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Influence of the Gut Microbiome, Diet, and Environment on Risk of Colorectal Cancer

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Abstract

Researchers have discovered associations between elements of the intestinal microbiome (including specific microbes, signaling pathways, and microbiota-related metabolites) and risk of colorectal cancer (CRC). However, it is unclear whether changes in the intestinal microbiome contribute to development of sporadic CRC or result from it. Changes in the intestinal microbiome can mediate or modify the effects of environmental factors on risk of CRC. Factors that affect risk of CRC also affect the intestinal microbiome, including overweight and obesity; physical activity; and dietary intake of fiber, whole grains, and red and processed meat. These factors alter microbiome structure and function, along with the metabolic and immune pathways that mediate CRC development. We review epidemiologic and laboratory evidence for the influence of the microbiome, diet, and environmental factors on CRC incidence and outcomes. Based on these data, features of the intestinal microbiome might be used for CRC screening and modified for chemoprevention and treatment. Integrated prospective studies are urgently needed to investigate these strategies.

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Colorectal cancer (CRC) is the third most common cancer and leading cause of cancer death in men and women in the United States (US), despite the increasing uptake of colonoscopy screening.[1] In 2019, approximately 145,600 new cases of CRC and 51,020 deaths were estimated to occur. Moreover, although CRC incidence and mortality have decreased steadily in the past few decades among adults older than 65 years, an opposing trend has occurred in adults younger than 50 years,[2] for whom routine screening has not been recommended. The incidence of colon cancer has increased by 2.4% per year in adults 20–29 years old and by 1.0% per year in adults 30–39 years old from the mid-1980s through 2013, and began increasing in adults 40–49 years old (1.3% per year) and 50–54 years old (0.5% per year) since mid-1990s.[2] A prolonged and steeper increase has been observed for rectal cancer cases. This alarming trend in young adults coupled with the continued burden of CRC in the overall population indicates a need to develop new prevention strategies to complement screening.

Over the past few decades, migration studies and prospective cohort studies have established the important effects of diet and lifestyle in the development of CRC.[3] Approximately 50%–60% of incident cases of CRC in the US are estimated to be attributable to modifiable risk factors[4, 5] such as smoking; heavy consumption of alcohol; overweight and obesity; physical inactivity; high consumption of red and processed meat; and low consumption of dietary fiber, whole grains, and other healthful nutrients.

The microbiome (including bacteria, virus, fungi et al) regulates health and alterations can contribute to disease. Increasing data indicate that changes in the intestinal microbiome allow environmental risk factors to initiate and promote CRC.[6, 7] This could be because changes of the microbiome affect metabolism and immune function. The intestinal microbiome might therefore be modified as part of CRC preventative strategies. Studies have identified differences in compositions of intestinal microbiomes between CRC cases and healthy individuals (controls) as well as individual microbes that are enriched or depleted in microbiomes of patients with CRC. Moreover, there is evidence that changes in the gut microbiome occur during early stages of colorectal carcinogenesis and can be used to identify individuals at risk for colorectal adenoma, the precursor lesion to CRC. Changes in the microbiome might therefore be used as biomarkers for early detection of CRC, to improve screening strategies. The intestinal microbiome can also influence the efficacy or toxicity of therapeutic agents, including immunotherapies.[8]

Although there have been numerous reviews of association between the gut microbiome and CRC, most have focused on specific microbes,[9] mechanistic pathways,[10–12] or individual risk factors.[6] We review the interactions among the gut microbiome, environmental risk factors, and CRC based on findings from epidemiologic and laboratory research. We also discuss the potential of integrating analyses of the gut microbiome into CRC screening, chemoprevention, and treatment.

Intestinal Dysbiosis in Patients With CRC

The number of microbial species in human intestine is estimated to exceed 2000.[13] The human intestinal microbiome primarily comprises Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Intestinal microbes metabolize indigestible ingredients from food, synthesize nutrients such as vitamins, detoxify metabotypes, modulate the immune response, provide signals for epithelial cell renewal and maintenance of mucosal integrity, and secrete antimicrobial products.[14] Dysbiosis is defined as pathogenic changes in microbiome profile and functions. Alterations in abundance of healthy intestinal microbes can promote chronic inflammatory conditions and production of carcinogenic metabolites, leading to neoplasia.

Patients with CRC have a less diverse microbiome than healthy individuals.[15] However, a meta-analysis of metagenomic data from different cohorts and populations found higher richness in microbiomes of CRC than controls, partly due to expansions of species typically derived from the oral cavity.[16, 17] Differences in abundance of individual microbes have been observed in comparisons of tumor and adjacent non-tumor mucosa, and between stool specimens collected from patients with CRC vs controls. Specific changes in the microbiome and metabolome occur during different stages of colorectal neoplasia, from adenomatous polyps to early-stage cancer to metastatic disease, supporting an etiological and diagnostic role for the microbiome.[18–20] We summarize results from epidemiologic studies of microbes that have been associated with CRC (Tables 1 and 2).

Fusobacterium nucleatum

Two independent studies reported increased levels of Fusobacterium DNA and RNA sequences in tumor compared with non-tumor specimens.[21, 22] Numerous studies, of multiple cohorts, of patients with CRC worldwide have found similar associations.[23–27] In support of the colorectal carcinogenic effect of *Fusobacterium nucleatum*, a higher abundance of *F nucleatum* (present in approximately 10%–15% of tumors) has been associated with advanced disease stage, higher risk of recurrence, and shorter patient survival times.[23, 26, 28] Moreover, levels of *F nucleatum* in tumor tissue have been associated with lower infiltration by T cells,[29, 30] supporting studies reporting that *F nucleatum* reduces the anti-tumor immune response. Epidemiologic studies of patients with CRC or premalignant lesions have associated *F nucleatum* with specific clinical and molecular features, such as right-sided anatomic location, mutations in *BRAF*, and hypermutation with microsatellite instability.[24–27] Given that these features characterize serrated neoplasia,[31] *F nucleatum* might contribute to the serrated pathway of CRC development.

A study associated *F nucleatum* with the consensus molecular subtype 1 of CRC,[32] which is characterized by microsatellite instability and upregulation of immune pathways.[33] More recently, among CRC patients with distant metastases, nearly identical, viable strains of *Fusobacterium* were found at similar relative abundances in paired primary tumors and metastases. So, *Fusobacterium* appears to be an important component of the tumor microenvironment.[34] In addition to studies of tumor tissues, studies of fecal microbiomes that used either 16s rRNA or shotgun metagenomic sequencing found that *F nucleatum* to be increased in fecal samples from patients with CRC or adenoma compared with controls (Table 1).[15, 18, 35–39]

Bacteroides fragilis

Enterotoxigenic *Bacteroides fragilis* (ETBF), which produces the *Bacteroides fragilis* toxin, has been associated with CRC. Although there is strong preclinical evidence for the association between ETBF and CRC, there is little evidence from epidemiologic studies. Only a few studies have examined ETBF in human tumor tissues.[40–43] Among them, 2 found significant enrichment of ETBF in tumor tissues compared to tissues from controls or adjacent normal tissues[40, 43]. However, the proportions of ETBF-positive colorectal tumors differed between these studies (26% vs 89%), possibly due to the differences in assays or sample processing methods.[40] Significantly higher proportions of late-stage vs early stage, and of right-side vs left-side, tumors were ETBF positive.[40, 43] Higher proportions of patients with familial adenomatous polyposis[42] or sporadic premalignant lesions had intestinal mucosa that tested positive for ETBF than of controls.[41]

Compared to mucosa, the abundance of ETBF in fecal samples is lower, because ETBF colonizes colon epithelial crypts.[40, 44] Moreover, different isotypes of ETBF colonize stool (*bft*-1) vs mucosa (*bft*-2). However, ETBF was reported to be enriched in fecal samples of patients with CRC compared with controls.[35, 44, 45] A meta-analysis of 4 case–control studies of metagenomes of patients with CRC found that *Bacteroides fragilis* was the only species that was consistently enriched in intestinal microbiomes of patients with CRC worldwide.[46]

Escherichia coli

Increases in mucosa-associated *E coli* have been observed in patients with inflammatory bowel diseases (IBD) and in patients with CRC, compared with healthy individuals. In patients with CRC, *E coli* invade the colonic mucosa and become intracellular.[47, 48] *E coli* strains with the polyketide synthase gene complex (*pks*) gene, which mediates production of genotoxin colibactin (called *pks*$^{+}$ *E coli*), are found at higher frequency in individuals with than without CRC,[49–52] in tumors than in adjacent non-tumor tissue,[51] and in late-stage tumors compared with early-stage tumors.[51] Levels of mucosa-associated and internalized *E coli* associate with the cell's proliferation index, assessed by Ki-67 expression.[51] However, only 1 fecal microbiome study found *E coli* to be enriched in samples of CRC patients.[18] This might be because *E coli* colonize the mucosa and reside within intestinal cells, rather than the lumen, so they are not shed into feces.

Oral microbiome

Besides *Fusobacterium*, other oral bacteria and markers of periodontal disease are enriched in colorectal tumors and feces of patients, including the genera of *Porphyromonas* and *Peptostreptococcus*, and *Parvimonas micra*.[15, 35, 37, 38, 53] Moreover, oral microbiome profiling studies identified several members of the oral biofilms with different abundances in patients with CRC compared with controls, including *Haemophilus, Parvimonas, Prevotella, Alloprevotella, Lachnoanaerobaculum, Neisseria,* and *Streptococcus*.[54, 55] These bacteria have been associated with distinct mucosal gene expression profiles, which might contribute to development of CRC. Interestingly, similar networks of oral bacteria were found samples from oral and colonic mucosal surfaces of individuals with colonic neoplasia and controls.[55]

These findings support the reported association of periodontal disease with CRC risk.[38, 56, 57] In addition, as a major risk factor for CRC, smoking might change the oral microbiome composition. Smokers have a decreased relative abundance of *Neisseria* and increased relative abundance of *Veillonellaceae* families.[58] Also, bacterial metabolic activities have pathophysiological consequences on oral and systemic health.[58] So, alterations to the oral and intestinal microbiomes might each contribute to colorectal carcinogenesis, via microbe dissemination and induction of inflammation.[54, 55, 59,60.]

Bacteria

Streptococus bovis or *gallolyticus* has been associated with CRC (see ref [61]). Serologic epidemiology studies (based on immunoassays) found the incidence of non-typhoid *Salmonella* infection to be much higher (about 600-fold) than reported,[62] ranging from 56 per 1000 person-years in Finland to 547 per 1000 person-years in Poland, from of 2003 through 2008 (ref [63]), and increasing from 13 per 1000 person-years to 217 per 1000 person-years in Denmark, from 1983 through 1999.[64] Furthermore, Salmonellosis, primarily caused by its major serotypes, *S* ser. Typhimurium and *S* ser. Enteritidis, has also been associated with development of disorders such irritable bowel syndrome[65] and IBD.[66] Studies from Scandinavian countries found that the probability of a new diagnosis of IBD following an episode of non-typhoid *Salmonella* infection (all subspecies combined), particularly within the first 10 years, increased significantly (2–3 fold) compared with general population.[67, 68] Antibody titers to *Salmonella* ser. Typhimurium were higher in CRC cases than controls in the US and the Netherlands. Smoking and dietary iron were identified as potential risk factors, indicating a link between non-typhoid *Salmonella* infection and intestinal tumoigenesis[69]. This observation was confirmed in an independent population-based linkage study in the Netherlands.[70] The investigators analyzed cancer registry and public health surveillance data and found an increased incidence of CRC, compared with the general population, among residents who with reported enteric (not systemic) salmonellosis. Moreover, epidemiology studies associated a serologic response to *Helicobacter pylori* with increased risk of CRC among African Americans.[71]

Infection-associated CRC might develop via transformation of cells to premalignant lesions, adenomas, to malignancies. Strategies to detect specific bacteria or bacterial DNA sequences might be used in screening for early-stage colorectal adenomas or carcinomas.[60, 61]

Virome and mycobiome

The enteric virome and mycobiome (fungal microbiome) have also been linked with CRC.[72–74] Compared to controls, CRC cases had increased diversity in bacteriophage viromes, associated with reduced bacterial diversity, and modest enrichment of specific viral taxa, such as members of *Inovirus* and *Tunalikevirus*, whose bacterial hosts have been implicated in CRC (such as ETBF, *F nucleatum*, and *pks*$^{+}$ *E coli*). These findings indicate an interaction between the virome and bacteriome in risk of CRC.[72, 74] Distinct mycobiome profiles were identified in patients with CRC vs controls and in patients with early- vs late-stage CRC. Ecological analysis revealed more co-exclusive correlations between fungi and bacteria in fecal samples from patients with CRC than controls,[73] supporting the preclinical findings for an antagonistic relationship between bacteria and fungi during CRC development.[75]

However, given the limited data, further studies are needed to investigate how interactions among the enteric virome, mycobiome, and bacteriome might contribute to development of CRC.

Environmental Factors

The composition of microbiota is determined by genetic, environmental, and dietary factors. For example, variants in the gene encoding the vitamin D receptor affect the composition of the microbiome,[76] but it is still not clear whether genetic or environmental factors have a bigger effect on the gut microbiome.[77] Although there is no direct evidence, CRC-related risk factors have been associated with specific changes in the intestinal microbiota.

Overweight and obesity

A meta-analysis found that each 5-kg/m^2 increase in body mass index (BMI, calculated as body weight in kilograms divided by square of height in meters) is associated with 5% increase in risk of CRC.[78] Obesity might also be contributing to the increasing incidence of young-onset CRC.[79] Epidemiology studies have provided evidence for an association between obesity-related metabolic and inflammatory factors, including changes in insulin-like growth factor 1 signaling, adipokines, sex hormones, and systemic inflammation, and CRC risk.[80] Obesity-associated changes in the intestinal microbes and their metabolites may also contribute to carcinogenesis.

Obesity has been associated with a significant decrease in the diversity of the gut microbiota.[81, 82] Some[83] but not all[81, 84] cross-sectional studies found an enrichment of the phylum *Firmicutes* and depletion of the phylum *Bacteroidetes* in obese individuals compared with lean individuals. Although it is not clear whether alterations to the intestinal microbiome are a cause or consequence of obesity, dietary intervention studies have shown that changes in body weight affect the gut microbiota.[85] Obese individuals who lost weight on a fat-restricted or carbohydrate-restricted low-calorie diet had increased abundance of *Bacteroidetes* and decreased *Firmicutes*, irrespective of diet type.[83] A decrease in the ratio of *Firmicutes*:*Bacteroidetes* was also observed in individuals who lost weight in other trials.[86]

Bacteria that produce short-chain fatty acids (SCFAs) are important regulators of metabolic homeostasis.[87, 88] A lower abundance of SCFA-producing bacteria has been associated with higher risk of type 2 diabetes.[89] Some dietary intervention studies, paradoxically, associated weight loss with reduced abundance of SCFA-producing bacteria.[84, 90–93] However, most of the studied diets were low in total calorie and carbohydrate, an important substrate for SCFA synthesis, so the observed reduction in SCFA-producing bacteria might have resulted from carbohydrate restriction in the diet, rather than the weight loss itself. In patients on a non-hypocaloric low-fat intervention, weight loss was associated with an increase in SCFA-producing bacteria including *Clostridium Cluster IV, Bifidobacterium spp.,* and *Faecalibacterium prausnitzii*.[86] A bidirectional Mendelian randomization analysis provided genetic evidence that increased intestinal production of SCFAs improves patient responses to insulin and reduces abnormalities in the production or absorption of SCFAs and risk of type 2 diabetes.[87] Obesity might therefore increase risk of CRC by reducing the abundance of SCFA-producing bacteria and SCFA production in the intestine [94]. Amino acids such as

glutamate and deoxycholate have also been associated with obesity, intestinal dysbiosis, and metabolic disorders.[20, 95]

Akkermansia muciniphila might also provide a link between obesity and CRC,[96] although there is controversy over the role of *A muciniphila* in colorectal carcinogensis.[53, 97] Several studies of different diets associated weight loss with enrichment of *A muciniphila.*[86, 93, 98, 99] The bacteria correlated with better metabolic parameters, including lower fasting level of plasma glucose, lower level of plasma triglycerides, and improved insulin response.[100]

Obesity might contribute to systemic inflammation by altering intestinal barrier function. Leakage of microbial products, such as the endotoxin lipopolysaccharide (LPS), causes metabolic endotoxemia.[101] Higher BMIs have been associated with increased blood levels of LPS and LPS-binding protein (LBP), whereas weight loss reduces circulating LPS and LBP levels.[102–104] A cross-sectional study found that patients with adenomas had higher blood levels of LPS than controls, and that patients whose adenomas had villous features had higher levels of LPS than patients with only tubular adenomas.[105] A polymorphism in the *LBP* gene (rs2232596) was associated with higher risk of CRC.[106]

A large prospective study of mostly Caucasian persons associated higher levels of LPS with increased risk of CRC in men,[107] whereas a prospective study in a racially diverse cohort observed that, compared to individuals in the first quartile of plasma LBP, those in the third, but not fourth, quartile had an increased risk of CRC.[108] Given these limited data, further studies, preferably pooled analyses of multiple cohorts, are needed to examine the relationship of LPS with CRC risk, according to sex and adiposity.

A recent meta-analysis assessed the effect of the gut microbiome on the relationship between obesity and increased CRC risk.[109] The study reported that the association between BMI and CRC risk was only slightly attenuated when several CRC-associated taxa were added to the analytic model, indicating a weak effect of these taxa. Although this meta-analysis was the first effort to analytically assess the mediating effect of the gut microbiome on the relationship between obesity and CRC, it could not establish that these taxa contributed to development of CRC, due to the cross-sectional design of the included studies —particularly the contemporary assessment of BMI and gut microbiome in patients with CRC vs controls. Prospective studies that assess BMI and the gut microbiome and then follow patients to see which ones develop CRC are needed to better understand the role of the gut microbiome in obesity-associated increase in CRC risk.

Physical activity

Individuals with the highest level of physical activity have a 19% lower risk of colon cancer than individuals with the lowest level, but physical activity has not been associated with rectal cancer.[78] Several cross-sectional studies have reported the effects of exercise on the composition of the intestinal microbiota and its functions.

Two studies compared the fecal microbiome and metabolomes of 40 elite professional rugby players with those of 46 male controls.[96, 110] Because the athletes tended to have a higher

BMI, to minimize the influence of BMI, the study included 2 control groups: 1 with BMIs ≤25 and the other with BMIs >28. The athletes were found to have a more diverse gut microbiome than the controls. Among the individual species that differed between athletes and controls, *A muciniphila* was found to be enriched by 16s rRNA and metagenomic sequencing analyses, as well as in the metabolic pathway analysis. These findings support the role of *A muciniphila* in metabolic regulation. Although dietary factors are often correlated with the metagenomic pathways, exercise and high protein intake also correlate with the metagenomic profiles of athletes. For example, levels of SCFAs were significantly higher in athletes compared with controls, although it is not clear whether this is due to higher fiber intake or the more intensive exercise by athletes. Cardiorespiratory fitness has also been associated with higher fecal levels of butyrate and increased abundances of butyrate-producing taxa, including *Clostridiales, Roseburia, Lachnospiraceae*, and *Erysipelotrichaceae*.[111] Similar findings were observed in a study that compared active vs sedentary women.[112]

Two intervention studies reported effects of exercise on intestinal microbiomes.[113, 114] In the first study, 32 sedentary adults underwent 2 weeks of baseline analysis, 6 weeks of endurance-based exercise intervention, and 6 weeks of washout, during which participants were instructed to refrain from exercising.[113] Exercise training increased fecal concentrations of SFCAs in lean but not obese participants, and increased butyrate-producing bacterial taxa that associated with parallel shifts in body composition in lean individuals. Although these findings support the role of exercise in regulation of SCFA production, it is unclear why the benefit was restricted to lean individuals. It could be that lean participants are more likely to comply with the intervention and achieve a higher intensity of exercise than obese individuals. Notably, the study also found that a return to sedentary lifestyle for 6 weeks reversed changes in the gut microbiota observed after exercise training, again indicating the sensitivity of the gut microbiome to physical activity.

In the second study, 8 weeks of mixed aerobic and resistance exercise training improved cardiorespiratory fitness and body composition, but did not produce significant changes in the fecal microbiome[114] or metabolites. Interestingly, exercise was associated with reduced levels of phenylacetylglycine and trimethylamine N-oxide (TMAO) in urine. TMAO is a gut microbiota-derived metabolite of dietary choline and L- carnitine obtained from consumption of red and other types of meat. Give the finding of metagenomic enrichment of choline-metabolizing pathways in patients with CRC, [16] these findings support the benefits of physical activity in reducing production of TMAO and risk of CRC.

Dietary fiber and whole grains

The hypothesis that higher intake of dietary fiber protects against CRC originated from the observation of the substantially low rates of CRC in Africans, who consume a high-fiber diet.[115] Although numerous epidemiologic studies have tested this hypothesis, none have produced conclusive results. According to the recent meta-analysis of 21 prospective studies, no linear association was found between fiber intake and CRC risk.[78] However, there was substantial heterogeneity among studies. In contrast to most US studies, which reported no association,[116–119] the European Prospective Investigation into Cancer and Nutrition cohort

consistently found an association between fiber intake and reduced risk CRC.[120–122] This might be due to differences in the food sources of fiber between European and American diets (mostly cereals vs fruits and vegetables) and the relatively low fiber intake in the US cohorts, which might not have achieved an effective threshold.[3] In contrast, several studies have reported associations between whole grains and reduced risk of CRC. A meta-analysis found a 17% reduction in CRC risk per 90 g/day increase in consumption of whole grains.[78]

The anti-CRC effects of fiber could be related to its effects on the gut microbiota.[6] Fiber is fermented by bacteria to produce SCFAs, which regulate the immune system and metabolism and reduce risk of CRC.[6, 88, 94, 123–125] Some but not all cross-sectional studies associated higher fiber intake with increased fecal levels of SCFAs and enrichment of SCFA-producing bacteria, such as *Eubacterium rectale*, *Roseburia* spp., and *Faecalibacterium prausnitzii*.[126, 127] Randomized controlled trials have examined the effect of supplementation with fiber or related prebiotics on the gut microbiota and metabolome in healthy adults. These findings were summarized in a meta-analysis[128] of 64 trials of various types of supplements (such as resistant starch, inulin, arabinoxylan-oligosaccharide, and short-chain fructo-oligosaccharides) with sample sizes ranging from 8 to 84 participants and durations of 1 to 6 weeks. Fiber supplementation was found to enrich *Bifidobacterium and Lactobacillus spp.*, whereas no effect was found on the other common SCFA producers except for increases in *Roseburia spp.* and *F prausnitzii* in parallel (rather than crossover) trials.

In support of the relevance of these findings to CRC, several studies have shown that compared with controls, patients with CRC had lower abundances of *Bifidobacterium and Lactobacillus spp*,[16, 18] and other SCFA-producing bacteria, such as the genera *Clostridium* and *Roseburia*, the family *Lachnospiraceae*, and *Faecalibacterium prausnitzii*.[15, 35, 37, 45, 53, 129] *Bifidobacterium and Lactobacillus spp.* produce lactate and acetate and can also increase production of butyrate through cross-feeding interactions with the butyrate-producing species, such as *Eubacterium rectale*.[130–132] Fiber supplementation increased the concentration of butyrate in fecal samples.[128] Furthermore, in support of the benefit of butyrate for CRC, a few cross-sectional studies reported that patients with CRC had a lower abundance of butyrate-producing species and lower fecal levels of butyrate than controls (Table 1).[97, 133–135]

In some[136, 137] but not all[138, 139] randomized controlled trials, increased consumption of whole grains was associated with higher abundance of SCFA-producing bacteria, such as *Roseburia* and *Lachnospira*, lower abundance of proinflammatory *Enterobacteriaceae*, and higher levels of fecal SCFAs through anti-inflammaion.[136] Whole grains contain other beneficial nutrients, including polyphenols and flavonoids, which are also important modulators of the gut microbiome. Consumption of whole-grain wheat increases the fecal concentration of ferulic acid (the most abundant phenolic compound in whole grains) and serum concentration of dihydroferulic acid (a metabolite derived from ferulic acid by *Lactobacillus* and *Bifidobacterium*).[136] Given the shared microbes in the synthesis of SCFA and dihydroferulic acid, synergistic mechanisms might augment the production of beneficial metabolites. These might account for ability of whole grains to reduce CRC risk compared with other food sources of dietary fiber. A large prospective study found that the association

of dietary fiber with reduced risk of CRC had a larger effect on tumors with detectable *F nucleatum* than those without *F nucleatum*.[140]

Red and processed meat

Higher intake of red and processed meat has been associated with increased incidence of CRC. A meta-analysis associated each 100 g/day intake of red and processed meat with a 12% higher risk of CRC.[78] The carcinogenic effect might be mediated by preservatives in red and processed (such as nitrates and nitrites), other additives (such as emulsifiers), chemicals produced during meat processing and cooking (such as heterocyclic amines and polycyclic aromatic hydrocarbons), or nutrients enriched in meats (such as heme iron, sulfur, and choline).[3] Some of these elements can be metabolized by the gut bacteria to produce metabolites that have been implicated in CRC.

Meat, particularly red meat, has high content of choline and carnitine, which are precursors to gut microbiota-mediated formation of TMA and TMAO.[141] A randomized controlled trial found that chronic ingestion of red meat, but not white meat or non-meat, increased plasma and urine levels of TMAO.[142] High levels of TMAO have been associated with increased risk of cardiovascular disease and mortality.[143–145] Several lines of evidence indicate the potential role of choline–TMAO pathway in development of CRC. A meta-analysis of metagenomes of fecal samples[146] found patients with CRC to have higher levels of 2 bacterial genes that regulate TMA synthesis: choline TMA-lyase (*cutC*) and choline TMA-lyase-activating enzyme (*cutD*). Several taxonomic features associated with sequence variants of *cutC* were also found to be enriched in patients with CRC, including *Hungatella hathewayi*, *Clostridium asparagiforme, Klebsiella oxytoca*, and *E coli*.

Some,[147, 148] but not all,[149–151] prospective studies have associated higher dietary and plasma levels of choline with increased risk of colorectal neoplasia. A higher plasma level of TMAO was associated with an increased risk of CRC,[151] but this finding was not replicated.[152] Choline is required for DNA methylation and synthesis, along with other nutrients (such as folate and vitamin B12),[3] so the ultimate effect of choline might depend on its distribution in the circulation for 1-carbon metabolism vs in the gut for microbial production of TMAO. In support of this hypothesis, the association between plasma level of TMAO and risk of CRC was restricted to patients with low plasma levels of vitamin B12.[151] Higher levels of choline and TMAO have been linked to a spectrum of metabolic disturbances that might increase CRC risk, including higher BMI, greater visceral adiposity, insulin resistance, diabetes, and fatty liver.[153, 154] Changes in TMAO and choline associated with increased insulin sensitivity in a weight-loss intervention study of obese individuals.[155]

Studies in the 1990s reported higher circulating levels of deoxycholic acid in patients with colorectal adenomas than controls.[156, 157] A large prospective study of serum metabolomes associated concentration of glycochenodeoxycholic acid with CRC risk in women but not in men.[158] Moreover, the *bai* operon, encoding 7α-dehydroxylation in *Clostridium spp.* required for synthesis of secondary bile acids, was found to be enriched in metagenomes of fecal samples from patients with CRC.[146] Patients with gallstone disease and cholecystectomy, who are believed to have higher concentrations of secondary bile acids due to continuous flow of bile acids to the bowel, have an increased risk of CRC.[159] Secondary

bile acids might contribute to development of CRC because they generate reactive oxygen and nitrogen species that cause DNA damage and promote resistance to apoptosis.[160] Changes in bile acid composition and concentrations have been associated with metabolic disorders and IBD, which increases risk for CRC.[161, 162] A recent analysis of 8 geographically and technically diverse fecal shotgun metagenomic studies found evidence for increased production of secondary bile acids in patients with CRC, indicating a metabolic link between cancer-associated microbes and a fat- and meat-rich diet. [146]

Hydrogen sulfide (H_2S) is generated in the gut either by sulfur-reducing bacteria from inorganic sulfur (sulfate and sulfite) that is routinely used as a preservative in processed meat or by fermentative bacteria that metabolize organic sulfur compounds that are enriched in animal products such as red meat.[3] Higher intakes of sulfur and sulfate were associated with increased risk of IBD[163], and fecal samples from patients with colon cancer have higher concentrations of H_2S than controls.[164] Several sulfidogenic bacteria were found to be enriched in tissue samples from patients with CRC, including *Fusobacterium*, *Bilophila wadsworthia*, and the genera *Lactococcus, Porphyromonas, Odoribacter, Bilophila,* and *Pyramidobacter*.[165, 166] H_2S-producing pathways are increased in fecal samples from patients with CRC.[20] Interestingly, African Americans have substantially higher abundances of sulfur-reducing bacteria and *B wadsworthia* than Caucasians, even after adjusting for dietary variables. Sulfidogenic bacteria might therefore contribute to the higher incidence of CRC in African Americans than Caucasians.[166]

In a cohort of elderly men (mean age of 71 years), higher dietary intake of organic sulfur was associated with increased fecal abundance of H_2S-producing *Clostridium clostridioforme*.[167] Consistent with the finding that processed red meat increases risk for distal colon cancer, in particular,[168, 169] we associated dietary patterns with higher fecal abundances of sulfur-producing and increased risk of distal CRC, but not proximal colon cancer.[167]

Mechanisms

The intestine must maintain commensal microbes and a high load of bacterial products, but swiftly respond to pathogens that threaten its integrity. Analyses of gut microbiota of mice raised in germ-free or gnotobiotic conditions and transgenic mice, using techniques such as next-generation sequencing, microbial gene mutations, and microbial RNA-sequencing, have identified mechanisms by which the intestinal microbiota might contribute to or prevent development of CRC (Figure 1). For example, obesity might promote colorectal carcinogenesis via LPS-mediated systemic inflammation and depletion of SCFA-producing bacteria. Red and processed meat might increase CRC risk by increasing bacterial production of secondary bile acids, H_2S, and TMAO. Physical activity and dietary fiber might reduce risk of CRC by increasing the abundance of *A muciniphila* and SCFA-producing bacteria. For reviews on the mechanisms by which the gut microbiome contributes to colorectal carcinogenesis, see refs [9, 11, 12].

Metabolites

The pathogenic mechanisms of bacteria associated with CRC (such as *F nucleatum*. *Bacteroides fragilis, E coli, and* inflammatory *Enterobacteriaceae)* have been investigated in animal models. Chronic inflammation, dysfunction of immunity, increased serum levels of LPS, secondary bile acids, and leaky gut are representative pathways that link the gut microbiota with CRC (Figure 1).

Although many types of bacteria have been identified that might contribute to colorectal carcinogenesis, we have also identified bacterial metabolites that affect CRC risk. High levels of secondary bile acids and SCFAs have opposing effects on colon inflammation.[170] High fat content and consumption of red and processed meats increase secretion of primary bile acids that can be metabolized by the gut bacteria to secondary bile acid, including deoxycholic acid, lithocholic acid, and glycochenodeoxycholic acid. Sulfidogenic bacteria are increased in tissue samples from patients with CRC.[165, 166] Sulfur is another compound in red and processed meat that is closely linked to the gut microbiota.

SCFA-producing bacteria, such as *Roseburia* and *Lachnospira*, reduce risk of CRC. Higher levels of fecal SCFAs were associated with increased numbers of memory T cells in blood, [171] reduced levels of inflammatory cytokines (such as tumor necrosis factor), and increased levels of anti-inflammatory cytokines (such as interleukin 10).[136] Butyrate increases intestinal expression of vitamin D receptor mRNA and protein, reduces intestinal dysbiosis, and activates the autophagy response of Paneth cells to inhibit chronic inflammation.[172]

Contribution of diet to colitis and colon cancer

Fiber regulates the immune response and levels of *F nucleatum*. It is possible that higher intake of fiber could reduce the carcinogenic effects of *F nucleatum* by restoring effective immunosurveillance.[173]

In *Il10*$^{-/-}$ mice and mice without disruption of this gene, a diet high in saturated (milk-derived) fat (MF) promoted expansion of the immunogenic sulfite-reducing pathobiont *Bilophila wadsworthia*, a member of the Deltaproteobacteria.[174] The *Bilophila wadsworthia* expansion resulted from a MF diet-induced shift in hepatic conjugation of bile acids, from glycocholic to taurocholic acid, which helps solubilize the more hydrophobic MF diet. H_2S-producing bacteria might promote intestinal inflammation to increase the risk for colitis-associated cancer.

Infection

Although dysbiosis is associated with chronic inflammation and production of carcinogenic metabolites, [175, 176] there is limited evidence to support a direct link between specific intestinal bacteria, their virulence factors, and sporadic CRC. Increases in population-wide exposure to antibiotics [177] might contribute to high rates of bacterial infection.[178] More than 1 million people in the US acquire *Salmonella* infection annually as a foodborne illness mainly from eggs, meats, dairy, and other contaminated non-animal foods.[178] In C57BL/6J mice, recurrent infection with *Salmonella enterica* promoted intestinal inflammation and progressively disabled protective mechanisms, inducing endogenous neuraminidase activity

to reduce the abundance and protective effects of intestinal alkaline phosphatase.[179] The protein AvrA, produced by *Salmonella*, promotes inflammation and colon tumor development by activating beta-catenin signaling to STAT3.[36, 180] Expression of AvrA protein, detected by immunohistochemistry, was significantly higher in pre-cancer colon tissues than in normal human colon or tumor tissues, based on pathology differences.[181] Mice with *Citrobacter rodentium*-induced colonic crypt hyperplasia have alterations in beta-catenin and Notch signaling, intestinal barrier function, and fecal dysbiosis, resulting in development of colon tumors.[182, 183] *F nucleatum* induce expression of microRNA 21 by CRC cells by activating TLR4 signaling via MYD88 and nuclear factor-kappa B. This increased proliferation of the CRC cells.[184]

Specific strains of bacteria therefore appear to disrupt the intestinal microbiome and promote inflammation to increase risk for IBD and colon carcinogenesis. The epithelial and metabolic changes that occur during development of CRC might provide a competitive advantage to a subset of intestinal bacteria.[185] Genetic variants, along with environmental factors, also contribute to contribute to dysbiosis and development of CRC. Microbial pathogens and chronic inflammation can compromise barrier function and enhance permeability. Translocation of microbial products, metabolites, increased serum LPS, and immune activation promote dysbiosis, barrier failure, and inflammation.

Screening

Alterations in the fecal microbiomes of patients with CRC have also been observed in patients with colorectal adenoma—these might be used in screening for individuals at risk for CRC. Fecal microbiome analysis identifies patients with adenomas with reasonable levels of accuracy (area under receiver operating curves ranging from 0.55 to 0.67 in validation studies), although this is a lower value than for detection of CRC.[16, 18, 186–188] Combining the fecal microbiome data with scores from risk factor-based models or results of screening tests (such as fecal occult blood test and fecal immunochemical test) increases the accuracy of detection for advanced adenomas.[35, 186, 187] For example, addition of fecal *F nucleatum* quantitation to fecal immunochemical test, which has suboptimal sensitivity in detecting adenomas, doubled the sensitivity for detection of advanced adenomas. A similar improvement was observed in an independent validation cohort.[186]

Several questions must be answered before fecal microbiomes can be used in CRC screening. First, studies have identified and used different microbial features to construct their analysis models. It is unclear to what extent the heterogeneity among studies reflects the true differences in the ability to detect CRC based on different microbial patterns or variations in the technical aspects of studies (such as stool collection methods, timing of bowel preparation for colonoscopy, and sequencing and analysis methods). Therefore, it is not clear whether there is one specific microbial signature that can be used to identify individuals with CRC or its precursors in diverse populations. Second, despite the discriminatory accuracy, the reliability and predictivity of the gut microbiome-based classifiers must be established in prospective studies. Most screening methods have limited abilities to detect proximal lesions (such as the fecal immunohistochemical test, flexible sigmoidoscopy, and colonoscopy), so it is important to determine whether analyses of fecal

microbiomes can improve the sensitivity of detection of proximal colon neoplasias. Other practical issues must be evaluated before fecal microbiome analysis can be used in CRC screening, such as determination of cost effectiveness, affordability, and acceptability by patients and physicians, compared with established screening strategies.

Therapy

In addition to affecting CRC development, the gut microbiota modulates the response to cancer therapy and susceptibility to toxic side effects, although there is only limited evidence from patients (for reviews, see [8, 189]). In 2013, Iida et al[190] reported that alterations in the intestinal microbiota can affect the efficacy of an immunotherapy (CpG-oligonucleotide) and oxaliplatin, a platinum compound used in chemotherapy for CRC and other cancers. Both therapies had reduced efficacy in mice given antibiotics or germ-free mice, which had lower production of cytokines and less tumor necrosis after CpG-oligonucleotide administration and reduced production of reactive oxygen species and cytotoxicity after chemotherapy compared. Bacterial metabolism was found to affect the efficacy of the anti-pyrimidine drugs 5-fluorouracil and 5-fluoro-20-deoxyuridine and the topoisomerase I inhibitor camptothecin in *Caenorhabditis elegans* [191, 192]. Different bacterial species increased the response to 1 drug and decreased the effect of another. Bacterial ribonucleotide metabolism affected the cytotoxic effects 5-fluorouracil and 5-fluoro-20-deoxyuridine by altering production of regulatory metabolites had synergistic effects with drug-induced DNA damage. However, no patients or mouse models of CRC were used in these studies.

F nucleatum was promotes resistance of CRC cells to chemotherapy.[28] Patients with post-chemotherapy recurrence had a higher abundance of *F nucleatum* in tumor tissues.[28, 193] Bioinformatic and functional studies indicated that *F nucleatum* activates innate immune responses, via TLR4 and MYD88, resulting in loss of specific microRNAs. This resulted in activation of autophagy activation and promotion of chemoresistance in patients with CRC.[28]

Several studies have indicated that the gut microbiota can determine the efficacy of cancer immunotherapy, although there is no evidence for its effects on immunotherapy for CRC. For example, *Bacteroides thetaiotaomicron* and *B fragilis* have been associated with higher efficacy of agents that block cytotoxic T-lymphocyte associated protein 4 (CTLA4), possibly by affecting interleukin 12-dependent T-helper 1 cell-mediated immune responses. *Bifidobacterium* increased the response of tumors to antibodies against programmed cell death 1 (PDCD1), which increased dendritic cell function, priming of CD8+ T cells, and their accumulation in the tumor microenvironment.[194] *A muciniphila*[195] and *Faecalibacterium*[196] have been associated with greater efficacy of PDCD1 blocking agents—possibly by increasing recruitment of T cells to tumors and their functions there. Moreover, specific bacteria have been associated with resistance to the development of immune checkpoint inhibitor-associated colitis,[197] and fecal microbiota transplants might be used to treat this form of colitis.[198]

Dietary interventions can be used to modulate the gut microbiota in patients receiving cancer therapy. Higher intake of nutrients such as fiber,[199] marine omega-3 fatty acid,[200–202] vitamin D,[203–205] or calcium,[203] or coffee[206, 207] or a plant-based low-carbohydrate diet[207] has been associated with increased survival times of patients with CRC. Many of these factors have immunomodulatory and microbiota-modifying effects, so they might increase the efficacy and reduce the adverse effects of immunotherapies or other therapeutic agents.[208]

Future Directions

Epidemiologic evidence for the effects of the gut microbiome on CRC risk comes from retrospective case–control studies of biospecimens collected from patients with established CRC or precursor lesions. It is not clear whether the identified microbial alterations are a cause or consequence of colorectal carcinogenesis.[209] Moreover, clinical studies have been limited by small sample sizes (mostly <100 cases), likely underpowered to identify predictive signals from hundreds to thousands of reads counts generated by high-throughput sequencing. Prospective studies, with large-scale microbial biospecimen collection and long-term follow up, are urgently needed to determine which microbes or collections of microbes contribute to CRC development discover and identify signatures that can be used in screening.[210]

Data on environmental factors that alter gut microbiome are mostly derived from cross-sectional or short-term interventional studies. Cross-section studies cannot determine whether changes in diet and lifestyle cause changes in the intestinal microbiota, its metabolism, or its effects on the immune response. Correlations among individual dietary and lifestyle factors and the gut microbiome are difficult to make due to confounding factors. The short duration of most intervention studies makes it impossible to examine the effects of alterations to the gut microbiome of long-term exposures that affect risk for CRC. Prospective studies with detailed diet and lifestyle data collected long before participants develop CRC are needed to better characterize the long-term influence of environmental exposures on the gut microbiome and their effects on CRC prevention. Furthermore, we need to uncover the specific mechanisms by which these diet and lifestyle factors influence the gut microbiome and risk of CRC.

There are few human data on the role of effects of alterations in the gut microbiota in CRC treatment. Including microbiota specimen collection into clinical trials, along with assessments of diet and other environmental factors, could provide information on how they affect treatment outcomes and survival times of patients with CRC. It is important to elucidate the immune and metabolic pathways that mediate the effects of the gut microbiota and dietary factors on treatment for CRC and survival times.

Researchers have generated exciting preliminary data for the role of the microbiota in CRC development, prevention, and treatment. Integrated prospective studies will open new avenues for studies of intestinal microbiota and its manipulation in CRC screening, prevention, and treatment.

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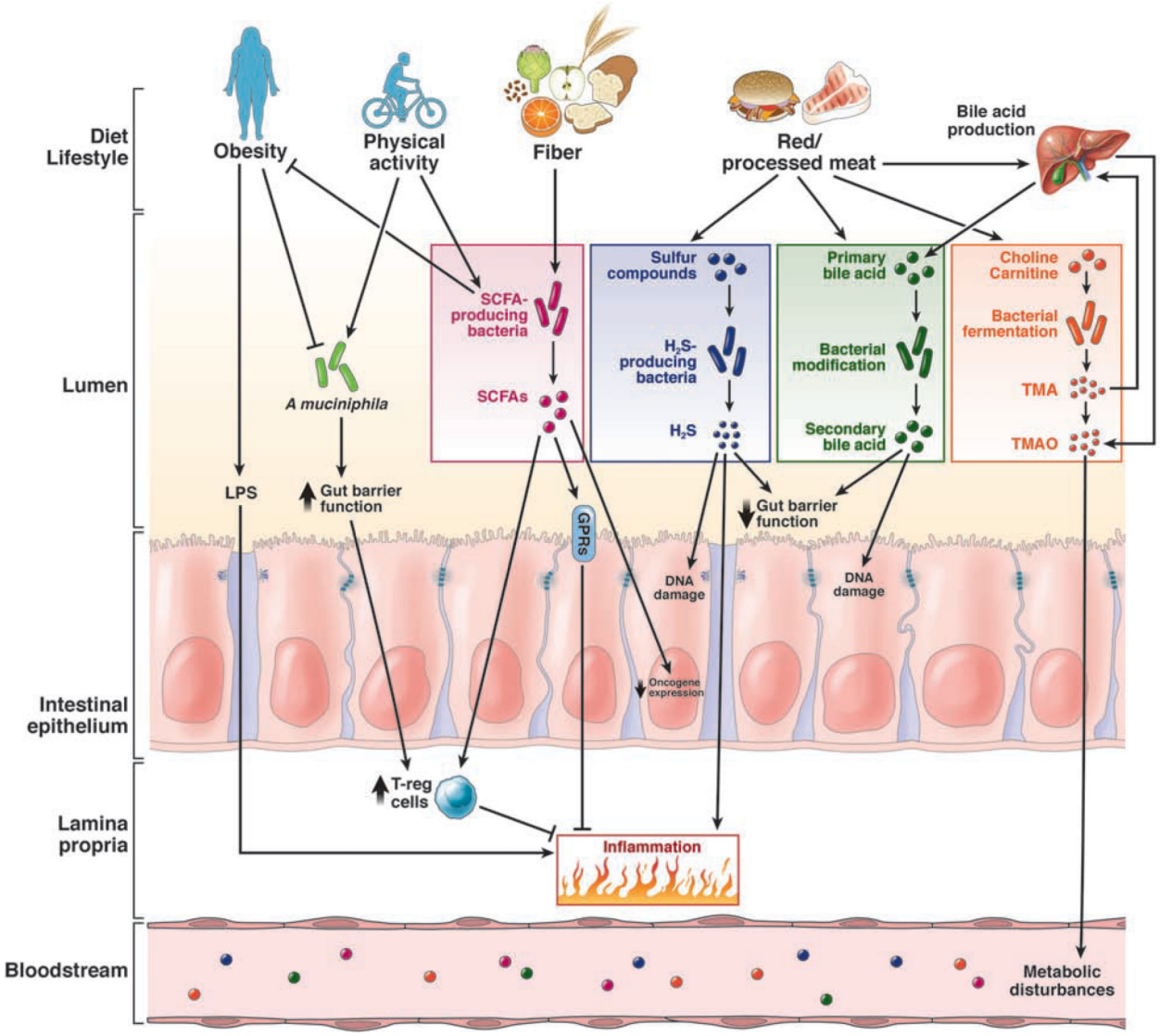

Figure 1.

Pathways by which dietary and environmental factors affect the intestinal microbiome and their roles in colorectal carcinogenesis. Obesity may promote CRC through LPS-mediated systemic inflammation and depletion of *A muciniphila* and SCFA-producing bacteria, whereas physical activity might protect against CRC by increasing the abundance of *A muciniphila* and SCFA-producing bacteria. The benefit of dietary fiber might be mediated by enrichment of SCFA-producing bacteria and increased production of SCFAs that inhibit CRC development, modulation of the immune and metabolic response. Red and processed meat may increase CRC risk by increased bacterial production of secondary bile acids, H_2S, and TMAO.

Abbreviations: H_2S, hydrogen sulfide; T-reg cell, T-regulatory cells.

Table 1.

Association of the Intestinal Microbiome with CRC

Author, Year	Country	Study design	Sample Size	Biospecimen type	Sequencing method	Main findings comparing cases to controls
Scanlan, 2008[211]	Belgium	case–control	20 cancers / 20 polyps / 20 controls	Stool	16s rRNA sequencing	↑ diversity of the *Clostridium leptum* and *C. coccoides* subgroups
Sobhani, 2011[212]	France	case–control	60 cancers / 119 controls	Stool	16s rRNA sequencing	↑ *Bacteroides/Prevotella*
Castellarin, 2012[21]	Canada	case–control	11 cancers with adjacent normal tissue	Tissue	RNA sequencing	↑ *Fusobacterium*
Kostic, 2012[22]	USA	case– control	95 cancers with adjacent normal tissue	Tissue	16s rRNA sequencing	↑ *Fusobacterium*
Sanapareddy, 2012[213]	USA	case–control	33 adenomas / 38 controls	Tissue	16s rRNA sequencing	↑ bacteria from 87 taxa, including potential pathogens such as Pseudomonas,Helicobacter and, Acinetobacter; ↓ bacteria from 5 taxa.
Chen, 2012[214] Wang, 2012[45]	China	case–control	46 cancers / 56 controls	Tissue, stool, and swab	16s rRNA sequencing	↓ diversity; ↑ *Bacteroides fragilis, Lactobacillales, Fusobacterium, Porphyromonas, Peptostreptococcus, and Mogibacterium;* ↓ *Bifidobacterium, Faecalibacterium, Blautia* butyrate-producing bacteria
Ahn, 2013[15]	USA	case–control	47 cancers / 94 controls	Stool	16s rRNA sequencing	↓ diversity; ↓ *Clostridia*; ↑ *Fusobacterium*, *Porphyromonas*;
Dejea, 2014[215]	USA	case–control	23 cancers and 2 adenomas with paired normal tissues, and 22 controls	Tissue	16s rRNA sequencing	↑ *Fusobacterium*; difference by biofilm status
Zackular, 2014[35]	USA	case–control	30 cancers / 30 adenomas / 30 controls	Stool	16s rRNA sequencing	↑ *Bacteroides fragilis, Fusobacterium*, *Porphyromonas*; ↓ butyrate-producing bacteria
Zeller, 2014[36]	France	case–control	91 cancers / 42 adenomas / 358 controls	Stool	Metagenomic sequencing	↑ *Bacteroidetes*, *Fusobacteria* and *Proteobacteria*; ↓ *Actinobacteria* and *Firmicutes*
Burns, 2015[216]	USA	case–control	44 cancers with adjacent normal tissue	Tissue	16s rRNA sequencing	↑ diversity, *Fusobacterium* and *Providencia;* ↑ *virulence-related genes*
Feng, 2015[18]	Austria	case– control	41 cancers / 42 adenomas / 55 controls	Stool	Metagenomic sequencing	↑ *B dorei, B vulgatus, E coli, Fusobacterium;* ↓ *Lactobacillus* and *Bifidobacterium*
Lu, 2016[217]	China	case– control	31 adenomas / 20 controls	Tissue	16s rRNA sequencing	↑ diversity; ↑Lactococcus and Pseudomonas; ↓ Enterococcus, Bacillus, and Solibacillus. Similar composition between adenomatous and adjacent nonadenoma tissues
Vogtmann, 2016[37]	USA, France	case–control	52 cancers / 52 controls	Stool	Metagenomic sequencing	↑ *Fusobacterium, Porphyromonas, Clostridia*
Baxter, 2016[187]	USA, Canada	case–control	120 cancers / 198 adenomas / 172 no colonic lesions	Stool	16s rRNA sequencing	↑ *Porphyromonas assaccharolytica*, *Peptostreptococcus stomatis*, *Parvimonas micra*, and *F nucleatum*; ↓Lachnospiraceae
Hale, 2017[218]	USA	case–control	233 adenomas / 547 controls	Stool	16s rRNA sequencing	↑ Bilophila, Desulfovibrio, inflammatory bacteria in the genus Mogibacterium; ↓ Veillonella, Clostridia order, and Bifidobacteriales family

Author, Year	Country	Study design	Sample Size	Biospecimen type	Sequencing method	Main findings comparing cases to controls
Yu, 2017[38]	Denmark, France, Austria	case–control	74 cancers / 54 controls	Stool	Metagenomic sequencing	↑ *Peptostreptococcus stomatis*, *F nucleatum, Parvimonas micra, Solobacterium moorei*
Liang, 2017[39]	China	case– control	203 cancers / 236 controls	Stool	16s rRNA sequencing	↑ *F nucleatum, Clostridium hathewayi;* ↓ *B clarus*
Flemer, 2018[55]	Ireland	case–control	43 cancers / 37 controls	Stool and tissue	16s rRNA sequencing	↓ *Lachnospiraceae incertae sedis* and *Coprococcus*

Note: only studies with at least 1o cases (either CRC or precursors) are included. Pooled analyses of published individual studies are not included.

Table 2.

Microbes Associated With Increased or Reduced Risk of CRC

Microbe	Epidemiologic Evidence	Potential mechanisms	References
Associated with higher risk of CRC			
Fusobacterium nucleatum	Enriched tumor tissue; higher fecal abundance in patients with colorectal neoplasia than controls; associated with advanced cancer stage, lower infiltration by T cells, higher risk of recurrence, and poorer patient survival; correlated with the molecular characteristics of the serrated pathway.	Promotion of a tumor-permissive microenvironment through recruitment of myeloidderived suppressor cells and inhibition of antitumor defense by NK or T cells; modulation of Ecadherin/β-catenin.	21, 22, 140, 219
Enterotoxigenic *Bacteroides fragilis* (ETBF)	Enriched in tumor tissue and fecal samples of CRC patients; associated with advanced cancer stage and proximal colon tumor.	DNA damage	40, 220, 221
pks+ Escherichia coli	More frequently detected in individuals with than without CRC, more frequently in tumors than in normal flanking tissue, and more frequently in late-stage tumors than in early-stage tumors.	Promotion of intestinal inflammation	49, 222
Associated with lower risk of CRC			
SCFA-producing bacteria	Lower abundance in CRC patients than bacteria controls; higher abundance in Native Americans with a low CRC incidence; associated with improved immune response and better metabolic parameters.	Metabolic and immune modulation of SCFAs that protects against CRC.	124, 128, 223