# Unraveling the mechanisms underlying associations between the microbiome and gastrointestinal malignancies

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## Introduction

It was recognized decades ago that gastrointestinal (GI) microorganisms were associated with the development of malignancies, particularly colorectal cancers (CRC). As early as the 1950's it was noted that streptococcal endocarditis, caused by *Streptococcus gallolyticus* (formerly *S. bovis*) was associated with colonic lesions (1). However, we have only recently begun to unravel the complex interactions between the microbial milieu, human cells, and environmental exposures and diet. These recent advances have been driven in part by the Nobel Prize winning discovery that an infectious agent, *Helicobacter pylori* (*H. pylori*), caused inflammation resulting in peptic ulcers. Together with advances in sequencing technology, this discovery has led to intensive research efforts that have increased our understanding of microbiota-driven chronic inflammation and cancer. In a recent issue of the journal *Gastroenterology*, Abreu and Peek highlight these advances, with a particular focus on some of the recognized molecular interactions between *H. pylori* and host cells (2). They also present the evidence for involvement of other members of the gut microbial community in initiation and development of GI cancers and discuss multiple proposed mechanisms by which these interactions occur. While many reviews have focused on research identifying microbial signatures associated with malignancies, including changes in diversity or evenness of GI microbial communities and depletion or overrepresentation of particular bacterial groups; this comprehensive review emphasizes research that explores the cause rather than effects of microbe-malignancy relationships.

## *Helicobacter pylori* (*H. pylori*) and gastric cancer

The evidence for involvement of *H. pylori* in development of gastric cancer is compelling enough to warrant its classification as a class I carcinogen by the World Health Organization. However, not all individuals colonized by *H. pylori* develop malignancy and multiple bacterial and host factors involved in determining the outcome of this association have been identified. An important bacterial factor is the presence of a pathogenicity island that codes for the CagA effector molecule and components of a Type IV secretion system that facilitates delivery of these effector molecules into host cells (3). In addition, *H. pylori* produces a secreted toxin VacA, which results in varying alterations to gastric epithelial cells. Although it is produced by all *H. pylori* strains, there is a high level of genetic variation in specific regions of the gene for this toxin, and risk of developing gastric malignancy has been associated with particular allelic variants (4).

Effects of CagA and VacA molecules on host cells may be cell lineage specific. Based on data from several recent studies, Abreu and Peek present the hypothesis that cells carrying progenitor-like features may be particularly susceptible to carcinogenic effects of CagA from *H. pylori*. Supporting evidence includes human studies showing that *Lgr5+* stem cell populations are more susceptible to *H. pylori*-induced oxidative damage (5), and epithelial cells carrying a stem cell marker, CD44 variant 9, are unable to eliminate bacterial CagA as occurs in differentiated gastric epithelial cells (6). Host genetic factors may also be important in how an individual responds to the presence of carcinogenic strains of *H. pylori*. In particular,

polymorphisms present in the *IL1B* gene cluster resulting in higher levels of IL1B appear to be associated with increased risk of cancer development and this is greatly augmented in individuals harboring pathogenic variants of *H. pylori* (7).

Abreu and Peek also suggest that although the association between specific strains of *H. pylori* and gastric cancer risk is firmly established, compelling evidence suggests co-involvement of other members of the gastric microbial community. Antibiotic therapy targeted at *H. pylori* elimination has been shown to reduce the incidence of gastric cancers despite the fact that *H. pylori* returns or is not completely eliminated in about half of all treated individuals (8). This suggests that there may be other members of the microbial community, which facilitate *H. pylori*-mediated gastric cancer development, that are altered by this antimicrobial therapy. Evidence of lateral gene transfer between GI bacteria and human cancer cells also suggests the involvement of other bacteria. Bacterial DNA most frequently integrated in human somatic cells was more similar to sequences from *Pseudomonas* than to *Helicobacter* (9). Finally, several studies have shown that there are quantifiable differences in the community structure and membership of gastric microbiota between *H. pylori* infected and *H. pylori* free individuals.

## Microbiome and other malignancies

Identification of *H. pylori* as a carcinogenic infectious agent has led to speculation that other GI microorganisms are involved in cancer development and progression. Resident microbiota are important in numerous host processes, including digestion of food, influencing host gene expression, and development and modulation of the immune system. While these are all critical aspects of the proper development and functioning of the host, it also provides opportunities for these organisms to incite dysfunction leading to the genesis of malignancies, among other issues. There are numerous mechanisms by which GI microbes can incite tumor development or encourage progression including facilitating DNA damage through oxidative stress, inducing chronic inflammation, producing mutagenic metabolites from dietary components, and altering the dynamics of stem cells. Evidence presented for the interaction between epithelial stem cells and *H. pylori*-produced CagA and VacA is a good example of the latter mechanism. Bacteria produce $H_2O_2$ and other molecules that can induce oxidative damage to cells, and microbe-associated molecular patterns (MAMPs), such as lipopolysaccharide and flagellin have been

shown to activate the host inflammasome through Toll-like (TLRs) and other receptors, inducing a chronic inflammatory state (10). Dietary components interact with the microbiota to influence cancer risk in two ways. One mechanism is through their direct metabolism by commensal microorganisms, for example the degradation of proteins in the distal colon to produce mutagenic amines. A less direct mechanism is through modulation of microbial populations and alterations in host metabolism through diet. Abreu and Peek present two interesting examples of this mechanism, including the release of excess bile acids after consumption of high fat diets, which can be modified by bacterial hydroxylases and other enzymes to form secondary bile acids. Many secondary bile acids, particularly deoxycholic and lithicolic acids have been associated with CRC (11), and bile acids may also exert large-scale influences on the composition of the microbial community, inducing microbial dysbiosis. The second example they present is the promotion of *Desulfovibrio vulgaris* by diets high in fat and protein, and the resultant production of genotoxic hydrogen sulfide by this organism.

The colon hosts the largest number of commensal organisms in the GI tract, and each of the mechanisms previously mentioned are thought to influence risk of developing CRC. To date no causal organisms have been identified with as strong an association to CRC, as has been demonstrated between *H. pylori* and gastric cancer. *Fusobacterium nucleatum*, *Bacteroides fragilis*, and *Enterococcus faecalis* are frequently overrepresented in samples from CRC patients and putative mechanisms have been proposed and are being explored (2). There have also been a number of microbiome studies in tissue and stool that consistently report microbial dysbiosis associated with CRC. Recently, overall decrease in the diversity of colonic microorganisms has also been identified in CRC patients (12). Several hypotheses including the "driver-passenger" and "α-bug" hypotheses have been proposed to explain the relationship between particular microbial groups and CRC (13,14), and these are briefly discussed in Abreu and Peek's review. Although the identification of a CRC-associated microbiome has remained elusive, identifying microbial signatures associated with CRC may have important implications in diagnosis and prognosis of this disease and could eventually be useful as preventive markers.

## Conclusions

It has become increasingly clear that the gut microbiome and its interactions with host cells and environmental

         

factors, particularly diet, strongly influence the risk of developing GI cancers. The role of *H. pylori* in development and progression of gastric cancer represents our most detailed understanding of the underlying mechanisms by which members of the GI microbiome can lead to malignancies. These detailed mechanistic studies provide a platform to advance our understanding of how other members of the microbiome interact with their host resulting in development and progression, or in some cases, suppression of tumors. Future studies exploring the association between particular microbes and malignancies should focus on the root causes of these relationships. Animal models, particularly Mongolian gerbils, mice with different genetic mutations, and rodents with "humanized" biota have been particularly useful in advancing our understanding of molecular and biochemical interactions underlying these associations. However, before the microbiome can be targeted for its diagnostic, prognostic, and therapeutic potential prospective human studies examining the alteration or elimination of particular microbes on disease incidence or progression are needed.

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