{100} found a continual increase in Firmicutes and decrease in Bacteroidetes species at 6 months, 12 months, and 18 months after infection, respectively. Bacteroidetes and Firmicutes are associated with the regulation of lipids and bile acid metabolic processes. Disturbances in
these balances have been implicated in metabolic disorders and obesity. Long-term perturbations of the gut microbiota due to \textit{H pylori} infection may therefore have further implications for host immune response and recovery.

\textbf{Epstein-Barr Virus}

EBV-associated gastric carcinoma (EBVaGC) is a distinct subtype that has very distinguished molecular characteristics compared with other GC subtypes defined by The Cancer Genome Atlas study. Although some of the molecular mechanisms underlying EBVaGC are still unclear, several studies have already demonstrated the genomic features of EBVaGC. These studies provide an important understanding of EBV involvement in gastric carcinogenesis.

\textbf{Latent infection in gastric epithelial cells}

Previous studies reveal that EBV can readily infect B lymphocytes through viral receptor CD21. Because epithelial cells lack the expression of CD21, the efficiency of EBV infection is much lower. Coculturing epithelial cells with EBV-producing B-lymphoblastoid cells will significantly increase infection efficiency, and direct cell-to-cell contact with B lymphocytes might be the major model of EBV infection within epithelial cells. After infection is established in epithelial cells, EBV will maintain a latent infection status and only express a limited set of viral genes, such as EBERs, EBNA-1, and BARTs. The functions of these viral genes were investigated in several studies and were found to have interactions with host proteins that affect cell proliferation, apoptosis resistance, and production of autocrine growth factors. The resulting DNA damage and promotion of cell survival contribute to the development of GC.

\textbf{Epigenetic alterations}

Besides direct manipulating cell proliferation and migration, EBV also can disrupt host epigenetic machinery to affect the host epigenome on its path to malignancy. EBVaGC exhibits the global but nonrandom CpG island hypermethylation at various tumor suppressor genes such as PTEN. By expressing LMP1 and LMP2A viral oncoproteins to upregulate host DNMTs, EBV not only can inactivate these host tumor suppressors but also may have its own DNA methylated to evade host immune response. However, the target specificity for these processes remains unclear. Different studies have reported several sets of genes that are hypermethylated in EBVaGC rather than other GC subtypes, but there is no single gene present in all 3 major studies on the topic.

\textbf{Viral microRNAs}

The EBV genome encodes 25 microRNA (miRNA) precursors and 44 mature miRNAs. Among them, only 22 miRNA precursors from BART cluster are expressed in EBVaGC. Several studies revealed that a set of EBV miRNAs, ebv-miR-BART1-3p, 2-5p, 3, 4, 5, 7, 9, 10-3p, 17-5p, and 18-5p, were expressed at relatively high levels in EBVaGC, and some of them could target the tumor suppressor gene PTEN. They also observed the downregulation of human cellular miRNAs, such hsa-miR-200a, hsa-miR-200b, and let-7 family, which are tumor suppressor miRNAs. Viral miRNA involvement in EBVaGC remains largely unknown. Besides regulating the gene expression, viral miRNAs might also interfere with intercellular communication and modify the tumor microenvironment. EBV miRNAs could be transferred from EBV-infected cells to noninfected cells through exosome transfer, and they accumulate in noninfected cells to induce apoptosis in Jurkat T cells.
Mechanisms of *Fusobacterium* sp

In addition to *H pylori*, another gastric bacterium that has recently been identified as significantly oncogenic is *Fusobacterium nucleatum*. *Fusobacterium* sp are gram-negative, non-spore-forming, anaerobic bacteria that are commonly isolated bacterial flora throughout the human GI tract. The species *F nucleatum*, however, is associated with several other disease manifestations such as periodontitis as well as tumor growth and carcinogenesis of both pancreatic and colorectal cancer. More recently, *F nucleatum* positivity has been linked to microsatellite instability (MSI-high) and CpG island methylator phenotype status in studies involving large US and Japanese colorectal cancer cohorts. MSI and other molecular characteristics of colorectal cancer influence T-cell-mediated adaptive immunity. Mechanistically, *F nucleatum* in colorectal tumors appears to have the capacity to expand myeloid-derived immune cells and inhibit T-cell proliferation, inducing T-cell apoptosis. As such, this bacterium may prove to have more serious immune implications in other regions of the GI tract, such as the stomach, and its impact on the gastric microenvironment has yet to be thoroughly investigated.

Other Species

Given the relatively recent discovery of *H pylori* and *F nucleatum* as known gastric bacteria with tumorigenic potential, it is likely that with improved surveillance and additional focus on the microbiome as an indicator for immune health, there are several other microbes with significant tumorigenic mechanisms yet to be fully elucidated in the gastric environment.

Several bacterial species are also coming to light with regard to gut-derived cancers that may be connected to the stomach microbiota as well. Retrospective studies have shown associations between *Streptococcus* sp and both oral and colorectal cancer, whereas *Salmonella typhi* has shown capacity for the manipulation of host signaling pathways leading to gallbladder carcinomas, respectively. *Streptococcus* species are frequently isolated within the stomach, and their carcinogenic potential therein has not yet been fully explored.

Notable temporal and spatial links between periodontal disease–causing bacteria and oral and esophageal cancers have also recently emerged, begging the question whether this association also may extend to microbial inhabitants of the stomach and other carcinogenic mechanisms throughout the body. *H pylori* and *Fusobacterium* have already been isolated from periodontal pockets, sometimes forming biofilms and coaggregating to form dental plaques. The relationship between this co-occurrence and further infection potential within the greater GI tract has yet to be fully understood.

Chronic inflammation caused by other orally derived species, such as *Campylobacter concisus*, *Porphyromonas gigivalis*, and *Prevotella melaninogenica*, has been linked to periodontal lesions as well as increased expression of biomarkers and cytokines for carcinogenesis, potentially promoting the development of esophageal and oral cancers as well as some cases of pancreatic cancer. These relationships will likely only prove more important as we continue to build on the understanding of oral microbiota and the spatial and temporal relationships between inflammation and immune response throughout the GI tract.

“COMMUNITY AS PATHOGEN”—COMMUNITY DYNAMICS AND PATHOBIONTS

The microbiome as a whole is defined as the sum total genome of all bacteria, archaea, fungi, protozoa, and viruses inhabiting the human body (see Fig. 1). In many ways,
these communities are analogous to isolated ecosystems with defined trophic architecture, ecological niches, and community assemblages that determine overall environmental health and stability. Much as a rainforest or reef system experiences disturbances, community assemblage overturn, and invasive species, which may alter entire species composition and ultimate homeostasis of a given environment, so too might this occur within the gut. Rather than identifying single pathogens as the causative agent for chronic inflammation and tumorigenesis, it is becoming increasingly evident that several environmental and community factors often come into play to potentiate these effects. These factors include conditions promoted by pathogenic symbionts or “pathobionts” of otherwise commensal bacteria within the human gut. When, for example, H pylori lowers the acidity profile of the gastric environment, it is an ecological disturbance that in turn affects community structure and perhaps the ability for pathobionts to proliferate and impact other aspects of host health (Fig. 2). This ecological disturbance could be observed within the esophageal environment as well. Unlike gastric malignancies, which have been correlated to specific infections by EBV or H pylori, GERD, esophageal adenocarcinomas, and other malignancies have been attributed to chronically disrupted microbiome profiles and dysbiosis.132

Microbial profile variations as well as community interdependence between and within individuals may offer valuable clues to the origins of cancer development,
disease prognosis, and immune system health. Current evidence that the microbiome may be linked to immunotherapy response within the colon and infections with *H pylori* leads to long-term perturbations of gut stability further illustrates the importance of understanding these relationships. Preliminary studies have shown positive correlations between abundance of certain commensal gut bacteria within the colon and antitumor responses. Specifically, colonization by various *Bifidobacterium* sp has demonstrated an improved response to anti-PDL1 therapy. However, precisely what effect a given homeostasis or biodiversity may have on chronic inflammation, immune status, and several other factors on the human body has yet to be fully understood.

**CHALLENGES AND ONGOING RESEARCH**

Gastric microbiota is strongly associated with many upper GI diseases, including cancer. *H pylori* itself is estimated to contribute to 5.5% of all cancer cases and more than 60% of GC cases. Gastric microbiota is still ignored by most GC studies, because stomach has been considered a sterile environment for a very long period. Hebert and colleagues revealed that only less than 10% National Cancer Institute–supported grants for GC research incorporated microbiome analysis, indicating lack of attention toward gastric microbiome study. Among all *H pylori*–infected individuals, only 1% to 3% develop GC. Gastric tumorigenesis studies should incorporate host genetic characteristic and virulence diversity of *H pylori* strains as well as the entire microbiome community. Notably, GC development might take longer than 20 years, and ideal design of the study should include long-term observations on microbiome dynamics and spontaneous host genetic changes over the time, such as mutation accumulation.

**REFERENCES**


