



Research
Microecology—Review

The Composition of Colonic Commensal Bacteria According to Anatomical Localization in Colorectal Cancer

Liuyang Zhao^a, Xiang Zhang^a, Tao Zuo^a, Jun Yu^{a,b,*}

^a Institute of Digestive Disease, Department of Medicine and Therapeutics, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China

^b Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen 518057, China

ARTICLE INFO

Article history:

Received 23 November 2016

Revised 20 January 2017

Accepted 22 January 2017

Available online 21 February 2017

Keywords:

Microbiota

Colorectal cancer

Luminal commensal bacteria

Translocation

Biomarker

ABSTRACT

Colorectal cancer (CRC) is a multistage disease resulting from complex factors, including genetic mutations, epigenetic changes, chronic inflammation, diet, and lifestyle. Recent accumulating evidence suggests that the gut microbiota is a new and important player in the development of CRC. Imbalance of the gut microbiota, especially dysregulated gut bacteria, contributes to colon cancer through mechanisms of inflammation, host defense modulations, oxidative stress, and alterations in bacterial-derived metabolism. Gut commensal bacteria are anatomically defined as four populations: luminal commensal bacteria, mucus-resident bacteria, epithelium-resident bacteria, and lymphoid tissue-resident commensal bacteria. The bacterial flora that are harbored in the gastrointestinal (GI) tract vary both longitudinally and cross-sectionally by different anatomical localization. It is notable that the translocation of colonic commensal bacteria is closely related to CRC progression. CRC-associated bacteria can serve as a non-invasive and accurate biomarker for CRC diagnosis. In this review, we summarize recent findings on the oncogenic roles of gut bacteria with different anatomical localization in CRC progression.

© 2017 THE AUTHORS. Published by Elsevier LTD on behalf of the Chinese Academy of Engineering and Higher Education Press Limited Company. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide [1]. Classic CRC is a malignant disease caused by a variety of factors, including genetic mutations, epigenetic changes, chronic inflammation, diet, and lifestyle [2,3]. However, the molecular mechanisms involved in CRC tumorigenesis and progression are not yet fully understood. Accumulating evidence suggests that the gut microbiota contributes to CRC development [4–7].

The gut commensal microbiota, as a mutualistic ecosystem, plays multiple roles in maintaining host health and inducing host diseases [8–10]. Balance of the microbiota can result in the production of essential nutrients, cause prompt and efficient host nutrient absorption, aid in the development of a mature and competent immune system of the host, and prevent pathogen colonization [11–17]. Dysbiosis of the gut microbiota can result in inflammation, barrier fail-

ure, mucosal tissue damage, and an altered microenvironment that favors the development of colon cancer [8,18]. A number of studies have found that the microbiota can drive colorectal carcinogenesis by causing DNA damage, oncogene expression, and gene silencing [4,10,19,20]. Due to the newly realized importance of the microbiota, the new model of CRC development takes the function of the microbiota into account.

Next-generation sequencing technologies (especially 16S ribosomal DNA sequencing and metagenomics sequencing) and other culture-independent methodologies have largely advanced our knowledge of the gut microbiota in both humans and mice [21,22]. Thanks to these rapidly evolving technologies, the origin of the gut microbiota and the landscape of its evolution, as well as its relatedness to human physiology, are gradually being unraveled. The gastrointestinal (GI) tract, especially the terminal ileum and large intestine, is the major source of commensal microbiota and contains

* Corresponding author.

E-mail address: junyu@cuhk.edu.hk

<http://dx.doi.org/10.1016/j.eng.2017.01.012>

2095–8099/© 2017 THE AUTHORS. Published by Elsevier LTD on behalf of the Chinese Academy of Engineering and Higher Education Press Limited Company. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

about 10^{14} archaeal and bacterial cells, as measured by 16S ribosomal DNA sequencing and by direct sequencing of genetic material [23,24]. The overall composition of the GI bacteria varies within and between individuals due to differences in pH [25], oxygen [26], antimicrobial peptide (AMP) gradients [27], short-chain fatty acids (SCFAs) [28], and intestinal motility [29]. The load of bacteria generally increases along the GI tract, ranging from 10^3 – 10^4 mL⁻¹ content in the stomach, duodenum, and jejunum (upper small intestine) to 10^8 mL⁻¹ in the ileum (lower small intestine) and up to 10^{11} mL⁻¹ in the colon [30,31]. Furthermore, the types of bacteria in the GI tract also vary—both longitudinally, from the small intestine to the large intestine [26,30], and cross-sectionally, depending on the anatomical location of the GI sections. In this review, we anatomically define gut commensal bacteria in the colon as four populations: ① luminal commensal bacteria, ② mucus-resident bacteria, ③ epithelium-resident bacteria, and ④ lymphoid tissue-resident commensal bacteria (Fig. 1). The dysregulated anatomical localization of colonic commensal bacteria is closely related to CRC [32]. In this contribution, we focus on the oncogenic roles of the aforementioned four categories of microbial populations in colorectal carcinogenesis.

2. Composition of gut commensal bacteria in the colon

2.1. Luminal commensal bacteria

In adult humans [33,34] and mice [35], the luminal commensal microbiota is typically dominated by bacteria. The majority of research on the role of the microbiota in CRC focuses on the luminal commensal microbiota. The luminal commensal bacteria form a huge and complex ecosystem, with up to 10^{12} commensal

bacteria comprising more than 1000 species [23,24]. Eckburg et al. [33] found that more than 90% of the luminal commensal bacteria belong to the phyla Firmicutes and Bacteroidetes, whereas Actinobacteria, Proteobacteria, and Verrucomicrobia are minor constituents. Arumugam et al. [36] consistently reported that Firmicutes (39%), Bacteroidetes (25%), Actinobacteria (9%), and Proteobacteria (4%) are the predominant bacteria in the human distal gut, based on an analysis of 39 healthy adults from six nations around the world (Table 1) [22–25,28,37–49]. As there is a huge inter-individual variability in the composition of the gut luminal microbiota [50], the functions of predominant bacteria, especially when integrated with the whole microbial community, remain largely unknown.

The phylum Firmicutes is a collection of Gram-positive, spore-forming, obligate anaerobes and cocci- or rod-shaped bacteria, including the Enterococcaceae and Lactobacillaceae families and the *Streptococcus* genus. Our study and others identify *Streptococcus bovis* (*S. bovis*), a member of the *Streptococcus* genus, as being enriched in CRC patients and highly associated with CRC [51–56]. The mechanism of *S. bovis* in promoting colorectal carcinogenesis is still unclear. However, Klein et al. [57] reported that most of the patients with *S. bovis*-induced endocarditis had colorectal adenomas or asymptomatic neoplasms, suggesting that *S. bovis* is involved in the early stage of CRC tumorigenesis. Similarly, another study found that the serum antigen levels of *S. bovis*-derived Rpl7/L12 were increased in colon polyps and in stage I/II CRC patients, but not in late-stage patients with lymph node or distant metastasis [58]. All these findings implicate *S. bovis* as an initiator in the development of CRC. Conversely, colorectal neoplastic lesions may provide a specific niche for *S. bovis*; either

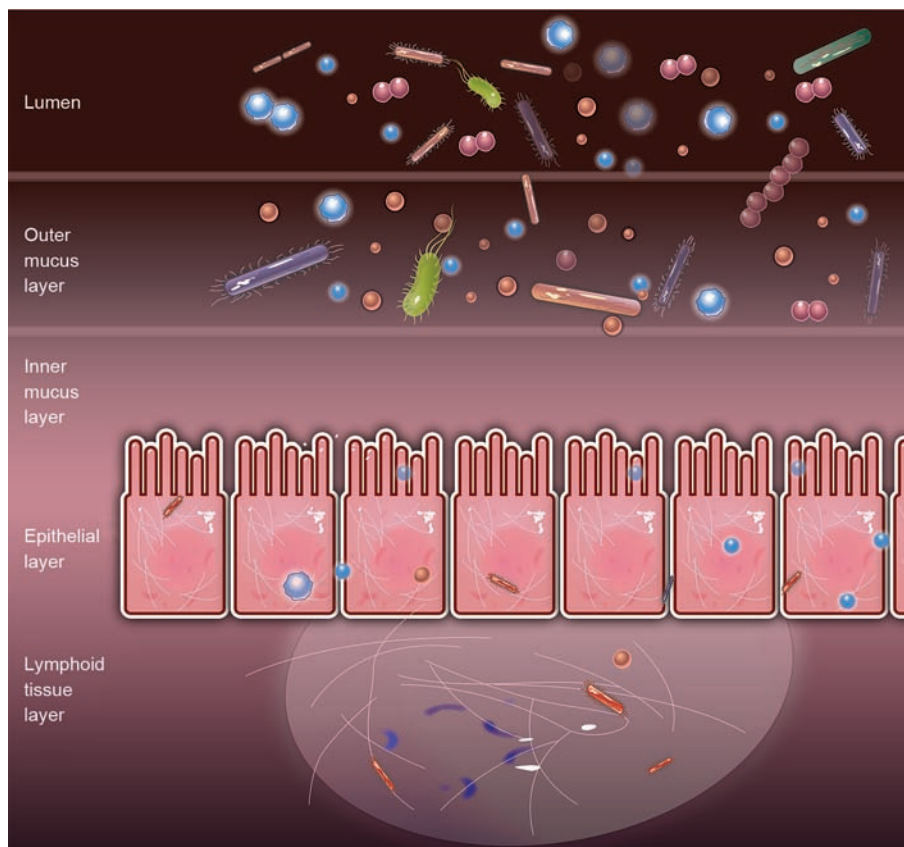


Fig. 1. Gut commensal bacteria are anatomically defined as four populations: luminal commensal bacteria, mucus-resident bacteria, epithelium-resident bacteria, and lymphoid tissue-resident commensal bacteria. Many species of bacteria are localized in the lumen and outer mucus layer, while the inner mucus layer is almost sterile. Few species of bacteria can move from the lumen and outer mucus to the intestinal epithelial cells (IECs) and lymphoid tissue.

Table 1

Composition of colonic commensal bacteria according to anatomical localization.

Populations		Major bacteria	Refs.
Luminal commensal bacteria	Phylum	Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia	[22–25,28,37–42]
	Order	Bacteroidales	
	Family	Rikenellaceae, Lactobacillaceae, Lachnospiraceae, Ruminococcaceae, Paraprevotellaceae	
	Genus	<i>Bacteroides</i> , <i>Prevotella</i> , <i>Mucispirillum</i> , <i>Lactobacillus</i> , <i>Ruminococcus</i> , <i>Oscillospira</i> , <i>Sutterella</i> , <i>Desulfovibrio</i> , <i>Fusobacterium</i>	
	Species	<i>Fusobacterium nucleatum</i>	
Mucus-resident bacteria	Phylum	Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia	[40–42]
	Order	Bacteroidales	
	Family	Rikenellaceae, Lactobacillaceae, Lachnospiraceae, Ruminococcaceae, Paraprevotellaceae	
	Genus	<i>Bacteroides</i> , <i>Prevotella</i> , <i>Mucispirillum</i> , <i>Lactobacillus</i> , <i>Ruminococcus</i> , <i>Oscillospira</i> , <i>Sutterella</i> , <i>Desulfovibrio</i>	
Epithelium-resident bacteria	Species	AIEC, SFB, <i>Enterococcus faecalis</i> , <i>Bacteroides fragilis</i> , <i>Clostridium</i> spp.	[43–47]
Lymphoid tissue-resident commensal bacteria	Species	<i>Achromobacter</i> spp., <i>Bordetella</i> spp., <i>Ochrobactrum</i> spp., <i>Serratia</i> spp.	[48,49]
		PP-DC: <i>Serratia</i> spp., SFB, <i>Ochrobactrum</i> spp., <i>Alcaligenes</i> spp.	
		MLN-DC: <i>Pseudomonas</i> spp., <i>Alcaligenes</i> spp.	

AIEC: adherent-invasive *Escherichia coli*; SFB: segmented filamentous bacteria; PP: Peyer's patch; DC: dendritic cell; MLN: mesenteric lymph node.

it or its antigens can stimulate the production of inflammatory cytokines such as interleukin-8 (IL-8), and promote the formation of hyper-proliferative aberrant colonic crypts [59,60]. In addition, *S. bovis* was shown to be capable of causing a chronic inflammation in the colon by producing IL-8 and prostaglandins E2 (PGE2), which can also promote colon cancer development when normal epithelial cells constantly sense abnormal signals from bacterial components [61].

Fusobacterium is a genus of Gram-negative, non-spore-forming, anaerobic bacteria. From metagenome-wide association studies on fecal samples from CRC patients and healthy controls, our group found that *Fusobacterium nucleatum* (*F. nucleatum*), a typically representative *Fusobacterium* spp., can be a selected non-invasive biomarker for CRC diagnosis—a finding that was further validated in several ethnically different cohorts [62]. Moreover, increasing evidence shows that a close relationship exists between *F. nucleatum* and CRC. A study performed on colitis-associated, *Apc^{min/+}*, and transgenic mouse models also indicated that *F. nucleatum* can accelerate colorectal tumorigenesis [63]. Accumulated interests were recently shared on the mechanism of this association. It was shown that a greater amount of *F. nucleatum* in colorectal carcinoma tissue is associated with high degrees of microsatellite instability (MSI-high) and CpG island methylator phenotype (CIMP) [64]. However, more evidence suggests that innate and adaptive immunity participates in the process of tumor development [37]. In addition, it has been found that *F. nucleatum* induces mucin secretion and inflammatory cytokine tumor necrosis factor (TNF)- α expression in direct contact with and/or during the invasion of colonic cells [65]. All these factors may predispose the host to adenomas or cancer development. *F. nucleatum* can also inhibit anti-tumor immunity and suppress the activities of natural killer cells, thereby promoting CRC development. A recent study identified fusobacterial lectin (Fap2) as a potent factor binding to tumor-expressed Gal-GalNAc and contributing to *F. nucleatum*-potentiated colorectal adenocarcinoma [38].

The phylum Bacteroidetes, formerly known as the *Cytophaga-Flavobacterium-Bacteroides* (CFB), is composed of Gram-negative, non-spore-forming, anaerobic, and rod-shaped bacteria. The genus *Bacteroides* is the predominant taxon among them [36]. *Bacteroides fragilis* (*B. fragilis*), a member of the genus *Bacteroides*, is detected in up to 80% of adults and children, and comprises only approximately 0.5%–1% of the fecal microbiota [39,66]. Recently, we performed metagenome-wide sequencing on fecal samples from 90 CRC patients and 78 healthy controls, and identified an

over-representation of *B. fragilis* in patients with CRC compared with healthy controls [62]. Furthermore, we analyzed the gut mucosal microbiome across different stages of colorectal carcinogenesis by 16S rRNA gene sequencing [67]. The abundance of *B. fragilis* was found to be significantly higher in the carcinoma mucosae and adenoma mucosae, compared with the adjacent normal mucosae [67]. These studies imply that luminal Bacteroidetes has a role in the development of CRC.

Research on the underlying mechanism of *B. fragilis* as a carcinogenic agent focuses on its secreted *B. fragilis* toxin (BFT) and on the structure of capsular polysaccharide A (PSA). BFT, a zinc-dependent metalloprotease toxin, stimulates structural changes and even dissolution of the zonula occludens (tight junction) and zonula adherents, electron-dense structures that regulate the permeability of epithelial monolayers [68]. BFT also induces proteolysis of the tumor-suppressor protein E-cadherin, resulting in the induction of β -catenin nuclear localization, up-regulation of proto-oncogene *c-myc* transcription and translation, and cellular proliferation of colonic epithelial cancer cells [69]. Dissolution of the zonula occludens, zonula adherens, and E-cadherin increases the permeability of polarized colonic epithelial cell monolayers, which, prior to tumor development, is an early pathophysiological change associated with incipient CRC [70]. According to the production of BFT or not, *B. fragilis* is commonly categorized into nontoxigenic *B. fragilis* (NTBF) and enterotoxigenic *B. fragilis* (ETBF). Accumulating evidence shows a positive correlation between an increased prevalence of ETBF in the GI tract and colorectal cancer [71–76]. Toprak et al. [71] reported that the enterotoxin (e.g., BFT) gene was more commonly identified in the stool of CRC patients compared with that of healthy individuals (38% vs. 12%, $P = 0.009$). Purified BFT from *B. fragilis* up-regulates spermine oxidase (SMO) in colonic epithelial cells, resulting in increased SMO-dependent generation of reactive oxygen species (ROS), epithelial release of pro-inflammatory effectors, and DNA damage [75].

The bacteria localized in the gut lumen can cooperate to form multicellular communities and compete with each other for limited environmental resources. Treatment with vancomycin, an antibiotic that selectively targets Gram-positive bacteria, can also eliminate a majority of bacteria from Gram-negative Bacteroidetes in the cecal lumina, indicating crosstalk between Gram-positive and Gram-negative bacteria [77]. Cooperation between the luminal bacteria Firmicutes and Bacteroidetes has also been reported in mouse intestines [78]. With the presence of

Bifidobacterium longum, a species of Actinobacteria phylum, the expression of glycoside hydrolases in *Bacteroides thetaiotaomicron*, a species of Bacteroidetes phylum, was increased [78]. Moreover, bacteria hold the potential to inhibit the growth of their competitors by pH modification, control of motility, nutrient depletion, and the production of antimicrobial substances such as bacteriocins [79]. The *Lactobacillus salivarius* strain UCC118, which belongs to the Firmicutes phylum, produces a two-component bacteriocin (Abp118) with broad-spectrum activity against the bacteria of the Bacteroidetes and Firmicutes phyla (including the food-borne pathogen *Listeria monocytogenes* [80] and methicillin-resistant *Staphylococcus aureus* [81]) in the colonic lumina.

2.2. Mucus-resident bacteria

The intestinal mucus is the first line of defense separating the luminal bacteria from underlying intestinal epithelium and systemic tissues. The thickness of colonic mucus is approximately 400 μm in humans [82] and 150 μm in mice [83]. Both of the two layers of colonic mucus are predominantly organized by various glycans and the large gel-forming mucin-2 (Muc2), which is secreted by goblet cells and Paneth cells [84]. The colonic inner mucus initially requires 6 weeks to become impenetrable when being challenged by bacteria in germ-free (GF) mice [85]. However, in both adult specific-pathogen-free (SPF) mice and GF mice, the 50 μm colonic inner mucus layer is constantly (hourly) renewed due to Muc2 secretion from goblet cells and Paneth cells, while the preformed mucus moves upward to become the outer mucus layer [86,87]. The underlying mechanism by which mucus migrates upward from the inner mucus layer to the outer mucus layer is still unknown. In addition to the barrier function of the inner mucus, the loose outer mucus is a direct source of nutrients for special commensal bacteria, which possess a large amount of catabolic glycosidic enzymes to disassemble complex mucus glycans [40]. Only these special commensal bacteria can create a specialized niche for mucus-resident bacteria such as *Akkermansia muciniphila* and *Mucispirillum* spp. [41,88]. This is probably a way for the host to geographically resist the luminal bacterial community, in part because the discriminant binding capacity of different bacteria to the loose mucus layer may be a determinant that contributes to the disparate spatial distribution of these bacteria species, thus maintaining microbial homeostasis in the colon.

Recently, Li et al. [42] found that outer colonic mucus-resident bacteria comprise more taxa from the Firmicutes phylum and the Deferribacteres phylum and less from the Bacteroidetes phylum, compared with luminal bacteria in C57BL6 mice by 16S sequencing. However, no significant difference exists between mucus-resident bacteria and luminal bacteria in the diversity of microbiotas. It is interesting that the species *Bacteroides thetaiotaomicron* and *Escherichia coli*, which are resident in the outer mucus, have a stronger potential to proliferate and to utilize resources (e.g., by recovering bioavailable iron and consuming mucus carbon sources), compared with the same species in the intestinal lumen [42]. Furthermore, the intrinsic mucus-resident bacteria can protect the host from pathogens by inhibiting physical contact between them [78,81].

2.3. Epithelium-resident bacteria

Intestinal epithelial cells (IECs) form an additional mono-layered, physical barrier underlying the two layers of mucus barriers, and play a key role in maintaining equilibrium between the gut commensals and the host [89]. IECs include absorptive IECs and secretory IECs. Absorptive IECs are adapted for metabolic and digestive functions. Secretory IECs include enteroendocrine cells, goblet cells, and Paneth cells [90], which secrete mucins and various AMPs to es-

tablish a physical and biochemical barrier that modulates microbial contact with the epithelial surface and underlying immune cells [27]. Although the layer of IECs is generally recognized as a sterile area, accumulating studies show that various bacteria can attach to and even invade IECs [43,44,91]. Bacterial attachment to and invasion of IECs are regulated by two main mechanisms, namely the zipper and trigger mechanisms, which rely on the modification of cytoskeletal rearrangements and membrane extensions, and the activation of reorganization of the actin cytoskeleton at the plasma membrane [45]. It is a multi-step process for bacteria to adhere to and invade IECs. A multitude of adjacent and invasive bacteria types exist; herein, we primarily focus on adherent-invasive *Escherichia coli* (AIEC), a category of invasive bacteria residing in the gut that cause innate immune responses (Table 1).

AIEC is capable of translocating into IECs and replicating intracellularly. Researchers have shown that AIEC is closely related to inflammatory bowel disease (IBD), and that its abundance in inflamed regions also correlates to the severity of diseases [92,93]. Moreover, AIEC plays a central role in the pathogenesis of CRC [46,94]. The obvious difference between AIEC and *S. bovis* is that the pathogenic cyclomodulin-positive AIEC is more prevalent on the mucosa of patients with stages III/IV CRC than those with stage I. This finding suggests that AIEC is highly likely to be involved in the progression of carcinoma, especially at the late stage, and may be a prognostic factor [47]. Unlike non-pathogenic strains, AIEC strains harbor flagella and usually include a variety of FimH adhesin variants, hence allowing them to bind and invade IECs more efficiently [95]. These polarized IECs can secrete the pro-inflammatory cytokine IL-8 and chemokine CCL20, leading to the recruitment of macrophages and dendritic cells to the sites of infection with further secretions of interferon (IFN)- γ and TNF- α [96,97]. Moreover, the binding of flagella to Toll-like receptor 5 (TLR5) in IECs is able to activate the classical NF- κ B pathway [98]. In turn, these molecular patterns cooperatively control the transcription of IL-8 and pro-angiogenic factors contributing to inflammation and vascularization, as well as tumorigenesis [99].

2.4. Lymphoid tissue-resident commensal bacteria

The gut-associated lymphoid tissues (GALTs) include Peyer's patches (PPs), isolated lymphoid follicles (ILFs), mesenteric lymph nodes (MLNs), and intestinal lamina propria (ILP) [100]. The underlying area of the intestinal epithelium was previously thought to be sterile in healthy mammals. Although pathogenic bacteria can penetrate the inner mucus, evade AMPs and immunoglobulin-A (IgA) killing, and translocate across the intestinal epithelium, they can still be quickly killed by lamina propria macrophages or other lymphoid cells in the GALTs. However, recent studies suggest that a special group of commensal bacteria can not only colonize the GALTs, but also replicate in the GALTs of healthy mammals by utilizing nutrients from lymphoid tissue [48,49,101,102]. Furthermore, the composition of lymphoid tissue-resident commensal bacteria (LRCs) is largely different from those of the lumen-resident bacteria and epithelium-associated bacteria [102]. Obata et al. [48] found that PPs are the main lymphoid tissue colonized by commensal bacteria. Absolutely different bacteria were found to populate the surface of the PPs (i.e., mainly segmented filamentous bacteria (SFB) and *Lactobacillus* spp.) compared with the interior of the PPs (i.e., mainly *Alcaligenes* spp. and *Ochrobactrum* spp.) (Table 1). Furthermore, *Alcaligenes* spp. was found to be the dominant bacteria in the PP-dendritic cells (DCs) and MLN-DCs [48]. GALTs regulate special LRCs (mainly *Alcaligenes*) to prevent the systemic inflammation associated with Crohn's disease and progressive hepatitis C virus infection [48,49,102]. In contrast, LRCs modulate the cytokine production of murine DC and promote the responses of tissue-specific Th17 cells and group 3 innate lymphoid cells [102].

3. Mechanism of the colonic microbiota in the progression of colorectal cancer

3.1. The colonic microbiota influences the progression of colorectal cancer through mucosal inflammation

One mechanism by which the colonic microbiota influences the progression of CRC is via the modulation of mucosal inflammation. The formation of CRC derived from normal colonic epithelia involves a series of inflammatory factors that enable and shape a tumorigenic microenvironment. Studies have shown that the development of dysplasia and CRC is profoundly influenced by the inflammatory state of the colon. In patients with IBD, constant inflammation of the colon increases the susceptibility to develop CRC [103,104]. These inflammation conditions have also been associated with gut microbiota dysbiosis. During the development of a tumor, the permeability of the physical barriers between the epithelium, which separate the microbiota from the lamina propria, is increased [89,105]. Barrier disruption results in bacterial translocation and leads to the exposure of microbial compounds to both antigen-presenting cells and epithelial cells; thus, the activation of immune-signaling pathways by bacterial stimuli contributes to a distortion of homeostasis that initiates a proneoplastic inflammatory milieu. Recognition of microbes and of microbial-derived molecules plays a pivotal role in the inflammation and induction of a pro-tumorigenic milieu in CRC. Among the mechanisms of action for the pro-inflammatory role of bacteria in CRC development, prominent mechanisms include inflammasome activation [106] and activation of the NF- κ B pathway [107], both of which respond to microbial stimuli promoting cell survival and proliferation. In addition to the bacterial-sensing mechanisms in epithelial cells, T cell subpopulations such as Th17 cells and regulatory T cells can modulate inflammation within the GI tract, thereby playing an important role in inflammation-associated CRC [108,109]. It is interesting that the proportion and function of these cells are affected by the gut microbiota, further substantiating the important role of microbiota-mediated inflammation in CRC development.

3.2. The colonic microbiota induces colorectal cancer progression through dysbiosis

The cause or effect relationship between the gut microbiota and CRC progression has come under debate. It is not possible to definitively conclude that a causal association exists, because much of the evidence merely implies a relation, without clearly indicating whether the dysbiosis is a primary cause or a secondary outcome. However, some studies have shown that mouse and rat models of intestinal tumorigenesis exhibited decreased tumor loads with the depletion of microbes, compared with mouse and rat models raised under conventional conditions [76,110,111]. Recent research has demonstrated that specific members of the gut microbiota contribute to the development of CRC [5,7]. It is notable that the tumor milieu is populated by immune cells, which play a role in both pro- and anti-tumor immunity. These immune cells can be influenced by the gut-resident microbiota as well, even after progression to CRC. Therefore, rather than being a causal relationship, the intricate interactions between the microbiota, the immune system, and CRC are a multifaceted network that warrants further investigation.

3.3. The colonic microbiota contributes to the progression of colorectal cancer through bacterial metabolites

Accumulating evidence suggests that not only the gut microbiota but also its metabolites contribute to the pathogenesis of CRC. The SCFAs acetate, propionate, and butyrate can function as suppression

factors in cancer, whereas other subsets of microbial metabolites, such as secondary bile acids, promote tumorigenesis. All these metabolites have been substantially reviewed in other literature [12,112]. In this section, we aim to briefly discuss the relations between microbial metabolism, diet, and CRC.

High protein intake results in an increase in the fermentation of diet-derived protein in the colon, as indicated by the increase in amino-acid-derived products such as branched-chain fatty acids and phenylacetic acid [113,114]. A small portion of the gut bacteria community, including some Bacteroidetes spp. and Firmicutes spp., can metabolize aromatic amino acids to produce bioactive compounds, consisting of indoles, phenols, *p*-cresol, and phenylacetic acid. These nitrogenous products, and *N*-nitroso compounds in particular, have the capacity to promote carcinogenesis through DNA alkylation that results in mutations. There is a positive correlation between the intake of dietary *N*-nitroso compounds and CRC [115]. Increases in fecal *N*-nitroso compounds have been observed in individuals with high-protein diets. Ammonia, another product of protein fermentation, is a carcinogenic agent as well, but at low concentrations; it has been shown to increase mucosal damage and the amount of colonic adenocarcinoma in a rat model [116]. Hydrogen sulfide is a product generated in the distal gut via the reduction of diet-derived sulfate and the metabolism of other compounds. Sulfate-reducing bacteria, such as *Desulfovibrio* spp., are detectable in low abundances in healthy individuals, and are capable of using lactate as a co-substrate for sulfide formation [117]. Sulfide is toxic to colonocytes and represses butyrate oxidation, leading to the disruption of the colonocyte barrier [118]. Sulfide is genotoxic to normal human cell lines, in which the mechanism of DNA damage engages ROS [75]. Polyamines, metabolites from bacteria or the diet, are also toxic and are associated with cancer. Oxidative stress due to polyamine catabolism is thought to be the underlying mechanism of this relation [119]. In addition, specific gut bacteria, *B. fragilis*, up-regulate polyamine production by host cells [120]. Excessive consumption of ethanol has been widely accepted as an important risk factor for cancer [121], and microbial metabolism may add to its toxicity. Many anaerobic bacteria can produce ethanol. Although ethanol itself is not regarded as a potent carcinogen, its oxidation product, acetaldehyde, is considered to be highly carcinogenic, causing an array of effects ranging from the degradation of the vitamin folate to DNA damage [122].

4. The translocation of commensal bacteria in colorectal cancer

In addition to dysbiosis, the dysregulated localization of commensal bacteria plays a crucial role in CRC development. A common bacteria translocation route is from the gut to MLNs [104]. In a prospective cohort study on 158 CRC patients for 5 years, CRC patients with bacterial translocation in the mesenteric lymph nodes had a worse rate of disease-specific survival and disease-free survival than those without [32]. Moreover, bacterial translocation is a specific predictor of the survival of CRC patients [32]. Lescut et al. [123] reported that the dysregulated bacteria in the pericolonic lymph nodes adjacent to the cancer are the major bacterial translocation resources in CRC patients. Moreover, CRC patients with bacterial translocation are prone to exhibit cachexia [124]. These studies indicate that targeting the dysregulated localization of commensal bacteria is a promising approach for CRC prevention and treatment.

5. Fecal bacteria as a biomarker in colorectal cancer diagnosis

Other studies by our group recently indicated that stool-based CRC-associated bacteria could serve as a non-invasive biomarker for CRC diagnosis [125,126]. Using probe-based duplex quantitative polymerase chain reaction (qPCR) assays, we examined CRC-related bacteria (*F. nucleatum*, *Bacteroides clarus* (*B. clarus*), *Roseburia intestinalis*

(*R. intestinalis*), *Clostridium hathewayi* (*C. hathewayi*), and m7) in stool samples of 203 CRC patients and 236 healthy controls. The combination of *F. nucleatum* + *C. hathewayi* + m7 + *B. clarus* showed high diagnostic ability, with an area under the receiver operating curve (AUC) of 0.886 [125]. By the qPCR analysis of 104 patients with CRC, 103 patients with advanced adenoma, and 102 healthy controls, we also identified that the combination of *F. nucleatum* + fecal immunochemical test (FIT) showed high sensitivity (92.3%) and an AUC of 0.95 in detecting CRC [126]. High abundances of *F. nucleatum* and *B. fragilis* have been identified as independent indicators of poor survival in CRC patients [127]. FadA, the unique adhesin of *F. nucleatum*, has also been reported to be a potential diagnostic target for CRC. The expression levels of the *fadA* gene in colon tissue from patients with adenomas and adenocarcinomas are significantly higher than those in normal individuals [128]. These studies suggest that probing the gut microbiota may provide a non-invasive, accurate, and affordable diagnosis of CRC.

6. Conclusions and perspective

The four populations of gut commensal bacteria manifest different compositions and various functions in their differential contributions to the interactions between host and microbes. The whole GI tract is a huge mutualistic ecosystem. Changes in each bacteria group could potentially form a cascade reaction with other bacteria groups. By dividing the GI commensal bacteria into more groups, we can understand the molecular mechanisms of action for special bacteria groups more explicitly. Dysregulated localization of bacteria is closely associated with CRC, and fecal bacteria may be valuable diagnosis markers for CRC. Furthermore, the gut microbiota may be a therapeutic target to inhibit CRC proliferation and metastasis.

Acknowledgements

This project was supported by RGC-GRF Hong Kong (766613, 14106145), the National Basic Research Program of China (2013CB531401), the National Natural Science Foundation of China (NSFC) (81201963, 81372600), the Shenzhen Municipal Science and Technology R&D fund (JCYJ20120619152326450), and the Shenzhen Virtual University Park Support Scheme to CUHK Shenzhen Research Institute.

Compliance with ethics guidelines

Liuyang Zhao, Xiang Zhang, Tao Zuo, and Jun Yu declare that they have no conflict of interest or financial conflicts to disclose.

References

- [1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016;66(1):7–30.
- [2] Hou TY, Davidson LA, Kim E, Fan YY, Fuentes NR, Triff K, et al. Nutrient-gene interaction in colon cancer, from the membrane to cellular physiology. *Annu Rev Nutr* 2016;36:543–70.
- [3] Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. *Annu Rev Genom Hum G* 2002;3(1):101–28.
- [4] Garrett WS. Cancer and the microbiota. *Science* 2015;348(6230):80–6.
- [5] Mima K, Nishihara R, Qian ZR, Cao Y, Sukawa Y, Nowak JA, et al. *Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. *Gut*. Epub 2015 Aug 26.
- [6] Bhattacharya N, Yuan R, Prestwood TR, Penny HL, DiMaio MA, Reticker-Flynn NE, et al. Normalizing microbiota-induced retinoic acid deficiency stimulates protective CD8⁺ T cell-mediated immunity in colorectal cancer. *Immunity* 2016;45(3):641–55.
- [7] Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, et al. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* 2015;42(2):344–55.
- [8] Brennan CA, Garrett WS. Gut microbiota, inflammation, and colorectal can-

- cer. *Annu Rev Microbiol* 2016;70:395–411.
- [9] Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* 2014;157(1):121–41.
- [10] Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 2012;338(6103):120–3.
- [11] Irrazábal T, Belcheva A, Girardin SE, Martin A, Philpott DJ. The multifaceted role of the intestinal microbiota in colon cancer. *Mol Cell* 2014;54(2):309–20.
- [12] Sears CL, Garrett WS. Microbes, microbiota, and colon cancer. *Cell Host Microbe* 2014;15(3):317–28.
- [13] Collins JW, Keeney KM, Crepin VE, Rathinam VAK, Fitzgerald KA, Finlay BB, et al. *Citrobacter rodentium*: infection, inflammation and the microbiota. *Nat Rev Microbiol* 2014;12(9):612–23.
- [14] Chung HC, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, et al. Gut immune maturation depends on colonization with a host-specific microbiota. *Cell* 2012;149(7):1578–93.
- [15] Duerkop BA, Vaishnava S, Hooper LV. Immune responses to the microbiota at the intestinal mucosal surface. *Immunity* 2009;31(3):368–76.
- [16] Geuking MB, Cahenzli J, Lawson MAE, Ng DCK, Slack E, Hapfelmeier S, et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* 2011;34(5):794–806.
- [17] Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998;66(11):5224–31.
- [18] Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, et al. The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation. *Immunity* 2015;43(5):998–1010.
- [19] Wang X, Yang Y, Moore DR, Nimmo SL, Lightfoot SA, Huycke MM. 4-hydroxy-2-nonenal mediates genotoxicity and bystander effects caused by *Enterococcus faecalis*-infected macrophages. *Gastroenterology* 2012;142(3):543–51.
- [20] Yang Y, Wang X, Huycke T, Moore DR, Lightfoot SA, Huycke MM. Colon macrophages polarized by commensal bacteria cause colitis and cancer through the bystander effect. *Transl Oncol* 2013;6(5):596–606.
- [21] Metzker ML. Sequencing technologies—the next generation. *Nat Rev Genet* 2010;11(1):31–46.
- [22] Schuster SC. Next-generation sequencing transforms today's biology. *Nat Methods* 2008;5(1):16–8.
- [23] Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010;464(7285):59–65.
- [24] Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol* 2016;14(8):e1002533.
- [25] Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ, Hardcastle JD. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut* 1988;29(8):1035–41.
- [26] He G, Shankar RA, Chzhan M, Samouilov A, Kuppusamy P, Zweier JL. Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of living mice with spatial and spectral EPR imaging. *Proc Natl Acad Sci USA* 1999;96(8):4586–91.
- [27] Gallo RL, Hooper LV. Epithelial antimicrobial defence of the skin and intestine. *Nat Rev Immunol* 2012;12(7):503–16.
- [28] Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009;461(7268):1282–6.
- [29] Rao SS, Kuo B, McCallum RW, Chey WD, DiBaise JK, Hasler WL, et al. Investigation of colonic and whole-gut transit with wireless motility capsule and radiopaque markers in constipation. *Clin Gastroenterol Hepatol* 2009;7(5):537–44.
- [30] Wlodarska M, Kostic AD, Xavier RJ. An integrative view of microbiome-host interactions in inflammatory bowel diseases. *Cell Host Microbe* 2015;17(5):577–91.
- [31] Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, et al. An estimation of the number of cells in the human body. *Ann Hum Biol* 2013;40(6):463–71.
- [32] Chin KF, Kallam R, O'Boyle C, MacFie J. Bacterial translocation may influence the long-term survival in colorectal cancer patients. *Dis Colon Rectum* 2007;50(3):323–30.
- [33] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. *Science* 2005;308(5728):1635–8.
- [34] Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature* 2012;489(7415):220–30.
- [35] Hill DA, Hoffmann C, Abt MC, Du Y, Kobuley D, Kirn TJ, et al. Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. *Mucosal Immunol* 2010;3(2):148–58.
- [36] Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. *Nature* 2011;473(7346):174–80.
- [37] Nisho K, Sukawa Y, Adachi Y, Ito M, Mitsuhashi K, Kurihara H, et al. Association of *Fusobacterium nucleatum* with immunity and molecular alterations in colorectal cancer. *World J Gastroenterol* 2016;22(2):557–66.
- [38] Abed J, Emgård JE, Zamir G, Faroja M, Almogy G, Grenov A, et al. Fap2

- mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host Microbe* 2016;20(2):215–25.
- [39] Huang JY, Lee SM, Mazmanian SK. The human commensal *Bacteroides fragilis* binds intestinal mucin. *Anaerobe* 2011;17(4):137–41.
- [40] Corfield AP. Mucins: a biologically relevant glycan barrier in mucosal protection. *Bba-Gen Subjects* 2015;1850(1):236–52.
- [41] Robertson BR, O'Rourke JL, Neilan BA, Vandamme P, On SLW, Fox JG, et al. *Mucispirillum schaedleri* gen. nov., sp. nov., a spiral-shaped bacterium colonizing the mucus layer of the gastrointestinal tract of laboratory rodents. *Int J Syst Evol Microbiol* 2005;55(3):1199–204.
- [42] Li H, Limenitakis JP, Fuhrer T, Geuking MB, Lawson MA, Wyss M, et al. The outer mucus layer hosts a distinct intestinal microbial niche. *Nat Commun* 2015;6:8292.
- [43] Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. T_H17 induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013;500(7461):232–6.
- [44] Brook I, Myhal ML. Adherence of *Bacteroides fragilis* group species. *Infect Immun* 1991;59(2):742–4.
- [45] Cossart P, Sansonetti PJ. Bacterial invasion: the paradigms of enteroinvasive pathogens. *Science* 2004;304(5668):242–8.
- [46] Martin HM, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singh R, et al. Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. *Gastroenterology* 2004;127(1):80–93.
- [47] Bonnet M, Buc E, Sauvanet P, Darcha C, Dubois D, Pereira B, et al. Colonization of the human gut by *E. coli* and colorectal cancer risk. *Clin Cancer Res* 2014;20(4):859–67.
- [48] Obata T, Goto Y, Kunisawa J, Sato S, Sakamoto M, Setoyama H, et al. Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. *Proc Natl Acad Sci USA* 2010;107(16):7419–24.
- [49] Sonnenberg GF, Monticelli LA, Alenghat T, Fung TC, Hutnick NA, Kunisawa J, et al. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. *Science* 2012;336(6086):1321–5.
- [50] Wu GD, Chen J, Hoffmann K, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011;334(6052):105–8.
- [51] Cordeiro J, Alonso MP, Coira A, Casariego E, Arias C, Alonso D, et al. Characteristics of *Streptococcus bovis* endocarditis and its differences with *Streptococcus viridans* endocarditis. *Eur J Clin Microbiol Infect Dis* 2008;27(4):285–91.
- [52] Lazarovitch T, Shango M, Levine M, Brusovansky R, Akins R, Hayakawa K, et al. The relationship between the new taxonomy of *Streptococcus bovis* and its clonality to colon cancer, endocarditis, and biliary disease. *Infection* 2013;41(2):329–37.
- [53] Gupta A, Madani R, Mukhtar H. *Streptococcus bovis* endocarditis, a silent sign for colonic tumour. *Colorectal Dis* 2010;12(3):164–71.
- [54] Harrison S, Benziger H, Koerner R. *Streptococcus bovis* infections, colorectal cancer and liver dysfunction. *ANZ J Surg* 2011;81(11):762–3.
- [55] McMahon AJ, Auld CD, Dale BA, Walls AD, McCormick JS. *Streptococcus bovis* septicaemia associated with uncomplicated colonic carcinoma. *Br J Surg* 1991;78(7):883–5.
- [56] Wending GK, Metzger PP, Dozois EJ, Chua HK, Krishna M. Unusual bacterial infections and colorectal carcinoma—*Streptococcus bovis* and *Clostridium septicum*: report of three cases. *Dis Colon Rectum* 2006;49(8):1223–7.
- [57] Klein RS, Catalano MT, Edberg SC, Casey JL, Steigbigel NH. *Streptococcus bovis* septicemia and carcinoma of the colon. *Ann Intern Med* 1979;91(4):560–2.
- [58] Boleij A, Roelofs R, Schaeps RM, Schülin T, Glaser P, Swinkels DW, et al. Increased exposure to bacterial antigen Rpl7/L12 in early stage colorectal cancer patients. *Cancer* 2010;116(17):4014–22.
- [59] Ellmerich S, Schöller M, Duranton B, Gosse F, Galluser M, Klein JP, et al. Promotion of intestinal carcinogenesis by *Streptococcus bovis*. *Carcinogenesis* 2000;21(4):753–6.
- [60] Tjalsma H, Schöller-Guinard M, Lasonder E, Ruers TJ, Willems HL, Swinkels DW. Profiling the humoral immune response in colon cancer patients: diagnostic antigens from *Streptococcus bovis*. *Int J Cancer* 2006;119(9):2127–35.
- [61] Biarc J, Nguyen IS, Pini A, Gosse F, Richert S, Thierse D, et al. Carcinogenic properties of proteins with pro-inflammatory activity from *Streptococcus infantarius* (formerly *S. bovis*). *Carcinogenesis* 2004;25(8):1477–84.
- [62] Yu J, Feng Q, Wong SH, Zhang D, Liang QY, Qin Y, et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* 2017;66(1):70–8.
- [63] Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013;14(2):207–15.
- [64] Tahara T, Yamamoto E, Suzuki H, Maruyama R, Chung W, Garriga J, et al. *Fusobacterium* in colonic flora and molecular features of colorectal carcinoma. *Cancer Res* 2014;74(5):1311–8.
- [65] Dharmani P, Strauss J, Ambrose C, Allen-Vercoe E, Chadee K. *Fusobacterium nucleatum* infection of colonic cells stimulates MUC2 mucin and tumor necrosis factor alpha. *Infect Immun* 2011;79(7):2597–607.
- [66] Macfarlane S, Woodmansey EJ, Macfarlane GT. Colonization of mucin by human intestinal bacteria and establishment of biofilm communities in a two-stage continuous culture system. *Appl Environ Microbiol* 2005;71(11):7483–92.
- [67] Nakatsu G, Li X, Zhou H, Sheng J, Wong SH, Wu W, et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat Commun* 2015;6:8727.
- [68] Chambers FG, Koshy SS, Saidi RF, Clark DP, Moore RD, Sears CL. *Bacteroides fragilis* toxin exhibits polar activity on monolayers of human intestinal epithelial cells (T84 cells) *in vitro*. *Infect Immun* 1997;65(9):3561–70.
- [69] Wu S, Rhee KJ, Zhang M, Franco A, Sears CL. *Bacteroides fragilis* toxin stimulates intestinal epithelial cell shedding and gamma-secretase-dependent E-cadherin cleavage. *J Cell Sci* 2007;120:1944–52.
- [70] Soler AP, Miller RD, Laughlin KV, Carp NZ, Klurfeld DM, Mullin JM. Increased tight junctional permeability is associated with the development of colon cancer. *Carcinogenesis* 1999;20(8):1425–32.
- [71] Toprak NU, Yagci A, Gulluoglu BM, Akin ML, Demirkalem P, Celenk T, et al. A possible role of *Bacteroides fragilis* enterotoxin in the aetiology of colorectal cancer. *Clin Microbiol Infect* 2006;12(8):782–6.
- [72] Boleij A, Hechenbleikner EM, Goodwin AC, Badani R, Stein EM, Lazarev MG, et al. The *Bacteroides fragilis* toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin Infect Dis* 2015;60(2):208–15.
- [73] Sobhani I, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, et al. Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS One* 2011;6(1):e16393.
- [74] Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J* 2012;6(2):320–9.
- [75] Goodwin AC, Destefano Shields CE, Wu S, Huso DL, Wu X, Murray-Stewart TR, et al. Polyamine catabolism contributes to enterotoxigenic *Bacteroides fragilis*-induced colon tumorigenesis. *Proc Natl Acad Sci USA* 2011;108(37):15354–9.
- [76] Housseau F, Sears CL. Enterotoxigenic *Bacteroides fragilis* (ETBF)-mediated colitis in Min (*Apc*^{+/−}) mice: a human commensal-based murine model of colon carcinogenesis. *Cell Cycle* 2010;9(1):3–5.
- [77] Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, et al. Vancomycin-resistant *Enterococcus* domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest* 2010;120(12):4332–41.
- [78] Sonnenburg JL, Chen CT, Gordon JL. Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. *PLoS Biol* 2006;4(12):e413.
- [79] Thompson-Chagoyán OC, Maldonado J, Gil A. Aetiology of inflammatory bowel disease (IBD): role of intestinal microbiota and gut-associated lymphoid tissue immune response. *Clin Nutr* 2005;24(3):339–52.
- [80] Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci USA* 2007;104(18):7617–21.
- [81] Dunne C, Murphy L, Flynn S, O'Mahony L, O'Halloran S, Feeney M, et al. Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. *Antonie van Leeuwenhoek* 1999;76(1):279–92.
- [82] Johansson MEV, Gustafsson JK, Holmén-Larsson J, Jabbar KS, Xia L, Xu H, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut* 2014;63(2):281–91.
- [83] Johansson MEV, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci USA* 2008;105(39):15064–9.
- [84] Johansson MEV, Hansson GC. Immunological aspects of intestinal mucus and mucins. *Nat Rev Immunol* 2016;16(10):639–49.
- [85] Johansson ME, Jakobsson HE, Holmén-Larsson J, Schütte A, Ermund A, Rodríguez-Piñero AM, et al. Normalization of host intestinal mucus layers requires long-term microbial colonization. *Cell Host Microbe* 2015;18(5):582–92.
- [86] Johansson MEV. Fast renewal of the distal colonic mucus layers by the surface goblet cells as measured by *in vivo* labeling of mucin glycoproteins. *PLoS One* 2012;7(7):e41009.
- [87] Jakobsson HE, Rodríguez-Piñero AM, Schütte A, Ermund A, Boysen P, Beemark M, et al. The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Rep* 2015;16(2):164–77.
- [88] Derrien M, Collado MC, Ben-Amor K, Salminen S, de Vos WM. The mucin degrader *Akkermansia muciniphila* is an abundant resident of the human intestinal tract. *Appl Environ Microbiol* 2008;74(5):1646–8.
- [89] Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol* 2014;14(3):141–53.
- [90] Yang Q, Bermingham NA, Finegold MJ, Zoghbi HY. Requirement of *Math1* for secretory cell lineage commitment in the mouse intestine. *Science* 2001;294(5549):2155–8.
- [91] Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 2009;31(4):677–89.
- [92] Rolhion N, Darfeuille-Michaud A. Adherent-invasive *Escherichia coli* in inflammatory bowel disease. *Inflamm Bowel Dis* 2007;13(10):1277–83.
- [93] Baumgart M, Dogan B, Rishniw M, Weitzman G, Bosworth B, Yantiss R, et al. Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *ISME J* 2007;1(5):403–18.
- [94] Swidsinski A, Khilkin M, Kerjaschki D, Schreiber S, Ortner M, Weber J, et al. Association between intraepithelial *Escherichia coli* and colorectal cancer. *Gastroenterology* 1998;115(2):281–6.

- [95] Dreux N, Denizot J, Martinez-Medina M, Mellmann A, Billig M, Kisiela D, et al. Point mutations in FimH adhesin of Crohn's disease-associated adherent-invasive *Escherichia coli* enhance intestinal inflammatory response. *PLoS Pathog* 2013;9(1):e1003141.
- [96] Eaves-Pyles T, Allen CA, Taormina J, Swidsinski A, Tutt CB, Jezek GE, et al. *Escherichia coli* isolated from a Crohn's disease patient adheres, invades, and induces inflammatory responses in polarized intestinal epithelial cells. *Int J Med Microbiol* 2008;298(5–6):397–409.
- [97] Subramanian S, Rhodes JM, Hart CA, Tam B, Roberts CL, Smith SL, et al. Characterization of epithelial IL8 response to inflammatory bowel disease mucosal *E. coli* and its inhibition by mesalamine. *Inflamm Bowel Dis* 2008;14(2):162–75.
- [98] Mimouna S, Goncalves D, Barnich N, Darfeuille-Michaud A, Hofman P, Vouret-Craviari V. Crohn disease-associated *Escherichia coli* promote gastrointestinal inflammatory disorders by activation of HIF-dependent responses. *Gut Microbes* 2011;2(6):335–46.
- [99] Hoessel B, Schmid JA. The complexity of NF- κ B signaling in inflammation and cancer. *Mol Cancer* 2013;12(1):86.
- [100] McWilliams M, Phillips-Quagliata JM, Lamm ME. Characteristics of mesenteric lymph-node cells homing to gut-associated lymphoid-tissue in syngeneic mice. *J Immunol* 1975;115(1):54–8.
- [101] Kunisawa J, Kiyono H. *Alcaligenes* is commensal bacteria habituating in the gut-associated lymphoid tissue for the regulation of intestinal IgA responses. *Front Immunol* 2012;3:65.
- [102] Fung TC, Bessman NJ, Hepworth MR, Kumar N, Shibata N, Kobuley D, et al. Lymphoid-tissue-resident commensal bacteria promote members of the IL-10 cytokine family to establish mutualism. *Immunity* 2016;44(3):634–46.
- [103] Beaugier L, Itzkowitz SH. Cancers complicating inflammatory bowel disease. *N Engl J Med* 2015;372(15):1441–52.
- [104] Deitch EA. Bacterial translocation or lymphatic drainage of toxic products from the gut: what is important in human beings? *Surgery* 2002;131(3):241–4.
- [105] Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, et al. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* 2012;491(7423):254–8.
- [106] Elinav E, Nowarski R, Thaïs CA, Hu B, Jin CC, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat Rev Cancer* 2013;13(11):759–71.
- [107] Karin M, Greten FR. NF- κ B: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 2005;5(10):749–59.
- [108] Liang J, Nagahashi M, Kim EY, Harikumar KB, Yamada A, Huang W, et al. Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. *Cancer Cell* 2013;23(1):107–20.
- [109] Madia F, Grossi V, Peserico A, Simone C. Updates from the intestinal front line: autophagic weapons against inflammation and cancer. *Cells* 2012;1(3):535–57.
- [110] Vannucci L, Stepankova R, Kozakova H, Fiserova A, Rossmann P, Tlaskalova-Hogenova H. Colorectal carcinogenesis in germ-free and conventionally reared rats: different intestinal environments affect the systemic immunity. *Int J Oncol* 2008;32(3):609–17.
- [111] Li Y, Kundu P, Seow SW, de Matos CT, Aronsson L, Chin KC, et al. Gut microbiota accelerate tumor growth via c-jun and STAT3 phosphorylation in APC^{Min/+} mice. *Carcinogenesis* 2012;33(6):1231–8.
- [112] Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014;12(10):661–72.
- [113] Ou J, Carbonero F, Zoetendal EG, DeLany JP, Wang M, Newton K, et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am J Clin Nutr* 2013;98(1):111–20.
- [114] Russell WR, Gratz SW, Duncan SH, Holtrop G, Ince J, Scobbie L, et al. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr* 2011;93(5):1062–72.
- [115] Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KT. N-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *Am J Clin Nutr* 2011;93(5):1053–61.
- [116] Windey K, De Preter V, Verbeke K. Relevance of protein fermentation to gut health. *Mol Nutr Food Res* 2012;56(1):184–96.
- [117] Marquet P, Duncan SH, Chassard C, Bernalier-Donadille A, Flint HJ. Lactate has the potential to promote hydrogen sulphide formation in the human colon. *FEMS Microbiol Lett* 2009;299(2):128–34.
- [118] Roediger WE, Moore J, Babidge W. Colonic sulfide in pathogenesis and treatment of ulcerative colitis. *Dig Dis Sci* 1997;42(8):1571–9.
- [119] Di Martino ML, Campilongo R, Casalino M, Micheli G, Colonna B, Prosseda G. Polyamines: emerging players in bacteria-host interactions. *Int J Med Microbiol* 2013;303(8):484–91.
- [120] Pegg AE. Toxicity of polyamines and their metabolic products. *Chem Res Toxicol* 2013;26(12):1782–800.
- [121] Wiseman M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc Nutr Soc* 2008;67(3):253–6.
- [122] Homann N. Alcohol and upper gastrointestinal tract cancer: the role of local acetaldehyde production. *Addict Biol* 2001;6(4):309–23.
- [123] Lescut D, Colombel JF, Vincent P, Cortot A, Fournier L, Quandalle P, et al. Bacterial translocation in colorectal cancers. *Gastroenterol Clin Biol* 1990;14(11):811–4.
- [124] Klein GL, Petschow BW, Shaw AL, Weaver E. Gut barrier dysfunction and microbial translocation in cancer cachexia: a new therapeutic target. *Curr Opin Support Palliat Care* 2013;7(4):361–7.
- [125] Liang JQ, Chiu J, Chen Y, Huang Y, Higashimori A, Fang JY, et al. Fecal bacteria act as novel biomarkers for non-invasive diagnosis of colorectal cancer. *Clin Cancer Res*. Epub 2016 Oct 3.
- [126] Wong SH, Kwong TN, Chow TC, Luk AK, Dai RZ, Nakatsu G, et al. Quantitation of faecal *Fusobacterium* improves faecal immunochemical test in detecting advanced colorectal neoplasia. *Gut*. Epub 2016 Oct 24.
- [127] Wei Z, Cao S, Liu S, Yao Z, Sun T, Li Y, et al. Could gut microbiota serve as prognostic biomarker associated with colorectal cancer patients' survival? A pilot study on relevant mechanism. *Oncotarget* 2016;7(29):46158–72.
- [128] Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/ β -catenin signaling via its FadA adhesin. *Cell Host Microbe* 2013;14(2):195–206.