Gastrointestinal Malignancy and the Microbiome


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Microbial species participate in the genesis of a substantial number of malignancies—in conservative estimates, at least 15% of all cancer cases are attributable to infectious agents. Little is known about the contribution of the gastrointestinal microbiome to the development of malignancies. Resident microbes can promote carcinogenesis by inducing inflammation, increasing cell proliferation, altering stem cell dynamics, and producing metabolites such as butyrate, which affect DNA integrity and immune regulation. Studies in human beings and rodent models of cancer have identified effector species and relationships among members of the microbial community in the stomach and colon that increase the risk for malignancy. Strategies to manipulate the microbiome, or the immune response to such bacteria, could be developed to prevent or treat certain gastrointestinal cancers.

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Human beings are colonized by a myriad of microbes including bacteria, archaea, eukaryotes, and viruses, although bacteria are the most abundant and well-studied component. The composition of the gastrointestinal (GI) microbiome is shaped by a variety of factors including diet, additional environmental elements, and the genetic background of the host. The GI microbiota is a complex ecosystem that contains more than 3 million genes, encoding enzymes that generate metabolites that can influence health as well as disease.[1,2]

Cancer of the GI tract is a leading cause of morbidity and mortality in the United States. Genetic factors have been identified that clearly increase cancer risk, including adenomatous polyposis coli (*Apc*) mutations that cause familial adenomatous polyposis and E-cadherin mutations that lead to hereditary diffuse-type gastric cancer. However, nongenetic factors more broadly affect most GI cancers. Microbial species participate in the genesis of a substantial number of malignancies worldwide; in conservative estimates, more than 15% of all cancer cases can be attributed to infectious agents, for a neoplastic burden of 1.2 million cases per year.[3] Residential microbes in the GI tract can alter cancer risk by inducing oxidative and nitrosative DNA damage in response to chronic inflammation, increasing cell proliferation, altering stem cell dynamics, and producing mutagenic metabolites such as butyrate. The GI microbiota also can constrain or facilitate tumor growth by altering immune surveillance mechanisms and affecting the metabolism of chemotherapeutic agents.[1,2] In this review, we discuss emerging concepts and provide specific examples for the role of the GI microbiome in the development of malignancies that arise within this niche. We focus on gastric and colonic malignancies because these have been investigated most thoroughly, and also briefly discuss esophageal cancers.

Gastric Cancer

Gastric adenocarcinoma is the second-leading cause of cancer-related death in the world.[4,5] *Helicobacter pylori* is a gram-negative bacterial species that selectively colonizes gastric epithelium; chronic infection with this organism is the strongest identified risk factor for gastric adenocarcinoma, prompting the World Health Organization to designate *H pylori* as a class I carcinogen. Approximately 660,000 new cases of gastric cancer per year are attributable to *H pylori*, making this pathogen the most common infectious agent linked to any malignancy.[3] However, only a percentage of colonized persons develop neoplasia. Risk is associated with *H pylori* strain, variations in host responses

Abbreviations used in this paper: **AOM, azoxymethane; Apc, adenomatous polyposis coli; ASF, altered Schaedler's flora; CRC, colorectal cancer; DSS, dextran sulfate sodium; GERD, gastroesophageal reflux disease; GI, gastrointestinal; HPV, human papilloma virus; IL, interleukin; INS-GAS, Insulin-Gastrin; JC, John Cunningham; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; PAI, pathogenicity island; PRR, pattern recognition receptor; SCFA, short-chain fatty acid; SPF, specific pathogen-free; TLR, Toll-like receptor.**

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governed by genetic diversity, and/or specific interactions between host, microbial, and environmental determinants.

One *H pylori* determinant that influences cancer risk is the cytotoxin associated gene (*cag*) pathogenicity island (PAI). Genes within the *cag* PAI encode an antigenic effector protein (CagA) as well as proteins that form a type IV bacterial secretion system that exports CagA from adherent *H pylori* into host cells.[6–9] *H pylori* strains that harbor the *cag* PAI (*cag*$^+$ strains) are associated with a significantly increased risk of distal gastric cancer compared with *cag*$^-$ strains.[10] After translocation, CagA undergoes tyrosine phosphorylation by Src and Abl kinases; phospho-CagA subsequently interacts with and activates several host cell proteins, including SHP2 a Src homology 2 (SH2) domain containing non-transmembrane phosphatase, leading to morphologic alterations such as cell scattering and elongation.[6,11,12] Nonphosphorylated CagA also exerts effects within host cells that affect oncogenesis. Unmodified CagA directly binds partitioning-defective 1B (PAR1b), which regulates cell polarity, and inhibits its kinase activity—an interaction that promotes loss of polarity.[13] Nonphosphorylated CagA associates with the epithelial tight-junction scaffolding protein zonula occludens-1 and the transmembrane protein junction adhesion molecule-A to cause ineffective assembly of tight junctions at sites of bacterial attachment.[14] Unmodified CagA also activates β-catenin, leading to transcriptional up-regulation of genes implicated in cancer.[15–17] The CagA protein of certain *H pylori* strains can stimulate expression of interleukin (IL)8 by activating the transcription factor nuclear factor-κB (NF-κB),[18] thereby contributing to neutrophil infiltration within the gastric mucosa. CagA also induces DNA damage in vitro and in rodent models of infection—results that have been validated in human subjects colonized with *H pylori cag*$^+$ strains.[19] Contact between *cag*$^+$ strains and host cells therefore activates multiple signaling pathways that may increase the risk for malignant transformation during prolonged colonization.

Another *H pylori* constituent linked to the development of gastric cancer is the secreted vacuolating cytotoxin A (VacA) toxin.[20] VacA causes a wide assortment of alterations in gastric epithelial cells, including vacuolation, altered plasma and mitochondrial membrane permeability, autophagy, and apoptotic cell death.[20] All *H pylori* strains possess *vacA*, but there is marked variation in *vacA* sequences among strains. The regions of greatest diversity are localized to the 5' region of the gene, which encodes the signal sequence and amino-terminus of the secreted toxin (allele types s1a, s1b, s1c, or s2), an intermediate region (allele types i1 or i2), and a midregion (allele types m1 or m2).[21,22] Strains that contain type s1, i1, and m1 forms of *vacA* are associated with a higher risk of gastric cancer than strains that contain type s2, i2, and m2 forms.[22]

VacA and CagA also can counter-regulate each other to affect host cell responses. Specifically, CagA antagonizes VacA-induced apoptosis and activates a cell survival pathway mediated by the mitogen-activated protein kinase extracellular regulated kinase and the anti-apoptotic protein myeloid cell leukemia 1 (MCL1).[23] CagA also activates nuclear factor of activated T cells (NFAT) and epidermal growth factor receptor signaling—processes that are inhibited by VacA.[23]

Recently, exciting data have shown that the opposing effects of CagA and VacA may be cell-lineage specific. The Wingless (Wnt) target gene leucine-rich repeat containing G protein coupled receptor (*Lgr5*) encodes an orphan G-protein–coupled receptor and marks a self-renewing, multipotent stem cell population responsible for long-term renewal of gastric epithelium.[24] Lgr5$^+$ epithelial cells have higher levels of oxidative DNA damage than Lgr5-negative cells in *H pylori*–infected persons with gastric cancer,[25] indicating that *H pylori* specifically target Lgr5$^+$ epithelial cells. In transgenic mice that overexpress Le^b, *H pylori* adhere directly to gastric epithelial cells.[26] Genetic ablation of parietal cells in Le^b-expressing transgenic mice permits the gastric epithelial stem cell population to expand, which is accompanied by increased *H pylori* colonization and inflammation within glandular epithelium.[27,28] Delineation of this stem cell transcriptome has identified several pathways that are over-represented and of particular importance for carcinogenesis, including Wnt activation of β-catenin,[29] which is, in turn, activated by CagA-mediated signaling.[15,16] In differentiated gastric epithelial cells, binding of VacA to low density lipoprotein receptor-related protein (LRP1), a specific receptor on the epithelial cell surface, leads to autophagic elimination of CagA.[30] Importantly, gastric epithelial cells that express a stem cell marker, CD44 variant 9, fail to degrade CagA; these findings have been verified in vivo.[30] A subpopulation of host cells with progenitor-like features therefore appear to be uniquely susceptible to the effects of a microbial oncoprotein, which may lower the threshold for carcinogenesis.

Host genetic factors also influence the risk of gastric cancer among *H pylori*–infected persons. IL1β is an inflammatory cytokine that inhibits gastric acid secretion, and production of IL1β is increased in the gastric mucosa of infected vs uninfected persons.[31] Polymorphisms within the *IL1β* gene cluster that increase IL1β production are associated with significant increases in the risk for hypochlorhydria, gastric atrophy, and distal gastric adenocarcinoma compared with low expression IL1β genotypes.[32–34] The presence of a virulent strain of *H pylori* in a genetically susceptible person further augments the risk for gastric cancer. Persons harboring high-expression *IL1β* alleles who are infected with *H pylori cagA*$^+$ or *vacA* s1-type strains have 25-fold or 87-fold increases in risk, respectively, for gastric cancer compared with uninfected persons.[33] A recent genome-wide association study has identified new targets for studies of gastric carcinogenesis, showing that Toll-like receptor 1 (*TLR1*) and Fc fragment of IgG low affinity IIa receptor (*FCGR2A*) loci are associated with *H pylori* seroprevalence. However, the relationship between these genetic loci and disease was not determined.[35] Dietary factors recently were shown to accelerate gastric carcinogenesis in rodent models of *H pylori* infection; specifically, iron depletion accelerates the development of gastric dysplasia and cancer by promoting assembly and function of the *H pylori cag* PAI.[36]