The gut microbiome and colorectal cancer: a review of bacterial pathogenesis

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Contributions: (I) Conception and design: CA Kistler; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: JD Dahmus, DL Kotler; (V) Data analysis and interpretation: JD Dahmus, DL Kotler; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Abstract: Colorectal cancer (CRC) is the third most common newly diagnosed cancer in both men and women in the United States. Colonoscopy has become increasingly popular in CRC screening and represents the gold standard for detecting and removing pre-cancerous lesions. Although colonoscopy is considered a relatively safe procedure, it is invasive and bowel preparation can be challenging for patients. As interest in the gut microbiome has expanded, there have been new links established between bacteria and the development of CRC. These developing associations could prove to be a useful adjunct to colonoscopy for CRC screening in the future. This review examines current research evaluating multiple proposed pathogenic microorganisms including sulfidogenic bacteria such as Bilophila wadsworthia, as well as Streptococcus bovis, Helicobacter pylori, Bacteroides fragilis, and Clostridium septicum. This discussion primarily focuses on bacterial pathogenesis, evidence of association with CRC, and the proposed mechanisms of carcinogenesis.

Keywords: Gut microbiome; colorectal cancer (CRC); microbiome

doi: 10.21037/jgo.2018.04.07
View this article at: http://dx.doi.org/10.21037/jgo.2018.04.07

Introduction

In 2017, there were an estimated 135,430 new cases of colorectal cancer (CRC) in the United States, making it the third most common cancer in both men and women (1). CRC was also responsible for over 50,000 deaths (1). Survival from CRC is directly related to stage of presentation, with stage I disease associated with 5-year survival of 90.1% (2). Screening lowers the incidence of CRC, shifts the detection of CRC to earlier stage disease, and lowers CRC mortality (3). Multiple societies and respective guidelines recommend CRC screening starting at age 45 to 50 with multiple modalities available. Family history and patient characteristics have been the primary means of risk stratification for CRC, and this is the basis of what age to begin, which screening methods are appropriate, and the interval with which screening is performed.

The human intestine houses over 100 billion bacteria (4), with the largest proportion of these bacteria located in the colon (5). The microbiome serves immunological, structural and metabolic functions (6). One area within the gut microbiome that has developed accumulating data is its role within cancer pathogenesis. Animal studies have shown that bacteria may potentially contribute to CRC development through direct interaction with the host's immune system, production of cancer-associated metabolites, and release of genotoxic virulence factors (7-11). These studies have also identified certain organisms that may play more significant roles within CRC development.

In this review, we explore the relationship between bacteria and CRC, focusing on sulfidogenic bacteria and five distinct bacteria: Streptococcus bovis, Fusobacterium
nucleatum, Helicobacter pylori, Bacteroides fragilis, and Clostridium septicum. Each section will introduce the organism, discuss its association with CRC, and describe its proposed mechanism of carcinogenesis.

**Sulfidogenic bacteria**

Sulfidogenic bacteria, such as Fusobacterium, Desulfovibrio and Bilophila wadsworthia, have been implicated in CRC development through the production of hydrogen sulfide. Hydrogen sulfide is a genotoxic compound that has been shown to damage DNA leading to genomic or chromosomal instability (12). Genomic instability, indicating a high frequency of mutations in a cell line’s genome, is found in over 80% of sporadic CRCs (13). Attene-Ramos et al. demonstrated that in colonocyte cell lines with the inhibition of DNA repair function, there is significant genotoxicity at sulfide concentrations found in the colon (12). This suggests that hydrogen sulfide, produced by sulfidogenic bacteria in the colon, may contribute to the development of CRC when combined with another mutation effecting DNA repair in a multistep carcinogenic process. The cellular mechanism is incompletely understood, but it is hypothesized that hydrogen sulfide diffuses into intestinal epithelial cells and interferes with mitochondrial function, ultimately leading to hyperproliferation via the Ras/MAPK pathway (14).

The Ras/MAPK pathway is a known mechanism of carcinogenesis in many malignancies, including CRC.

In addition to a correlation with CRC, concentration of sulfidogenic bacteria correlated positively with a diet high in fat and animal protein. A diet high in animal fat is a known CRC risk factor. A systematic review and meta-analysis of the relationship between dietary patterns and CRC demonstrated that a traditional Western diet high in red meat and low in fiber, as compared to a diet high in fruits and vegetables, was associated with a 29% increased risk of CRC (15). The dynamic nature of the microbiome is demonstrated by a study in which a dietary intervention rapidly changed the gut bacterial population in African-Americans. The microbiome of an urban African-American group consuming a high fat low fiber diet was compared to the microbiome of a rural Black South African group consuming a low fat, high fiber diet. Notably, B. fragilis, which has been implicated in CRC (16), was seven times more abundant in the baseline African-American microbiome compared to the baseline South African microbiome. Their respective baseline diets were then reversed, and within two weeks a dramatic change in the microbiome was observed, including B. fragilis abundance doubling in the South African group consuming a high fat low fiber diet. Furthermore, this change translated to a reduction in inflammation of biopsied colon tissue demonstrated by histological changes and immunochemical biomarkers (17).

An additional study used polymerase chain reaction (PCR) to quantify the concentration of specific bacterial DNA in colonic tissue biopsies and showed that African-Americans with CRC had higher concentrations of sulfidogenic bacteria compared to non-Hispanic whites (18). Furthermore, African-Americans with CRC had a higher abundance of sulfidogenic bacteria compared to African-Americans without CRC.

**Streptococcus bovis (S. bovis)**

*Streptococcus bovis*, a Lancefield group D Gram positive organism, was one of the first bacteria to be linked with CRC. Descriptions of this association appear as early as 1966 (19) which has resulted in the accumulation of more significant research.

*S. bovis* bacteremia appears to correlate with increased rates of both adenomas and carcinomas (20,21). Of note, Burns et al. showed *S. bovis* in the stool specifically correlated with an increased rate of villous and tubulovillous adenomas (22). Studies vary greatly regarding the likelihood of concomitant CRC in patients with *S. bovis* bacteremia, ranging from 6–71% (20). However, when stratified by subtype, an association with a narrower range (33–71%) emerges with *S. bovis* biotype I, also known as *Streptococcus gallolyticus* (23). A study comparing bacteremia with *S. gallolyticus* compared to all other *S. bovis* subtypes, observed an incidence of CRC of 71% for *S. gallolyticus* versus 17% for all other subtypes (21). When CRC specimens were evaluated for bacterial DNA, *S. gallolyticus* DNA was present in 49% of cancer specimens as compared to 8% of healthy colon tissue (24). Even without detectable bacteremia, the presence of *S. gallolyticus* IgG antibodies in the serum is associated with higher rates of colorectal adenomas and carcinomas (25,26). In general, serum antibodies to gut flora would raise the concern for CRC-induced mucosal barrier destruction. In order to eliminate this possibility as a confounding factor, Abdulamir et al. demonstrated that the presence of serum antibodies to *B. fragilis*, another gut bacterium, did not correlate with the rate of adenomas or carcinomas (25). In their study,
they found that 68% of CRC patients were positive for *S. gallolyticus* serum antibodies, 78% of adenoma patients were positive, and 17% of control patients were positive. Given this data, *S. gallolyticus* serum antibody testing could have a role in future CRC screening algorithms. Stool testing has shown less promise, as a 17-year longitudinal study showed no correlation between the presence of fecal *S. bovis* and increased incidence of colorectal neoplasia. However, this study did not analyze for specific subtypes such as *S. gallolyticus*, as the required genotyping technology was not available (27). In the future, repeating this type of study to specifically detect *S. gallolyticus* in the stool may demonstrate value in screening for carcinomas and villous or tubulovillous adenomas.

With 6% of all cases of infectious endocarditis attributable to *S. bovis* (28), this well-established relationship may provide insights regarding the mechanism underlying this bacteria’s association with CRC. Similar to that seen with CRC, *S. gallolyticus* in particular seems to have a disproportionately high association with endocarditis. In patients presenting with *S. bovis* bacteremia, 94% with *S. gallolyticus* have endocarditis compared to 18% presenting with other subtypes (21). Compared to other *S. bovis* subtypes, *S. gallolyticus* has greater affinity for collagen I and IV and is able to form a biofilm within extracellular matrices (29). Heart valves are rich in collagen I and IV which may explain the predilection of this bacterial pathogen to cause endocarditis. Collagen IV is one of the main components of the colon mucosa and is vital in maintaining the integrity of the basement membrane (30).

Besides attraction to the collagen rich milieu of colonic neoplasia, *S. bovis* may promote cellular proliferation and interfere with apoptosis. *S. bovis* induces production of proinflammatory cytokines NF-κB, IL-1 and IL-8 as well as COX-2 overexpression, all of which increase cellular proliferation and angiogenesis while decreasing apoptosis in malignant cells (24). Specifically, much of this may be attributed to the *S. bovis* cell wall antigen. This is highlighted in a three-arm study of rats with carcinogen-induced (azoxymethane) pre-neoplastic colorectal lesions receiving live *S. bovis*, cell wall antigen only, or neither. Receipt of isolated cell wall antigen as well as live *S. bovis* resulted in a 1.8-fold increase in the concentration of aberrant hyperproliferative crypts as compared to the controls. However, the progression to neoplasia differed between the two groups who developed hyperproliferative crypts; half of the rats given the cell wall antigen developed adenomas, while no adenomas were identified in the *S. bovis*-infected (31). The aforementioned studies indicate the complexity of the involvement of *S. bovis* in CRC. In aggregate, these studies may suggest a hypothetical self-perpetuating cycle of increased abnormal cellular proliferation secondary to *S. bovis* in a uniquely favorable microenvironment for *S. bovis* binding and growth. These studies suggest *S. bovis* may have a role in promoting the transition to colonic neoplasia and is also attracted to the collagen-rich neoplasia environment where it may accelerate progression of disease. Further research is needed to better elucidate these complex relationships.

*While at this time screening for asymptomatic *S. bovis* colonization or the presence of antibodies is not recommended in any algorithm for assessing CRC risk, any patient with *S. bovis* bacteremia or endocarditis should undergo colonoscopy (32).*

**Fusobacterium nucleatum (F. nucleatum)**

*Fusobacterium nucleatum* is a gram-negative anaerobe typically implicated in periodontal disease. With respect to the gut microbiome, sever studies have shown that patients with CRC have a much greater concentration (OR =4.11, 95% CI: 1.62 to 10.47) of *F. nucleatum* and decreased overall microbial community diversity compared to controls (33).

There may be a relationship between the concentration of *F. nucleatum* and CRC stage and prognosis at presentation. Higher tissue and fecal concentrations of the bacteria have been associated with later stages of CRC (34) and, in particular, portends a higher risk for lymph node involvement (35,36). In one study, lymph node metastases were present in 52/88 (59%) of patients with high concentrations of *F. nucleatum* and 0/13 patients with low concentrations of *F. nucleatum* (35). Other studies have found that moderate to high concentrations of *F. nucleatum* were associated with poorer survival related to CRC (37,38). Along the continuum of CRC development, *F. nucleatum* may also have a more significant role within the hyperplastic polyp pathway (36). Yu *et al.* showed that invasive *F. nucleatum* was significantly more prevalent in proximal hyperplastic polyps and sessile serrated adenomas compared to tubular adenomas. In addition, *F. nucleatum* was detected more frequently in proximal CRCs (89.6%) compared to distal CRCs (42.2%).

Unlike *S. bovis*, *F. nucleatum* bacteremia has not been associated with a greater likelihood of CRC. However, there may be an association between *F. nucleatum* bacteremia and other malignancies (39,40). These have included
gastrointestinal malignancies (stromal, esophageal, gastric) and extra-gastrointestinal (hematologic, breast).

Several mechanisms have been proposed to explain the role of F. nucleatum in the pathogenesis of CRC. These mechanisms are related to the bacteria’s ability to attach to the host cell’s surface, invade the host cell, promote immune cell migration through the generation of a proinflammatory microenvironment, and through the shuttling of other bacteria to the affected tissue. Additionally, this bacterium has been shown to produce hydrogen sulfide, as reviewed in the prior sulfidogenic bacteria section. Despite these studied mechanisms, it has not been fully elucidated whether F. nucleatum promotes tumorigenesis or promotes the optimal environment for already established tumor cell progression through enhanced oxidation and subsequent inflammatory disequilibrium.

F. nucleatum attaches to and invades epithelial cells through its surface virulence factor, FadA. FadA is an adhesin specific to F. nucleatum (10). It facilitates F. nucleatum attachment by binding to E-cadherin, a calcium-dependent cell adhesion glycoprotein present on human epithelial and CRC cell membranes. E-cadherin additionally binds cell cytoplasmic components like β-catenin, a human cytosolic protein involved in cell-to-cell adhesion and gene transcription. E-cadherin normally acts as a tumor suppressor however, through FadA binding and receptor modulation, its tumor suppressor activity is inhibited. The FadA-E-cadherin complex can be internalized into the cell’s cytoplasm through clathrin-mediated endocytosis, facilitating F. nucleatum invasion. Within the host cytoplasm, F. nucleatum may release its RNA, leading to detection by cytosolic RIG-1, thereby stimulating cytosolic NF-kB and promoting inflammation (41). F. nucleatum invasion is required to promote chronic inflammation within the host cell, as it cannot be promoted with F. nucleatum cell surface attachment alone. However, as seen by Kostic et al., it appears that a mechanism other than inflammation induction within host cells is responsible for F. nucleatum-related tumor development (9). Likely, F. nucleatum generation of a pro-inflammatory microenvironment outside of the tumor cell through recruitment of tumor-infiltrating immune cells is the primary mechanism.

Besides its ability to interact with E-cadherin, Fap2 can bind and stimulate T cell immunoglobulin and immunoreceptor tyrosine-based inhibition motif domain (TIGIT), an inhibitory receptor found on human natural killer cells and tumor-infiltrating lymphocytes, leading to enhanced inhibition, diminished cytotoxicity, and resulting immune suppression (42). This immune dysregulation allows further promotion an inflammatory microenvironment, potentially promoting further progression to CRC. Additionally, a positive correlation has been found between F. nucleatum concentration in human tissue and TNF-α and IL-10 abundance, two additional inflammation-producing factors (43).

F. nucleatum has the ability to form bacterial aggregates with other bacterial species. This has been demonstrated in periodontal disease in gingival plaques (41). This mechanism could thereby allow transportation of otherwise non-invasive bacteria into the host cell cytoplasm, which is another potential mechanism for CRC development that should be studied in future studies.

Although there is significant knowledge regarding the virulence of F. nucleatum, there are still outstanding gaps in understanding how a bacterium predominantly found in the oral cavity can migrate to the colon. Abed et al. have suggested that Gal-GalNAc, a carbohydrate moiety overexpressed on the epithelial surface of CRC cells and metastases, facilitates F. nucleatum binding (44). Transient periods of F. nucleatum bacteremia, such as during gingival manipulation, may allow Fap2 recognition and binding to Gal-GalNAc. Although E-cadherin-Fap2 binding is required for F. nucleatum invasion, E-cadherin is nonspecific to colonic epithelial cells. Gal-GalNAc, on the other hand, is specific to colonic epithelial cells and is overexpressed in dysplastic and neoplastic colonic lesions.

The relationship between diet, particularly one high in processed meats and refined grains and low in fiber, and the risk for CRC has been explored in studies focused on F. nucleatum (15,45). A large prospective cohort study utilizing data from the Nurses’ Health Study and the Health Professionals Follow-up Study evaluated the presence of F. nucleatum in CRC tissue samples in those with a Western diet versus a healthier diet rich in whole grains, fruits and vegetables. This high fiber diet was associated with a decreased risk of F. nucleatum-positive CRCs. However, there was no correlation between a healthier diet and F. nucleatum-negative CRC incidence (46). Therefore, the increased risk of CRC with a Western diet was only seen when F. nucleatum was involved. This supports the hypothesis that a healthier diet may decrease CRC risk by modifying the microbiome, specifically regarding bacteria such as F. nucleatum. When instituting dietary changes, the gut microbiome may be affected within a relatively rapid
Helicobacter pylori (H. pylori)

Helicobacter pylori is a small, spiral, gram-negative bacillus that has a well-established association with the development of gastric cancer and is considered a definite carcinogen by the World Health Organization (47). However, there is data to support a potential association between H. pylori and CRC, although the data remains more controversial. Zumkeller et al. reported a 1.4-fold increased risk of CRC in patients with H. pylori infection, however the article admits that publication bias could have contributed to the positive association (48). Additionally, a meta-analysis of studies conducted in the East Asian population found an increased risk in colonic adenoma development (OR =1.83, 95% CI: 1.35–2.51, P<0.01) but not in CRC (OR =1.08, 95% CI: 0.89–1.68) (49).

H. pylori has been shown to promote oxidative stress and induce gastritis in the stomach through surface infection of the host gastric epithelial cells and through alterations in the intra-gastric environment (50). Using its flagella, the bacterium burrows between the mucous layer of the stomach and the gastric epithelium, hiding itself from the acidic environment within the stomach. The bacterium binds, but does not invade, the epithelial cell surface using adhesins. It can then promote inflammation and carcinogenesis through multiple mechanisms. The first mechanism involves the bacterium’s virulence factor, the cagA oncoprotein, present in only certain H. pylori strains. The bacterium injects the oncoprotein into the epithelial cells. Once inside the cell, cagA undergoes tyrosine phosphorylation by gastric Src family kinases. Once phosphorylated, the protein is able to bind and activate gastric SHP2, a gastric epithelial cell phosphatase and oncoprotein. SHP2 then transmits positive signals for cell growth and motility, thus promoting carcinogenesis (50).

One study found an association between cagA seropositivity and an associated increased risk in gastric and colonic cancer. Shmuely et al. reported cag-positive H. pylori strains were associated with a 10.6-fold increased risk of CRC compared to cagA negative strains (51). The same study found a 5.8-fold risk of gastric adenocarcinoma with the protein present. However, this was a retrospective study and focused on subject’s serum IgG antibodies rather than tissue biopsy or urease breath test. The study also excluded any patient who had ever been treated for H. pylori. Therefore, it is unknown if cagA promoted CRC development or if it was coincidentally present in the patient’s serum without causing associated inflammation.

The second mechanism of oxidative stress production involves alteration of the intra-gastric environment through bacterial and neutrophilic production of reactive oxygen species (ROS), pro-inflammatory cytokines, and upregulation of COX-2 (50). This includes excessive production of ROS by the body’s neutrophils in an effort to eradicate the bacterium and H. pylori can produce superoxide (a ROS) itself. This excessive production of ROS is thought to cause gastric mucosal damage (47).

Despite multiple mechanisms studied related to inflammation induction as well as a relationship to gastric adenocarcinoma, research has yet to find an exact mechanism by which H. pylori induces gastric carcinogenesis and, in turn, whether this can be elucidated a connection with CRC. At this time, the mechanisms in which H. pylori may induce CRC remain hypothetical and need additional investigations.

Bacteroides fragilis (B. fragilis)

Bacteroides fragilis is a common anaerobic bacteria in the human body. It has two molecular subtypes, nontoxicigenic and enterotoxigenic, with the enterotoxigenic strain [Enterotoxigenic Bacteroides fragilis (ETBF)] causing diarrheal illness in humans (52). The B. fragilis toxin (BFT), a toxin encoded by the bft gene, is specific to ETBF. In murine models, ETBF has been shown to influence the development of CRC through the production of the metalloprotease toxin (53). This metalloprotease toxin binds to colonic epithelial cells, stimulating cleavage of the tumor-suppressor protein E-cadherin, a protein involved in intercellular adhesion of the zonula adherens, leading to increased epithelial cell permeability (54-56). E-cadherin stimulation augments cell signaling via the β-catenin/Wnt pathway, a pathway that has been shown to be active in certain CRC cases.

Although Bacteroides fragilis represents less than 1% of the gut microbiota (57), its abundance in CRC-affected mucosa has been shown to be an independent predictor of 3-year overall survival (58) and has been found to be present and more concentrated in the mucosa of later-staged CRC compared to nearby non-cancerous tissue (16). Boleij et al. found that all late-stage (stage III/IV) CRC mucosal samples in their study were positive for the bft gene, compared to
of the organism. As the organism grows, it induces necrosis via its alpha-toxin, leading to mucosal ulceration which allows for hematogenous spread. In the case of \textit{C. septicum} in hematologic malignancies, there is often concomitant neutropenic enterocolitis (66). \textit{C. septicum} is more commonly found in leukemia (especially those who are neutropenic) compared to lymphoma. Neutropenia, whether it is organic or secondary to chemotherapy, appears to be the greatest risk factor in hematologic malignancy patients developing \textit{C. septicum} infection (67).

Alpha-toxin, a necrotizing pore-forming cytolysin, induces cell death via mitochondrial dysregulation and cell membrane destruction. It also causes thrombocytopenia and hemolysis, which may contribute to its propensity to seed the blood through hemorrhaging of the tumor cells into systemic circulation. Histological analysis of \textit{C. septicum} myonecrosis shows a paucity of leukocytes in the affected tissue due to alpha toxin's ability to selectively induce apoptosis of neutrophils, thus interfering with the immune response (68). This is beneficial to the organism's growth, but it also may play a role in tumor growth. Depending on the tumor microenvironment, neutrophils can play a pro- or anti-tumorigenic role (69). In this case, it is theoretically possible that antitumor neutrophils, such as N1-tumor associated neutrophils, are prevented from suppressing tumor growth due to alpha toxin-mediated apoptosis. With the alpha-toxin creating a leukocyte-free microenvironment, there is no immune system modulation of hyper-proliferating malignant cells.

The relationship of \textit{C. septicum} and tumor growth provides a small window into the cellular and chemical complexities of the tumor microenvironment and indicates further research is needed. At this time, there is no evidence that screening for the presence of \textit{C. septicum} in the gastrointestinal tract would provide any benefit. However, given the relatively high incidence of malignancy in symptomatic \textit{C. septicum} infection, a thorough CRC screening evaluation is warranted in the setting of \textit{C. septicum} bacteremia or myonecrosis.

**Future aims**

The development of CRC in humans is multifactorial with increasing evidence that the gut microbiome may be a significant contributing factor. The bacteria reviewed in this article play crucial roles in carcinogenesis with future research needed to further clarify their specific roles. Future research may focus on whether the detection of certain...
bacterial concentrations within stool or biopsied polyps could serve as adjuncts to current screening modalities to help identify higher risk patients. In addition, faster and less expensive bacterial assays are needed to help facilitate the integration of the gut microbiome into routine medical testing. Additional analyses could also be employed to evaluate the occurrence of bacteremia associated with these colonic pathogens and subsequent CRC detection. This would be helpful in determining if the presence of a colonic organism bacteremia should warrant further endoscopic evaluation. It would also be interesting to determine whether early antibiotic treatment targeted towards the aforementioned pathogens has any effect on CRC development.

Acknowledgements
None.

Footnote
Conflicts of Interest: The authors have no conflicts of interest to declare.

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