A discussion of the gut microbiome’s development, determinants, and dysbiosis in cancers of the esophagus and stomach

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Abstract: The microbiome refers to a population of microbes that colonize the skin, nasopharynx, oral cavity, gastrointestinal tract, and urogenital tract. The human microbiome consists of bacteria, archaea, fungi, viruses, and phages. Recent advances in genomic sequencing have catalyzed a deeper understanding of complex microbe-microbe and host-microbe interactions. Dysregulation of these interactions, or dysbiosis of the gastrointestinal tract, has been implicated in a growing list of pathologies including nonalcoholic fatty liver disease, cardiovascular disease, obesity, diabetes, depression, Parkinson’s disease, autism, and various gastrointestinal cancers. Gastric and esophageal cancer, for example, continue to remain as two of the most common causes of cancer-related deaths worldwide, therefore there is an increased emphasis on investigating the role of dysbiosis on these cancers. In this review, we discuss the development and structure of the gut microbiome, its homeostatic and dysbiotic mechanisms, and the key microbes in esophageal and gastric carcinogenesis with a focus on bacterial biology. Further clarification of these pathways and discovery of diagnostic or therapeutic targets could have broad impacts on global subpopulations. It is important to understand the nature of the gastrointestinal tract microbiome and its potential risk factors for dysbiosis in order to tailor its application to the individual patient and create an era of highly personalized, precision medicine.

Keywords: Microbiome; gut; gastrointestinal tract; cancer; cancer prevention; human

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Introduction

The microbiome refers to a population of microbes that colonize the skin, nasopharynx, oral cavity, gastrointestinal tract, and urogenital tract in a ratio of at least one microbe cell to one human cell. Though the above estimate accounts for only bacterial cells, it should be noted that the microbiome includes archaea, fungi, viruses, and phages (1). More important than the actual number of cells or even the number of species inhabiting these microenvironments is the additional genetic diversity, which by some estimates, are orders of magnitude larger than the human genome (2). Though interactions amongst commensal microbiota and between microbiota and the host are complex, the underlying mechanisms are beginning to be elucidated and even manipulated. A healthy gut microbiome confers in humans the ability to digest complex dietary polysaccharides, synthesize essential amino acids, and absorb vitamins, metabolize certain drugs, develop the immune system, and to defend against pathobionts (3-7).
Table 1 Glossary of terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commensalism</td>
<td>A relationship between two organisms in which one organism benefits, whereas the other is not harmed or helped</td>
<td>(13)</td>
</tr>
<tr>
<td>Dysbiosis</td>
<td>An abnormality—in composition and/or function—of the host symbiotic microbial ecosystem that exceeds its restitutive capacity and has negative effects on the host</td>
<td>(14)</td>
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<tr>
<td>Enterotypes</td>
<td>Distinct microbiome configurations depending on composition or metabolic profile of component microbial organisms</td>
<td>(15)</td>
</tr>
<tr>
<td>Esotypes</td>
<td>Functionally distinct community types found in the esophageal microbiome depending on the relative abundances of microbial organisms</td>
<td>(13)</td>
</tr>
<tr>
<td>Genetic diversity</td>
<td>The variation in a DNA sequence between distinct individuals of a given species (or population)</td>
<td>–</td>
</tr>
<tr>
<td>Genetic variability</td>
<td>Genotype differences in a given population</td>
<td>–</td>
</tr>
<tr>
<td>Genome</td>
<td>The genetic product of an organism</td>
<td>–</td>
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<tr>
<td>Human Microbiome Project</td>
<td>A National Institutes of Health project to characterize the human microbial flora launched in 2007</td>
<td>(16)</td>
</tr>
<tr>
<td>Microbiome</td>
<td>The complex ecosystem of microbial cells including bacteria, archaea, eukaryotes, and viruses, that colonize the human body. The microbiome has the capability of encoding genetic products that affect health and disease in the human host</td>
<td>–</td>
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<tr>
<td>Microenvironment</td>
<td>The immediately local habitat of a microbial cell, usually referring to distinct space within an organ in the host</td>
<td>–</td>
</tr>
<tr>
<td>Mutualism</td>
<td>A relationship between two organisms in which both organisms benefit</td>
<td>(14)</td>
</tr>
<tr>
<td>Pathobiont</td>
<td>Normally innocuous microorganisms that can behave like pathogens if their abundance increases and/or their environmental conditions change</td>
<td>(13)</td>
</tr>
<tr>
<td>Pathogen</td>
<td>A microorganism that can cause disease</td>
<td>(14)</td>
</tr>
<tr>
<td>Phylum</td>
<td>A taxonomic classification that ranks in between kingdom and class</td>
<td>–</td>
</tr>
<tr>
<td>Taxon</td>
<td>A group of organisms</td>
<td>–</td>
</tr>
<tr>
<td>Taxonomic level</td>
<td>A relative level of a group of organisms</td>
<td>–</td>
</tr>
<tr>
<td>Virulence genes or virulence factors</td>
<td>Molecules expressed by pathogenic microorganisms that help them to gain a growth advantage in a specific ecosystem. These molecules are often responsible for disease manifestation in the host</td>
<td>(13)</td>
</tr>
</tbody>
</table>

Dysregulation of the microbiome, or dysbiosis, has been linked to a growing list of pathologies including nonalcoholic fatty liver disease, cardiovascular disease, obesity, diabetes, depression, Parkinson’s disease, autism, and various cancers (8-12) (Table 1).

Once regarded as the “forgotten organ”, the gut microbiome is now the subject of renewed fervor. In fact, more than 80% of all microbiome publications were published after 2012 (17,18). Given the dizzying pace of discovery in this field, it may be difficult for clinicians to keep up with the latest insights on gut microbiomes and the implications of dysbiosis in gastrointestinal malignancies. We will briefly discuss perturbations in the gastrointestinal microflora or gut “dysbiosis” focusing on the development and structure of gut microbiota, their reputed role in the protection against cancer, and the proposed mechanisms by which gut dysbiosis may contribute to some types of cancer such as esophageal and gastric cancers.

Recently, the International Cancer Microbiome Consortium released a consensus statement describing four key features of a healthy gut microbiome: (I) synergizing with the host for immune function; (II) metabolic mutualism; (III) resilience to temporary disturbances and adaptation; and (IV) tumor-suppression (14). The first
The development and structure of the gut microbiome

The prenatal intestinal tract was once thought to be sterile, though this notion has recently been challenged. Molecular surveys on infants have shown micro-organism colonization may occur in utero, suggesting that our microbiomes are older than we are (19). Neonatal gut-associated lymphoid tissue along with the rest of the immune system is not fully developed upon birth. Mucosal secretion of mucin, IgA, and antimicrobial peptides has not been established. Infants are inoculated with microbes during birth, and the composition of the early microbiome is affected by gestational age and mode of delivery (Figure 1). For example, preterm infants and infants delivered via c-section were found to have reduced diversity and a delayed colonization by commensals as compared to term infants or vaginal deliveries (20). Breastfeeding then provides the infant gut with a “continuous inoculum” of microbes along with human milk oligosaccharides and secretory IgA (21). Breastfed infants have a microbiota composed predominantly of Bifidobacteria spp. and Lactobacilli spp. which consume human milk oligosaccharides and produce short chain fatty acids (SCFAs) (i.e., acetate, propionate, and butyrate). SCFAs are substrates for energy production, downregulate pathogen virulence genes, and promote immunotolerance (21). The microbiome of formula-fed infants is more diverse. This discrepancy of SCFA production in infants has implications in allergic airway inflammation, arthritis, ulcerative colitis, and colic (22-25). Introduction of solid foods at around 6 months of age promotes a shift towards Bacteroides spp. which have better ability to metabolize complex carbohydrates. Diversification of the microbiome increases and adapts to the diet of the child. By age 3, gut microbiota approaches adult-level diversity (26,27).

The adult gut microbiome is composed of intestinal colonization niches in which bacterial microbiota are generally consistent at higher taxonomic levels (18). Firmicutes and Bacteroidetes are the major phyla within the intestinal tract. Stratifying the gut microbiome by just the relative abundance of organisms provides an overly simplistic view because significant variability occurs at lower taxonomic levels. The Human Microbiome Project examined 4,788 specimens from 242 screened and phenotyped healthy adult men and women in the US to characterize the microbial composition of 18 body habitats. The results revealed that microbial compositions were unique to each individual and remained stable over time. Interestingly, no taxa were observed to be universally
present among the body habitats or individuals (16). An analysis of monozygotic and dizygotic twins demonstrated that significant gut microbial variability exists even between identical twins (28). Thus, over 80% of individuals can be reliably identified by their “microbial fingerprint” for up to a year after collecting a stool sample (29).

Metabolic pathways of microbiota, however, remained stable with respect to the individual and the overall population, regardless of the taxonomic variance and geography, suggesting that a “core microbiome” is organized around the functional gene level and not just at the organismal level (16,28-30). Indeed, clustering models accounting for frequencies of both microbial genes and populations indicate reproducible patterns of variation driven by subsets of three taxa (Prevotella, Bacteroides and Ruminococcaceae) which appear to be mutually negatively correlated (15). These clusters, or “enterotypes”, suggest that the core metagenomic functions and structure of gut microbiomes are conserved. Therefore, it follows that the gut microbiome has the capacity to achieve homeostasis between the host, the gut microbiome, the component microbiota, the host’s environment, the host’s disease state, and pharmacology.

The gut microbiome in homeostasis, dysbiosis, and tumor suppression

The homeostatic drive of the gut microbiome can be illustrated through its protective role against pathogen colonization (Figure 2). Given that approximately 15% of all cancers have been attributed to infectious agents, the gut microbiome is a crucial shield against infection and the subsequent carcinogenic sequela (31,32). Commensals competitively inhibit pathogenic growth by consuming nutrients and residual oxygen, depleting the resources available to pathobionts. Commensals fortify the intestinal epithelial barrier function by upregulating mucin

Figure 2 Commensal microbiota inhibit colonization of exogenous pathogens and pathobionts through upregulation of host-immunity mediated resistance such as activating plasma B cells, macrophages, T-helper cells, and innate lymphoid cells. Commensal microbiota also competitively inhibit pathogens and pathobionts by uptake of nutrients, suppressing virulence factors, and consuming O₂ needed for pathogen and pathobiont survival.
production, inducing antimicrobial peptides (RegIIIβ), and promoting IgA secretion. Commensal microbiota enhance innate host immunity via the MyD88 pathway by upregulating intestinal macrophage production of pro-IL-1β, which is activated to rapidly recruit neutrophils in enteric infections. Additionally, resident commensals facilitate differentiation of T\textsubscript{H}17 cells and innate lymphoid cells which release IL-22 and through a MyD88 independent mechanism (18).

When the gut microbiome is unable to return to homeostasis, it exists in a state referred to as dysbiosis. It is tempting to define dysbiosis as an anomalous composition of resident microbes (i.e., bloom of pathobionts, loss of commensals, and loss of diversity) (33). Experts, however, define dysbiosis an “abnormality—in composition and/or function—of the host symbiotic microbial ecosystem that exceeds its restitutive capacity and has negative effects on the host” (14). Compositional and functional abnormalities probably coexist and propagate each other. Dysbiosis is not applicable beyond the context of the individual host and specific pathology. A sustained state of dysbiosis may lead to carcinogenesis (14). In fact, dysbiosis could promote carcinogenesis by several potential mechanisms. For example, studies on the vaginal microbiome have found that the human papillomavirus inserts its DNA into the host DNA in a strategy known as genomic integration. In genotoxicity, pathogens or their metabolites damage host DNA structure causing cell death, promoting oncogenes, or disabling tumor suppressor genes. Chronic inflammation by microbial virulence factors can affect host intracellular signaling pathways inducing cellular proliferation and deregulating apoptosis. Pathogens may weaken cancer immunosurveillance by targeting host immune cells. Even commensal metabolism can be manipulated to produce reactive metabolites or convert pro-carcinogens into carcinogens.

**Esophageal cancer**

The esophagus is relatively sterile when compared to the rest of the GI tract, harboring less than one trillionth of the bacterial population in the colon (17). Pei et al. demonstrated that approximately 100 species are endemic to the normal esophageal population within six phyla: Firmicutes (e.g., Streptococcus), Bacteroides (e.g., Prevotella), Actinobacteria (e.g., Rothia), Proteobacteria (e.g., Haemophilus), Fusobacteria (e.g., Fusobacterium), and TM7/Saccharibacteria (34). Deshpande et al. later performed cluster analyses on bacteria found in patients with a normal esophagus and described the existence of three clusters or “esotypes”. Esotypes were characterized by dominant organisms and had distinct functional signatures with the first type largely comprised of *Streptococcus* spp. (enriched pentose phosphate pathway, fructose/mannose metabolism), the second *Prevotella* spp. [enriched lipopolysaccharide (LPS) biosynthesis], and the third *Prevotella, Haemophilus*, and *Rothia* (enriched glycolysis pathway, SCFA metabolism) (35). Notably, the authors found that patients with gastroesophageal reflux disease (GERD) or Barrett’s esophagitis (BE) had a taxonomic shift towards gram-negative organisms including *Fusobacterium* and *Campylobacter* spp. with enhanced lactic acid production in the *TH* pathways. Yang et al. also concluded that patients with esophagitis or BE have a propensity for gram-negative bacterial colonization (36) (Table 2).

The shifts in the GERD/BE microbiome may contribute to malignant transformation into esophageal adenocarcinoma (EAC). Gram-negative bacteria could promote expression of inflammatory signals due to interactions between bacterial metabolites and inflammatory cells resulting in chronic inflammation (37). Toll-like receptors (TLRs) normally mediate host-microbiota interactions and are key players in recognition of pathogen-associated molecular patterns. TLR4 expression is abundant in normal esophageal epithelium and increases in BE/EAC (38). Gram-negative bacteria produce LPSs that bind to TLR4, thereby activating the NF-κB pathway and potentially upregulating oncogenes like COX-2 (39). The enhanced expression of lactic acid producing pathways and dominance of lactate fermenters could indicate EAC is a glycolytic tumor. In these tumors, oncogenic and tumor suppressor mutations result in a synchronized process to produce lactate continuously without regulation (40). The heavy concentration of lactate promotes an acidic microenvironment, potentially inhibiting competitor growth while selecting for other organisms with similar metabolic profiles (41).

The link between dysbiosis and esophageal squamous cell cancer has not been investigated as well as EAC. Microbial diversity, especially in neighboring microbiota, has been inversely associated with esophageal squamous dysplasia (42). Patients with squamous dysplasia and squamous cell cancer had higher proportions of *Clostridiales* and *Erysipelotrichales* spp. in their gastric microbiomes relative to healthy patients (43). Salivary concentrations of *Lautropia, Bulleidia, Catonella, Corynebacterium, Moryella, Peptococcus*, and *Cardiobacterium* spp. were lower in esophageal...
squamous cell carcinoma compared to healthy controls (53). In studies of the esophageal microbiome, patients with esophageal squamous cell carcinoma were more likely to infected with Porphyromonas gingivalis and presence of the microbe was correlated with severity of disease (44). In patients who underwent esophagectomy for ESCC, tissue analysis revealed that approximately 23% of specimens contained Fusobacterium nucleatum and presence was associated with shorter survival (45).

**Gastric cancer**

The stomach’s acidic microenvironment, release of antimicrobial enzymes, and its rapid intraluminal flow make it inhabitable to one billionth of the bacterial population in the colon. Taxa commonly found in the stomach include Streptococcus, Lactobacillus, Veillonella, Prevotella, Rothia, and Neisseria spp. representing a more diversified taxonomic profile than the esophagus (48). Fundamentally, colonizers...
of the healthy stomach must have the ability to resist acid degradation. Tumorigenic organisms possess the additional ability to incite chronic inflammation thereby inducing dysbiosis in susceptible hosts. The International Agency for Research in Cancer (IARC) has designated *Helicobacter pylori* (*H. pylori*) as a class I carcinogen with definite links to gastric cancer (54-56). Yet, only 3% of the people infected with *H. pylori* actually go on to develop gastric cancer (57). Furthermore, a nationwide effort to eradicate *H. pylori* in patients with chronic gastritis failed to reduce the incidence of gastric cancer in Japan over a 5-year period (58). This implies an indirect, non-causal relationship between *H. pylori* status and gastric cancer. Indeed, *H. pylori* could promote a microenvironment that leads to gastric carcinogenesis to varying degrees depending on virulence factors, bacterial and host genotypes, geography, and environmental factors.

*H. pylori* has coevolved with humans over the past 60,000 years and has only recently disappeared from the West’s microbiome for reasons that are not fully understood (59). The bacteria’s impact on the human gut microbiome is not well understood and is limited by sequencing techniques, interindividual variability, and small sample sizes. One study of 23 healthy subjects found that *H. pylori* status had no effect on phylotype distribution within the stomach. Clustering analysis did not demonstrate distinct clusters of microbes between positive and negative *H. pylori* groups (60). A more recent comparison between these groups found that *H. pylori* did affect microbiome composition. Relative abundance of *Proteobacteria* (excluding *H. pylori*) and *Acidobacteria* was higher in *H. pylori* infected patients, while relative abundance of *Actinobacteria* and *Firmicutes* was higher in *H. pylori* negative patients. *H. pylori* has been shown to significantly reduce gastric microbiome diversity, comprising 24-97% of sequences found in samples (60-62). How microbial diversity relates to gastric cancer is unclear, though the relative paucity of *H. pylori*, and a shift towards nitrosating organisms is associated with carcinogenesis (49,50,63-65).

When involved in antral gastritis, *H. pylori* induces gastrin secretion and local acid production contributing to peptic ulcer disease but protecting against gastric cancer. Strains causing corpus gastritis stimulate release of inflammatory mediators which degrade gastric glands resulting in deficient acid production and a more hospitable environment for pathobionts (66). Interestingly, *H. pylori* prefers normal gastric mucosa and the population dwindles in atrophic gastritis, allowing proliferation of tumorigenic bacteria (49,51,61,67,68). These lesions progress to intestinal metaplasia followed by dysplasia, and eventually gastric adenocarcinoma (69).

Additionally, some strains of *H. pylori* employ several mechanisms to colonize the stomach, destroy gastric epithelium, upregulate inflammatory cascades, disrupt host signaling, alter cell permeability, and promote epigenetic changes. *H. pylori* gains access to gastric mucosa by producing urease, an enzyme which catalyzes urea into ammonia for neutralization of acid and reaction with neutrophilic metabolites. Carcinogenic strains contain the cytotoxin-associated gene A (*CagA*) and deliver the oncoprotein into epithelial cell cytoplasm via type IV secretion. *CagA* is then phosphorylated by tyrosine kinase, binds to SHP2 and activates the Ras-Erk pathway (FGFR2, KRAS, EGFR, ERBB2, and MET), dysregulating downstream cell signaling, promoting cell proliferation, destroying the epithelial barrier, and dismantling the cytoskeleton. Vacuole-forming factor (VacA) induces vacuole formation by the host cell and ultimately compromise cellular membrane integrity. *H. pylori* adheres to epithelial cells with outer membrane proteins; the interaction triggers release of IL-6, IL-8, IL1-β, TNF-α. Activation of NF-κB prompts cytokine release and inhibits apoptosis. *H. pylori* also has been implicated in DNA methylation, histone changes, and production of reactive oxygen species (ROS) induced dsDNA breaks (52).

**Conclusions**

The “germ theory of disease” or the idea that microscopic pathogens are responsible for disease, has served as a foundational concept of our understanding of the human body for the past two centuries. Robert Koch and Louis Pasteur prompted the departure from miasma theory in which a poisonous vapor from an unhygienic environment was thought to be the culprit for infection. Koch is credited with formalizing criteria to establish the causation of a disease by a microbe: the pathogen must be present in all diseased subjects; the pathogen should be isolated from the diseased subject and grown in culture; when the cultured pathogen is introduced to a healthy experimental host, disease should be observed; the pathogen should be re-isolated from the experimental host and be identical to the originally isolated pathogen (13). In this model of disease, the single pathogen role is overemphasized, and the host factors are unaccounted for. Antoine Bechamp, a vocal rival of Pasteur, believed that “there is an independently living microanatomical element in the cells and fluids of
all living organisms” which he termed the “microzyma”. Bechamp posited that the disruption of the host’s inherent “microzymia” was a predisposition to disease (70). Though Bechamp’s “microzymia” was largely ignored by the scientific community then, it has remarkable conceptual similarities to what we now know as the microbiome.

The gut microbiome has been linked to gastric and esophageal cancers, which represent the third and sixth most common causes of cancer-related deaths worldwide (71,72). Both cancers have plausible explanations for carcinogenesis rooted in dysbiosis. Further clarification of these pathways and discovery of diagnostic or therapeutic targets could have broad impacts on global subpopulations (46). As sequencing technology becomes increasingly sophisticated and accessible, researchers will be empowered to elucidate causal, rather than just correlative relationships (47). By reframing the microbiome as a determinant of global health and tailoring its application to the individual patient, we have the power to usher in the era of highly personalized, precision medicine.

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