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REVIEW ARTICLE

# Gut microbiota, inflammation and colorectal cancer



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**Abstract** Although genes contribute to colorectal cancer, the gut microbiota are an important player. Accumulating evidence suggests that chronic infection and the ensuing inflammation contributes to tumor initiation and tumor progression. A variety of bacterial species and tumor-promoting virulence mechanisms have been investigated. Significant advances have been made in understanding the composition and functional capabilities of the gut microbiota and its roles in cancer. In the current review, we discuss the novel roles of microbiota in the progression of colon cancer. Although microbiota technically include organisms other than bacteria e.g., viruses and fungi, this review will primarily focus on bacteria. We summarize epidemiological studies of human microbiome and colon cancer. We discuss the progress in the scientific understanding of the interplay between the gut microbiota, barrier function, and host responses in experimental models. Further, we discuss the potential application in prevention, diagnosis, and therapy of colon cancer by targeting microbiota. We discuss the challenges lie ahead and the future direction in studying gut microbiome in colon cancer to close the gap between the basic sciences and clinical application.

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## Introduction

Colorectal cancer is the 3rd most common cancer in both males and females in the US and the 2nd leading cause of cancer deaths with the estimated new cases of nearly 133,000 and deaths of 50,000 in 2015.<sup>1</sup> Worldwide, 1,360,000 new cases and 694,000 deaths per year are estimated.<sup>2</sup> Cancer incidence in the large intestine is also known to be approximately 12-fold higher than that of the small intestine, which has been attributed to several magnitude greater bacterial density in the large intestine ( $\sim 10^{12}$  cells per ml) compared with that in the small intestine ( $\sim 10^2$  cells per ml).<sup>3</sup> With advance in metagenomic technology, growing evidence now suggests that dysbiosis, i.e., imbalance in of normal intestinal microbiota, can promote chronic inflammatory conditions and the production of carcinogenic metabolites, leading to neoplasia.<sup>4,5</sup>

Gut microbiota represents a complex ecosystem that develops in close parallel with hosts and depends on the physiological environment of hosts. Humans have coevolved with their microbes over thousands of years. The gut bacterial population stabilizes during the first years of life and then remains stable throughout our life in terms of the major populations. Human gut microbiota are dominated by four main phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. The corporate number of microbial species in human gut is estimated to be 1000–1150, with each individual harboring at least 160 (Qin, Li et al 2010). The number of genes of gut microbiota exceeds the number of genes in the human genome by 150 times. A large portion (38%) of the total gene pool is commonly shared from individual to individual. The “core human microbiome” refers to the central part of microbial gene pool existing in all or most of humans. The “variable human microbiome” is the microbial genes in a specific cohort of people, which is determined by a combination of host factors (Turnbaugh, Ley et al 2007). In the modern society, the host-microbial relationship is now being dramatically affected by shifts in the collective human microbiome resulting from changes in the environment and societal norms (Sun and Chang 2014).

In this review, we will discuss the roles of gut microbiota in colorectal cancer, summarizing both epidemiologic observations and the data from experimental animals. Although microbiota technically include organisms other than bacteria e.g., viruses and fungi, this review will primarily focus on bacteria, of which significant recent progresses have been made in understanding their role in human health. Specifically, understanding of the interplay between the gut microbiota, barrier function, and inflammatory responses will uncover new therapeutic targets in colorectal cancer. We will discuss the potential application in prevention, diagnosis, and therapy of colorectal cancer by targeting gut microbiota. Moreover, we will also discuss challenges lie ahead and the future direction in studying gut microbiome in cancer to close the gap between the basic sciences and clinical application.

## Epidemiological studies of microbiome and colorectal cancer

At least two approaches have been employed to study colorectal cancer-associated microbiome. One is the

targeted, more hypothesis-testing studies to examine whether exposure to specific bacteria species of interest increases the risk of colorectal cancer. The second type is studies aiming to identify differences in overall microbial composition by disease status. The latter has gained more popularity recently with advances in genomic technology for high throughput sequencing and discussed here first.

## Microbiome core structure, diversity, richness and colorectal cancer

Most common materials used in these types of investigation are fecal or mucosal biopsy/resection samples and have been analyzed primarily by pyrosequencing. But it is now clear that bacterial populations in feces and mucosa are distinct.<sup>6,7</sup> As summarized in Table 1, the majority of these studies have demonstrated beta diversity by principal coordinate or component analysis illustrating structural difference of gut microbiome, where samples belonging to different disease status (cancer, adenoma, or controls/normal adjacent tissue) cluster in different two dimensional spaces,<sup>7–12</sup> indicating the presence dysbiosis. Analysis of community diversity/richness indices based on 16S rRNA gene sequencing has shown significantly reduced microbial diversity in feces of colorectal cancer patients than in controls<sup>13</sup> and in cancer tissue compared with mucosa at least 10 cm apart from cancer.<sup>14</sup> On the contrary a richness index was higher in rectal mucosa of colorectal cancer patients than in that of control subjects<sup>7</sup> or in cancer tissues than paired normal tissue.<sup>11</sup> Others did not find differences in these alpha diversity indices.<sup>9,10,15,16</sup> With or without using additional quantitative PCR (qPCR), these studies have also found that specific bacterial groups were more common or less common in colorectal cancer cases than control specimens.<sup>7–16</sup> Because each study has used different taxonomic levels/classifications for the comparison, there have little consistency in changes associated with colorectal cancer. However, there were multiple studies reporting overrepresentation of *Fusobacterium* and *Porphyromonas* and underrepresentation of *Faecalibacterium* (Table 1). Yet, it should be noted that some of these studies were based on very small numbers of samples and control subjects were often not comparable with cases in terms of basic demographic factors (such as age). In summary, while these studies underscore marked differences in gut microbial membership between colorectal cancer patients and healthy controls, it is difficult to generalize characteristics of cancer associated gut microbiome.

## Individual bacterial species and colorectal cancer risk

### *Streptococcus bovis*

*Streptococcus bovis* (SB) is a gram-positive bacterium and lower-grade opportunistic pathogen that can cause systemic infections (endocarditis or bacteremia) in humans. It is a group D streptococcus with the specific ability to grow in 40 percent bile.<sup>17</sup> Intestinal mucosal lesions have been deemed to serve as a portal for these bacteria to the systemic circulation. Based on biochemical traits, DNA homology and divergence in 16S rRNA sequences, SB can be

**Table 1** Summary of 16rRNA pyrosequencing studies involving colorectal cancer (CRC) and control specimens addressing microbial community structure.

Authors (year)	Study subjects (N)	Type of specimens	16S rRNA region	Beta diversity	Alpha diversity	Overrepresentation	Underrepresentation
Sobhani et al (2011) <sup>8</sup>	CRC (60), colonoscopy control (119)	Stool	V3–V4	PCA	–	<i>Bacteroides/Prevotella</i>	
Ahn et al (2013) <sup>13</sup>	CRC (47), surgical control (94)	Stool	V3–V4	–	Shannon index down in CRC	<i>Fusobacterium, Porphyromonas</i>	<i>Clostridia</i>
Wang et al (2012) <sup>9</sup>	CRC (46), healthy volunteers (56)	Stool	V3	PCA	No difference in diversity and evenness	<i>Porphyromonas, Escherichia/Shigella, Enterococcus, Streptococcus, Peptostreptococcus</i>	<i>Bacteroides, Roseburia, Alistipes, Eubacterium, Parasutterella</i>
Wu et al (2013) <sup>10,65</sup>	CRC (19), healthy volunteers (20)	Stool	V3	PCoA	No difference in diversity and richness	<i>Bacteroides species, Fusobacterium, Campylobacter species</i>	<i>Faecalibacterium, Roseburia</i>
Weir et al (2013) <sup>15</sup>	CRC (11), healthy volunteers (10)	Stool	V4	–	No difference in diversity and richness	<i>Akkermansia muciniphila</i>	<i>Bacteroides, Prevotella, Ruminococcus</i>
Chen et al (2012) <sup>14</sup>	CRC (46), healthy volunteers (56)	Stool, rectal swab, cancer tissue, adjacent (2–5 cm and 10–20 cm apart) normal mucosa	V1–V3	–	Shannon index down in CRC tissue vs paired mucosa 10–20 cm apart	<i>Lactobacillales</i> (tumor), <i>Erysipelotrichaceae, Prevotellaceae, Coriobacteriaceae</i> (stool)	<i>Faecalibacterium</i> (tumor)
Mira-Pascual et al (2015) <sup>7</sup>	CRC (7), adenoma (11), healthy volunteer (10)	Tissue (tumor or rectal mucosa), stool	V1–V3	PCoA (tissue)	Richness up in cancer tissue	<i>Enterobacteriaceae</i> (cancer tissue)	
Geng et al (2013) <sup>11</sup>	CRC (8)	Paired tissue (cancer, normal)	V1–V2	PCoA	Richness up in cancer	<i>Roseburia</i>	<i>Microbacterium, Anoxybacillus</i>
Geng et al (2014) <sup>12</sup>	CRC (8), adenoma (10), healthy volunteer (10)	Normal and tumor tissue	V1–V2	PLS-DA	–	<i>Streptococcus, Porphyromonas, Veillonella</i> (cancer vs control)	
Kostic et al (2012) <sup>16</sup>	CRC (95)	Paired tissue (cancer, normal)	V3–V5	–	No difference in richness	<i>Fusobacterium</i>	<i>Bacteroides, Clostridia, Faecalibacterium</i>

PCA: Principal component analysis; PCoA: Principal coordinate analysis; PLS-DA; Partial least square discriminant analysis.

grouped into *Streptococcus gallolyticus* (SB biotype I and II/2) and *Streptococcus infantarius* (biotype II/1). Earlier studies suggest stronger association of *S. gallolyticus* with colorectal tumors<sup>18</sup> in contrast to stronger link of *S. infantarius* to non-colonic cancers, primarily in the pancreas and biliary tract.<sup>19</sup>

Although SB is a member of normal gastrointestinal flora in ruminants, e.g., cattle, sheep, horses, pigs, camels and deers, it is also found in human feces as well as gastric biopsy materials.<sup>20,21</sup> Approximately 10% of healthy individuals have been estimated to carry this bacterium asymptotically in their digestive tract.<sup>20</sup> While fecal–oral or oral–oral is a possible transmission route between humans, it may be acquired through dietary intake of ruminant-derived foods, such as unpasteurized dairy products,<sup>22</sup> red meat and animal organs.<sup>20</sup> In fact SB is a frequently detected contaminant in commercially available meat.<sup>23,24</sup> The correlation between SB and colonic disease has long been recognized. Besides case-reports for the patients who were diagnosed with asymptomatic colorectal neoplasia simultaneously with SB endocarditis or bacteremia,<sup>25–30</sup> investigators have reported increased prevalence of colorectal tumors (cancer and polyps) among patients diagnosed with SB endocarditis or bacteremia. The prevalence of colorectal tumors ranges from 10 to 60%,<sup>18,31–45</sup> although these are based on diverse study populations in terms of patient demographics and colorectal surveillance methods. These variations may also be due to the heterogeneous definition of the cases, as adenomas have been defined as diseased in some studies but not in the others.<sup>46</sup> A more recent study found that 52% of SB bacteremia patients had advanced adenoma/cancer, which was approximately 2.5 fold more frequent than colonoscopy controls.<sup>47</sup> Similar prevalence (60%) of advanced adenoma/cancer was reported in SB endocarditis patients by Sharara et al.<sup>48</sup>

The second set of evidence is derived from studies comparing SB prevalence among various patient groups with or without colonic diseases.<sup>49–56</sup> While 3 small studies including 13–46 controls and corresponding 11 colorectal cancer, 47 pediatric inflammatory bowel disease (IBD) and 56 polyp patients failed to show any association,<sup>52–54</sup> five other studies found that SB carriage (either in stool or antibodies) rates were significantly higher in cancer patients than in controls. Interestingly, 3 studies also showed that patients with premalignant lesions (IBD or polyps) had intermediate SB carriage rate between cancer cases and controls. In addition, stronger associations observed in studies by Darjee & Gibb, Tjalsma et al and Abdulmir et al<sup>51,55,56</sup> suggest that antibody assays may be a more powerful tool than fecal culture in assessing the associations between this bacterial infection and colorectal disease. Subsequent enzyme-linked immunosorbent assay (ELISA) based studies have demonstrated that seropositivity or higher antibody titer to specific SB antigens or their combinations was associated with early stage of colorectal cancer<sup>57,58</sup> or colorectal cancer diagnosed at younger age (<65 years),<sup>59</sup> yielding odds ratios of 1.5–8.0. In summary, despite these observations it remains elusive whether colorectal neoplastic sites provide a specific niche for SB resulting in sustained colonization and survival or whether SB infection itself promotes colorectal carcinogenesis, or a combination of both.

### *Helicobacter pylori*

*H. pylori* was designated as a group 1 human carcinogen by International Agency for Research on Cancer (IARC) in 1994 because an expert panel concluded that there was sufficient evidence in humans for the carcinogenicity of this bacterial infection and that its chronic infection causes non-cardia gastric adenocarcinoma and low-grade B-cell mucosa-associated lymphoid tissue lymphoma.<sup>60</sup> *H. pylori* is a gastric pathogen that infect more than a half of the adult population in the world.<sup>61</sup> Gastric carcinogenic pathway caused by *H. pylori* has been well documented, arising from stages of premalignant lesions, i.e., chronic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia, and then progressing to adenocarcinoma.<sup>62–64</sup> While the gold standard for diagnosis of active *Helicobacter* infection is histological detection in gastric biopsies, stool antigen tests have been clinically accepted as a non-invasive alternative, indicating *H. pylori* also resides in the large intestine. Although no *Helicobacter* induced intestinal pathologies have been established, a number of epidemiologic studies have been conducted to examine if HP infection increases the risk of colorectal cancer. A recent meta-analysis by Wu et al<sup>65</sup> summarized the data for 3488 colorectal cancer cases, 3792 colorectal adenoma and 10,598 and 4348 corresponding controls from 27 studies. They reported significantly increased summary odds ratios for both, 1.39 (1.18–1.64) for cancer and 1.66 (1.39–1.97) for adenoma. However, two prospective studies<sup>66,67</sup> with the nested case–control design did not find any indication of increased risk, while all others were either cross-sectional or retrospective case–control studies. It is noteworthy that except one study by Jones et al,<sup>68</sup> there was no histological confirmation of presence of *H. pylori* in colorectal mucosa as others used gastric histology, serology or breath test to assess *H. pylori* infection. Nevertheless, significantly increased risks of cancer and polyps were observed by Jones et al,<sup>68</sup> as well as in an additional study among children with hamartomous (juvenile) colorectal polyps,<sup>69</sup> respectively. Despite relatively consistent epidemiologic observations to date, there seems insufficient evidence to support causality of the events. Certain biases may be involved, such as publication bias as reported<sup>65</sup> as well as surveillance bias particularly for adenoma. In addition, there may be indirect consequences from gastric pathology, such as hypergastrinemia, which is common in patients with *Helicobacter* infection and has been hypothesized to stimulate colorectal tumor growth.<sup>70</sup>

### *Escherichia coli*

*Escherichia (E) coli* strains are aero-anaerobic Gram-negative bacteria in the normal intestinal flora. As a commensal, *E. coli* coexist harmoniously with their mammalian host, promote normal intestinal homeostasis and rarely cause disease. However, some virulent *E. coli* that have acquired pathogenicity islands can colonize the human gastrointestinal tract and induce disease.<sup>71</sup> Mucosa-associated *E. coli* have been identified more frequently in colon tissue from patients with adenocarcinomas than in controls.<sup>72–74</sup> Some *E. coli* strains harbor a ~54-kb polyketide synthases (pks) pathogenicity island that encodes multi-enzymatic machinery for synthesizing a peptide-polyketide hybrid genotoxin named Colibactin.<sup>75</sup> Carriage

of *E. coli* positive to the pks island or genes in the island has been recently found more common in the mucosa of colorectal cancer and IBD patients than that of control subjects.<sup>71,75,76</sup> Epithelial proliferation and *E. coli* colonization density were significantly correlated in the mucosa distant from cancer<sup>76</sup> and pks positive cancer specimens showed higher levels of DNA damage than its negative counterparts,<sup>77</sup> supporting potential causal link.

### ***Bacteroides fragilis***

The anaerobe *B. fragilis* is a colonic symbiote with an affinity for mucosal colonization that comprises a relatively small proportion of fecal microbiota (approximately 0.5%–1%). There are 2 molecular subtypes, nontoxigenic *B. fragilis* (NTBF) and enterotoxigenic *B. fragilis* (ETBF) and ETBF is now established as a cause of diarrheal disease.<sup>78</sup> ETBF pathogenicity is due to the *B. fragilis* toxin (BFT), a 20 kDa zinc-dependent metalloprotease toxin with 3 isotypes (BFT-1, BFT-2, and BFT-3) and the bft gene is unique, only identified in *B. fragilis*.<sup>78</sup> BFT binds to a specific colonic epithelial receptor activating Wnt and NF- $\kappa$ B signaling pathways with increased cell proliferation, epithelial release of proinflammatory mediators, and induction of DNA damage<sup>78,79</sup> and ETBF promotes tumor formation in experimental animals.<sup>79,80</sup> Despite these laboratory data, to date only limited data in humans support an association of ETBF with colorectal cancer. Ulger Toprak et al<sup>81</sup> reported that 38% of fecal samples from cancer patients were positive to bft gene while only 12% of those from control patients were positive ( $P = 0.009$ ). Boleij et al<sup>82</sup> recently revealed more frequent detection (~75%) of bft genes in colonoscopic biopsies, particularly among patients with no antibiotic pretreatment and the prevalence was significantly higher in cancer than controls.

### ***Fusobacterium (F) nucleatum***

*F. nucleatum* is a Gram negative, non-spore forming, obligate anaerobic of the *Fusobacteriaceae* family, which consists of 9 genera, including *Fusobacterium* and *Leptotrichia*. *Fusobacterium* genus includes at least 14 species, several of which (including *F. nucleatum*) are known pathogens.<sup>83</sup> *F. nucleatum* is perhaps best appreciated for its role as a component of oral plaque, where by virtue of its adhesive abilities it serves as a bridge organism between early and late colonizers of this biofilm and consequently is implicated in various forms of periodontal diseases.<sup>84</sup>

Until recently, *F. nucleatum* was thought to primarily be a component of the oral microbiota of humans and only an occasional resident of the gut. However, this premise was built on culture-based examination of stool, which usually does not contain high numbers of live, epithelium-associated bacteria. Using metagenomic approaches recently, growing number of studies have reported an over-representation of sequences from *F. nucleatum*<sup>16,85,86</sup> or genus *F*<sup>5,87,88</sup> in tumors relative to control specimens. Two of these by Castellarin<sup>86</sup> and by Warren<sup>87</sup> were based on RNA, representing transcribing bacteria. These observations were further confirmed by quantitative (q) PCR and in situ hybridization in tumor tissue.<sup>16,86,89</sup> Using qPCR, McCoy et al studied F counts in normal rectal mucosa of the cases with or without colorectal adenoma, revealing a 3 fold

increase in risk of adenoma among subjects with highest tertile of F counts.<sup>90</sup> Ito et al<sup>91</sup> also demonstrated that *F. nucleatum* detection in formalin-fixed paraffin-embedded tissue by qPCR progressively increased with malignant grades of the lesions from hyperplastic polyps to colorectal cancer. Several others found higher fecal carriage of genus *F*<sup>13,85,92</sup> or *Fusobacteriaceae* family<sup>10</sup> in colorectal cancer patients than in control subjects, pointing to a potential tool for colorectal cancer screening.

All *F. nucleatum* strains may not equal in their virulence potential. *F. nucleatum* is naturally co-aggregative and would likely exist in the human gut microbiota as a feature of a larger microbial grouping. The ability to adhere to other bacterial species could also enable gene transfer and thus some *F. nucleatum* strains may acquire genes through horizontal transfer leading to increased virulence,<sup>93</sup> which suggests that the involvement of *F. nucleatum* in disease may not be just a function of a direct result of its own virulence. Despite these accumulated evidences, however, whether this association is indeed involved in colorectal carcinogenesis, or simply the result of *F. nucleatum* exploitation of an ecological niche created as a result of the cancer/tumor microenvironment, remains to be tested in further studies.

### ***Salmonella enterica***

*S. enterica* is a Gram-negative, facultative anaerobe and an intracellular pathogen to both humans and animals, posing a major public health concern worldwide. Non-typhoidal *Salmonella* is a major foodborne pathogen, with an estimated 93.8 million cases and about 155,000 deaths globally per year.<sup>94a</sup> Common sources of infection include contaminated food, such as meat, eggs and produce.<sup>94b</sup> Outcomes of this bacterial infection vary widely, ranging from mild self-limiting gastroenteritis to the severe systemic infection that can be fatal. Some of these acute infections result in a chronic carrier state excreting the bacteria in stool and urine without symptoms, which represents another transmission mechanism of this bacterium to other humans. Salmonellosis has also been implicated in the development of various chronic sequelae, including reactive arthritis, irritable bowel syndrome, IBD<sup>95</sup> and even cancer.<sup>96</sup>

Two studies from Scandinavian countries have found that the probability of new IBD diagnosis significantly (2–3 fold) increases compared with general population following an episode of non-typhoid salmonella infection, particularly within the first 10 years.<sup>97,98</sup> Although data directly linking to colorectal cancer are still limited, *Salmonella typhi* carries status is well recognized to increase the risk of gallbladder cancer. A meta-analysis by Nagaraja et al demonstrated the summary odds ratio of 3–4<sup>96</sup> regardless of salmonella detection methods. Furthermore, Kato et al<sup>99</sup> recently reported that antibody against *Salmonella* flagellin was higher in colorectal cancer and pre-cancer cases than controls in two distinct populations in US and the Netherlands and that dietary intake is the one of the mediating factors, supporting a possible link of *Salmonella* to colorectal cancer.

### **Other miscellaneous**

Several other species of bacteria have received research interest because their bacterial metabolites have potential



detrimental effects against colorectal mucosa or may exert potentially beneficial or protective effect towards epithelial cells. These include *Desulfovibrio*, *Enterococcus faecalis* due to hydrogen sulfide and superoxide respectively,<sup>100</sup> *Faecalibacterium prausnitzii* and Bifidobacteria due to butyrate and lactate, respectively.<sup>101,102</sup> The presence or density/quantity of these bacteria in feces or mucosa has been primarily studied by qPCR. However, there have been only sporadic studies reporting a significant association with colorectal cancer itself,<sup>101,102</sup> while others found higher prevalence or density of these bacteria in IBD than in controls, which was further correlated with disease activity.<sup>4,103,104</sup> In addition to *F. nucleatum*, *Porphyromonas gingivalis*, another oral pathogen more tightly associated with periodontal disease has been linked to digestive tract cancer in a seroepidemiologic study. However, the study was too small to separate colorectal cancer from other cancers.<sup>105</sup> The potential association of this bacterium with colorectal cancer may be further corroborated by several other metagenomic studies that observed the overrepresentation of genus *Porphyromonas* or *Porphyromonadaceae* family in colorectal cancer specimens than control specimens.<sup>10,13,92</sup> Overall, the information available thus far for these bacteria is insufficient to address their etiological involvement in colorectal cancer.

### Interactions between colorectal cancer risk factors and gut microbiome

As discussed above, growing evidence now point to differential gut microbial compositions or differential prevalence of specific bacteria in colorectal cancer patients in comparison with control subjects. However, there are also abundant data supporting the associations between gut microbiota and several established risk factors for colorectal cancer. Thus, one should consider a possibility that observed difference in microbiota mirror at least in part changes associated with such risk factors. The best example is obesity. Obese and lean individuals are known to harbor different types of gut microbiota.<sup>106</sup> While low energy diet induces change in microbial compositions increasing gene richness,<sup>107</sup> microbiome itself also contributes energy harvest to the host, as demonstrated in mice models where transfer of obese microbiome to lean animals led to an increase in body adiposity in a diet dependent manner.<sup>108,109</sup> Other dietary risk factors for colorectal cancer include low fiber and high red meat intake.<sup>110</sup> Dietary fiber and resistant starch are well known to stimulate gut bacterial fermentation to generate short chain fatty acids (SCFA) as well as lactate and to increase relative abundance of bacterial groups with the relevant metabolic activities.<sup>111</sup> Although meat intake itself has been rarely studied, removal of animal products (vegan diet) was recently tested in a few clinical trials, showing changes in the Firmicutes/Bacteroidetes ratio and abundance of bacteria capable of triggering inflammation.<sup>112,113</sup> Moreover, as discussed above, meats are one of the suspected sources of acquisition of specific pathogens, e.g., *S. Bovis* ad *Salmonella enterica*. There has been relatively sparse information as to the associations between other risk factors, physical activity, smoking and alcohol, and gut microbiome.

A study from Ireland found that athletes had significantly higher microbial diversity than controls.<sup>114</sup> Alcoholics have been reported to carry greater abundance of Proteobacteria or its family *Enterobacteriaceae*<sup>115,116</sup> than control subjects. Smoking cessation led to changes in gut microbial composition, increasing some Firmicutes and decreasing some Bacteroidetes and Proteobacteria,<sup>117,118</sup> while Kato et al demonstrated a positive association between smoking and *Desulfovibrio* abundance.<sup>119</sup> Since these risks factors are postulated to be involved in multiple mechanistic pathways, contribution of microbial changes to colorectal carcinogenesis remains to be determined.

### Cautions in the interpretation of epidemiologic data

Despite the presence of certain biological mechanisms possibly contributing to colorectal carcinogenesis (discussed in later sections), the causal association cannot be inferred only from the data from retrospective or cross-sectional human studies. Except a few for *H. pylori*<sup>66,67</sup> and *P. gingivalis*,<sup>105</sup> all other studies identified the exposure, i.e., the presence bacteria or their antibodies to bacteria, was assessed at or after diagnosis of the disease. This makes it difficult to establish the temporal sequence of the events, which came first, bacteria or cancer. Moreover, the presence of the organism may no longer necessary once carcinogenic pathways are activated by infection as seen in the case of HP and gastric cancer. Serum antibody assays can capture past and current infection and have played a vital role in establishing infectious etiology of several types of cancer, including *H. pylori* and hepatitis viruses,<sup>120</sup> especially with use of prediagnostic blood samples from prospective cohorts. Thus, development of reliable serological assays is likely to greatly advance epidemiologic studies. However, due to the limitation of serology as well as fecal analyses, i.e., an inability to identify the location of colonization for the bacteria that can colonize at diverse anatomical sites, histological detection of bacteria in cancer and surrounding tissues would also be required to reinforce their causal involvement.

### Mechanisms for microbially induced/promoted colorectal cancer

A systemic review summarizes the original articles studying microbiota and colorectal cancer until November 2014. It showed that some bacteria are consistently augmented (such as Fusobacteria, Alistipes, Porphyromonadaceae, Coriobacteridae, Staphylococcaceae, Akkermansia spp. and Methanobacteriales), while other are constantly diminished in colorectal cancer (such as Bifidobacterium, Lactobacillus, Ruminococcus, Faecalibacterium spp., Roseburia, and Treponema). It is clear that bacteria metabolites amino acids are increased and butyrate is decreased throughout colonic carcinogenesis.<sup>121</sup>

Identification of components of the microbiota and elucidation of the molecular mechanisms of their action in inducing pathological changes or exerting beneficial activities could aid in our ability to influence the composition of the microbiota and to find bacterial strains and components

(e.g., probiotics and prebiotics) whose administration may aid in disease prevention and treatment.<sup>122</sup>

### Experimental animal models to study microbiome in colon cancer

To study the microbiome in colon cancer, researchers have developed various Experimental animal models: gnotobiological model, antibiotic treatment, inflammatory model with increased risk of colon cancer, inoculation of specific bacteria or products in genetic engineering mice.

Gnotobiological model is an indispensable tool for studying the consequences of bacterial colonization. Animals (such as zebrafish, mouse, rat, pig) can be maintained in sterile conditions and colonized with defined microbes. The effects of the germ-free state or the effects of colonization on disease initiation and maintenance can be observed in these experimental models for disease initiation and progression. Using this approach, researchers have demonstrated direct involvement of components of the microbiota (including non-cultivable commensal bacteria) in chronic intestinal inflammation, development of colonic neoplasia, and other diseases.

A variety of bacterial species and tumor-promoting virulence mechanisms have been investigated, using mouse models. There involve bacterial metabolic products, Pathogenic bacterial toxins/virulence factors, and Immune reaction/modulation.

### Bacterial metabolic products

*Firmicutes* and *Bacteroidetes* predominate the gut microbiota, followed by *Proteobacteria* and *Actinobacteria*, with minor contributors including *Verrucomicrobia* and *Fusobacteria*.<sup>123</sup> *Bacteroides* and *Ruminococcus* are consistent with enriched intake of animal sources, while a plant-based diet favors *Prevotella*.<sup>124</sup> *Prevotella* to *Bacteroides* ratio constitutes an important index for clinical diagnosis. Butyrate-producing bacteria, including *Clostridium* groups IV (*Faecalibacterium prausnitzii*) and XIVa, *Roseburia* spp., *Butyrivibrio*, and lactic acid bacteria (LAB), mainly *Lactobacillus* and *Bifidobacterium*, are believed to benefit the host through anti-inflammation, anti-tumorigenesis, and pathogen exclusion.<sup>125–127</sup> There is also a metabolic interplay between LAB and butyrate-producing bacteria due to the ability of the latter to feed on lactate.<sup>128</sup>

It is known that gut microbiota could produce an enormous quantity of molecules interacting with the host. The beneficial effects of gut microbiota on the host are mainly mediated by its metabolites. Short-chain fatty acid (SCFA), including acetate, propionate, and butyrate, are the major end-products of gut bacteria fermentation of dietary fiber. SCFAs, particularly butyrate, are the preferred source of energy for colonic epithelial cells. SCFA promotes and maintains colonic epithelial health through maintaining barrier function,<sup>129</sup> suppressing colonic cancer,<sup>130–132</sup> inhibiting intestinal inflammation (Wu et al 2014), modulating immune response,<sup>133</sup> regulating DNA methylation for proliferation,<sup>132</sup> and diminishing oxidative DNA damage.<sup>134</sup>

The balance between two phyla (*Firmicutes* and *Bacteroidetes*) appears to be critical to regulating disease

progression. Some bacterial species have been implicated in the development of colorectal carcinoma. Using culture methods, Moore and Moore observed that the abundance of *Bacteroides* and bifidobacteria was associated with increased risk of colon polyps, whereas *Lactobacillus* and *Eubacterium aerofaciens* were protective.<sup>135</sup> An association between the abundance of *Fusobacterium*, *E. coli*, hydrogen sulfide (H<sub>2</sub>S)-, and bile salt-producing bacteria was associated with increased risk of colon cancer.<sup>5,136</sup> Cancer is associated with reduced abundances of *Clostridium*, *Roseburia*, *Eubacteria* spp., and other butyrate-producing bacteria in fecal samples of adenoma subjects compared with healthy controls. Zeller et al<sup>85</sup> reported that a relative abundances of 22 gut microbial species, such as *Fusobacterium* collectively associated with CRC. This is the first paper based on the whole sequence of bacterial genes, not 16S. It also compared the bacterial markers with the results of the standard Hemocult FOBT routinely applied for CRC screening and an experimental CRC screening test based on methylation of the wif-1 gene, a Wnt pathway member. The authors believe that there is a potential to use fecal microbiota markers for early-stage detection of colorectal cancer.

### Pathogenic bacterial toxins/virulence factors

*Salmonella* infection in humans can become chronic which leads to low grade persistent inflammation.<sup>137</sup> These chronic infections increase the risk of several gastrointestinal<sup>138</sup> diseases, including chronic cholecystitis and gallbladder cancer.<sup>139,140</sup> Recently, Kato et al reported that antibody against *Salmonella* flagellin was higher in colorectal cancer and pre-cancer cases than controls in two distinct populations in US and the Netherlands and that dietary intake is the one of the mediating factors, suggesting a potential link of *Salmonella* to colorectal cancer.<sup>99</sup> In animal models, *Salmonella* and its derivatives have been observed invading transformed tissue more efficiently than normal tissue.<sup>141,142</sup> *Salmonella* AvrA is a multifunctional protein that influences eukaryotic cell pathways by altering ubiquitination and acetylation of target proteins.<sup>143–149</sup> We reported that AvrA acts as a deubiquitinase to stabilize  $\beta$ -catenin. By suppressing  $\beta$ -catenin degradation, AvrA enhances intestinal epithelial proliferation, thus promoting tumorigenesis.<sup>150</sup> We reported that AvrA-enhanced tumor multiplicity and tumor progression. Our studies could suggest biomarkers (such as AvrA level in gut) to assess cancer risk in susceptible individuals and infection-related dysregulation of  $\beta$ -catenin signaling in colon cancer. Another novel finding in our study was that the pathogenicity factor altered tumor distribution. Uninfected mice treated with AOM/DSS developed tumors in the distal colon.<sup>150</sup> In contrast, in mice infected with AvrA-expressing bacteria, tumors were found more in the proximal colon. AvrA alters the colonic milieu so as to enhance tumorigenesis in the right colon. Compared with the left colon, the cecum has a greater bacterial load and increased bacterial fermentation that we speculate contributes to this rightward shift in tumors. Increasing incidence in right-sided tumors has also been reported in the Western world. While increased endoscopic screening that probably clears

distal colonic lesions more effectively than proximal colonic lesions, based on our studies, this shift might also reflect changes in the microbiome. However, it remains unclear how many human CRC cases can be attributed to bacterial agents, how these exactly interact with the human host or the microbial community in the gut.

Gut microbiota metabolism could be linked with polyp formation, using mice genetic model.<sup>151</sup> A diet reduced in carbohydrates resulted in reduced polyp formation in APCMin/+ MSH2-/- mice. Butyrate, a bacterial product, induced aberrant proliferation and transformation of colon epithelial cells. Treatment with either antibiotics or a low-carbohydrate diet reduced cell proliferation as well as the number of tumors in the small intestines and colons. However as mice microbial ecology is different, compared to human, authors did not find *Fusobacterium*, which was shown to be link to CRC in humans.

A paper from *Journal of Experimental Medicine*<sup>152</sup> reported that antibiotics prevented polyp formation. Most of the tumor-dwelling bacteria belonged to the Clostridiales family and an upregulation of inflammatory molecules near the polyps. FMT from the untreated mice to the once germ-free mice, the previous germ-free mice developed polyps. If transplanted early embryos of the transgenic mice into females of another, cancer-free mouse strain. Inoculated at birth with the bacteria of their surrogate mothers, these transplanted mice did not develop tumors until 25 weeks, whereas the genetically identical controls had tumors by 12 weeks. This showed that small changes in the gut microbiota could have a large influence on tumor growth. This study indicates that the same genetic mutation in different individuals may have a different outcome.<sup>152</sup>

One environmental factor – a diet low in fiber – may impact the intestinal microbiota in a way that affects host cell physiology, cellular homeostasis, energy regulation, and/or metabolism of xenobiotics. This in turn may lead to chronic inflammation and CRC. Cancer is associated with reduced abundances of some butyrate-producing species. Transplanting feces from mice with CRC into germ-free mice leads to increased tumorigenesis.<sup>153</sup>

While emerging evidence suggests a link between the gut microbiota and colon cancer, it is hard to say that certain bacteria strain(s) play a causal role in CRC. Evidence is still needed to determine whether those bacteria enhance the development of the disease or might even play a causal role.

Cancer is fueled by deregulation of signaling pathways in control of cellular growth and proliferation. These pathways are also targeted by infectious pathogens en route to establishing infection. It is established that a single infectious agent, namely *H. pylori*, hepatitis B virus, plays a causal role in human gastric and hepatic cancers, respectively. The exact roles and mechanisms of microbes on the development of colon cancer in are still unknown and of great interests.

### Immune reaction/modulation

Although genes contribute to colorectal cancer (CRC), the gut microbiota are an important player. Accumulating evidence suggests that chronic infection and the ensuing

inflammation contributes to tumor initiation and tumor progression.<sup>137,154</sup> A variety of bacterial species and tumor-promoting virulence mechanisms have been investigated, using mouse models. A recent study in mice showed that adenomas cause barrier defects in the colonic epithelium allowing microbial products to drive IL-23/IL-17-mediated tumor growth.<sup>155</sup> Another study demonstrated that a human colonic commensal bacterium promoted tumorigenesis via activation of T helper type 17 T cell responses.<sup>80</sup>

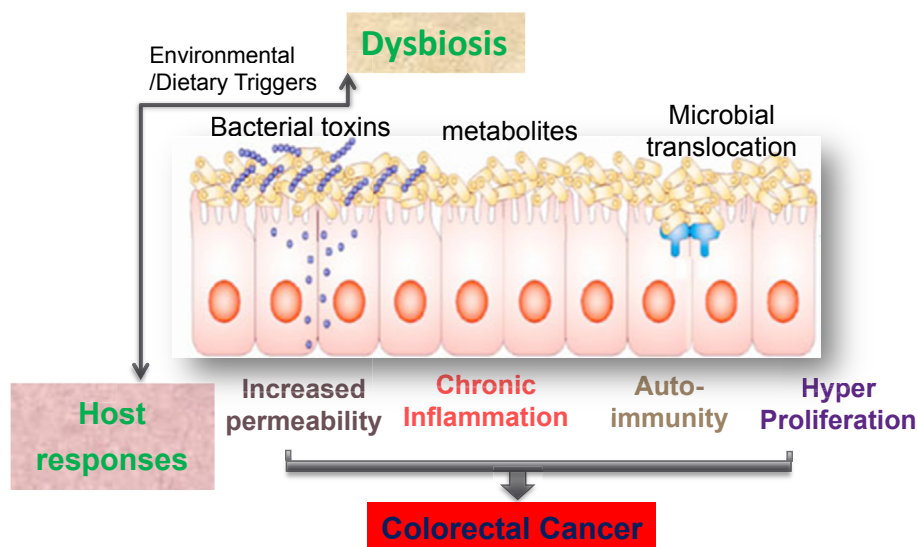
Colitis was shown to promote tumorigenesis by altering microbial composition and inducing the expansion of microorganisms with genotoxic capabilities.<sup>75</sup> Arthur et al reported the intestinal microbiota as a target of inflammation that affects the progression of CRC. Monocolonization with the commensal *E. coli* NC101 promoted invasive carcinoma in azoxymethane (AOM)-treated IL10-/- mice. Specifically, deletion of the polypeptide synthase genotoxin from *E. coli* NC101 decreased tumor load and tumor invasion in AOM treated IL10 knockout mice. *E. coli* NC101 mutant without the polyketide synthase (pks) genotoxic island decreased tumor multiplicity and invasion in AOM/IL10-/- mice. Mucosa-associated pks+ *E. coli* were found in a significantly high percentage of inflammatory bowel disease and CRC patients. These studies have highlighted the essential roles of bacteria and/or their products in colonic tumorigenesis.

SCFA is known to modulate immune responses in intestine.<sup>133</sup> Another bacterial product Peptidoglycan (PTGN) modulates peripheral immune function via a pattern-recognition receptor, oligomerization domain-containing protein-1 (NOD1) and depletion of the microbiota in mice.<sup>133</sup> Lower systemic PTGN concentration leads to less ability to kill certain bacterial pathogens. Polysaccharide A, produced by a commensal bacteria, increases local interleukin 10 by inducing Foxp3+ regulatory T-cell and this effect is mediated by Toll like receptor 2 signaling.<sup>156,157</sup> Although recent studies provide insights into the roles of the bacterial products, the molecular mechanisms of the beneficial effects are not fully elucidated yet.

Analysis of the functions that significantly differed between healthy participants and cancer patients revealed a global metabolic shift from predominant utilization of dietary fiber in the tumor-free colon to more host-derived energy sources in CRC.<sup>85</sup> They hypothesize that an increased degradation of host glycans might be related to the etiology of CRC. In healthy gut metagenomes, exclusively some fiber-degrading enzymes and fiber-binding domains are enriched, whereas in CRC metagenomes, the microbiota appears to exploit growth substrates derived from host cells to a much larger extent.<sup>85</sup>

In summary, the general mechanisms for bacteria – associated (or induced) GI tumorigenesis are through enhancing toxic bacterial products, decreasing beneficial bacterial metabolites, disrupted tissue barriers. Abnormal immunity, chronic inflammation, and hyperproliferation also contribute to the progression of cancer (Fig. 1). Microbial pathogens and intestinal inflammation can compromise intestinal barrier function and result in increased gut permeability, translocation of various microbial substances, and immune activation.<sup>158a</sup> Dysbiosis further enhances barrier failure and inflammation. The host factor, such as genetic defect, could enhance the dysbiosis





**Fig. 1** Working models of general mechanisms for bacteria – associated (or induced) colon cancer. Through enhancing toxic bacterial products, decreasing beneficial bacterial metabolites, disrupted tissue barriers, translocation of microbes, dysbiosis leads to abnormal immune activation, chronic inflammation, and hyperproliferation that contribute to the colorectal cancer. The host factor, such as genetic defect, could enhance the dysbiosis along with the environment trigger and change of dietary.

along with the environment trigger and change of dietary (Fig. 1). One unanswered question is how microbes affect the intestinal epithelium: Do the bacteria make it more permeable or just capitalize on its pre-existing weak spots?

### Target gut microbiota in prevention diagnosis, and therapy of GI cancers

Based on current understandings of the roles of microbiota in GI cancer, targeting the gut microbiota is a promising avenue in order to prevent cancer or at least stop the increase of cancerous cells. O’Keefe et al<sup>158b</sup> investigated the role of fat and fiber in this association by conducting 2-week-long food changes in volunteers from both populations: African-Americans received an African-style diet high in fiber and low in fat, while rural Africans received a high-fat, low-fiber ‘Western’ diet. They found the food changes led to remarkable reciprocal changes in mucosal biomarkers of cancer risk. The dietary switch also changed the microbiota and metabolism in ways known to affect cancer risk.<sup>158b</sup> This study suggests the potential of dietary intervention or use of prebiotics in colorectal cancer prevention.

Insights into microbiome and cancer risk also provide the opportunities to use of fecal microbial detection for mass screening and diagnosis. By comparing the fecal CRC data to those of IBD patients the researchers could confirm that the microbial characteristics found in the feces were really specific to CRC and not just indicative of inflammatory intestinal conditions in general. The use of fecal microbial CRC detection for mass screening will depend on the development of procedures that are more cost-effective than the ones we used for research purposes.<sup>85</sup>

The idea of using bacteria as a potent cancer fighting therapy traces its roots back to the early nineteenth

century, when French researchers first noticed that bacterial infections in people with cancer often led to shrinkage of their tumors. Increasing evidence has demonstrated that targeting microbiome can improve therapy effects of anti-cancer drugs. Wallace et al reported that inhibiting an enzyme beta-glucuronidase produced by gut microbiota can improve cancer therapy by preventing the intestinal metabolism of the anticancer drug irinotecan.<sup>159</sup> More studies have also shown that gut microbes make three anticancer therapies most effective.<sup>160a</sup> Melanoma growth in mice harboring distinct commensal microbiota and observed differences in spontaneous antitumor immunity, which were eliminated upon cohousing or following fecal transfer. Bifidobacterium is identified to be associated with the antitumor effects. Oral administration of Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy (checkpoint blockade).<sup>160b</sup> This study also indicates the importance of gut microbiota in other cancers beyond the GI cancer. Although different bacterial groups are implicated in enhancing cancer therapy, the same endpoint through different drugs and different bugs further indicate the novel role of gut microbiome in health and diseases.

Cachexia is a multifactorial condition characterized by systemic inflammation and severe wasting of skeletal muscle, with or without wasting of adipose tissue that causes considerable morbidity and mortality in cancer patients. Infections and inflammation can lead to cachexia and wasting of skeletal muscle and fat tissue by as yet poorly understood mechanisms. Gut colonization by a strain of *E. coli* prevents wasting triggered by infections or physical damage to the intestine.<sup>161</sup> During intestinal infection with *Salmonella Typhimurium* or pneumonic infection with *Burkholderia thailandensis*, the presence of this *E. coli* did not alter changes in host metabolism, caloric uptake, or inflammation but instead sustained signaling of

the insulin-like growth factor 1/phosphatidylinositol 3-kinase/AKT pathway in skeletal muscle, which is required for prevention of muscle wasting. This effect was dependent on engagement of the NLR4 inflammasome.<sup>161</sup> Therefore, commensal bacteria in gut promote tolerance to diverse diseases.

Compromised gut barrier function because of dysbiosis or intestinal inflammation can lead to translocation of microbial substances and the development of systemic inflammation with potential consequences for patients prone to cachexia. A recent study showed that non digestible oligosaccharides modulate the gut microbiota may constitute a new nutritional strategy to modulate gut microbiota with positive consequences on cancer progression and associated cachexia.<sup>162</sup> Research is needed to clarify the role of gut microbiota and systemic inflammation in the cause of cancer cachexia. Efforts to preserve the integrity of the gut epithelial barrier and/or limit intestinal inflammation in cancer patients may help avoid the serious metabolic alterations associated with cachexia. Multimodal treatment strategies that include interventions aimed at maintaining gut barrier function and correcting dysbiosis may be used to in controlling cachexia.

Microbiota-based cancer prevention, diagnosis, and therapy are beginning to emerge as researchers learn to 'decode' the meaning of human microbiota composition at different stages in cancer.

## Conclusion and future direction

Growing evidence suggests that human microbiota play novel roles in the progression of colon cancer. The advance of current experimental models and methods allow us to obtain the scientific understanding of the interplay between the gut microbiota, barrier function, and host responses. These insights will leads to uncover new therapeutic targets in cancer. Despite these gains, many challenges lie ahead that make it difficult to close the gap between the basic sciences and clinical application.

We believe the following steps are needed in order to move the current microbiota research into clinical practice. First, we need focus on gaining mechanistic insights. Microbiota functions will be important to be considered. We already generated huge information from microbiota analyses. Based on the genomic analyses, we need analyze the microbiota of individuals. Second, we need simple and low-cost tools to identify key bacteria in patients with colon cancer. For GI patients who will undergo therapy – surgery, chemotherapy – we should follow-up of these bacteria and try to understand why some of those will have very good response to therapy and some others will not. Last, identification of components of the microbiota and elucidation of the mechanisms of their action in inducing pathological changes or exerting beneficial, disease-protective activities could aid in our ability to influence the composition of the microbiota. Understanding gut microbiota in cancer will open a door for the prevention, diagnosis and therapy.

## Conflicts of interest

There are no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jendis.2016.03.004>.

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