

MICROBIOTA IN COLORECTAL CANCER: ADVANCES IN 2022

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Abstract – The colonic mucosa has a complex interaction and is likely the most effective barrier against the numerically more superior microbial community within the gut microbiota. Therefore, it is probably not surprising that colorectal cancer (CRC), one of the largest challenges in gastroenterology and oncology, may be triggered and modulated by gut-microbial interactions more than many other diseases. Intense effort has been made to uncover the host:microbial interplay and provide simple, population-applicable and effective tools in the prevention of CRC with substantial scientific advances are made over the past year. As part of the series Year in Microbiota, this review provides comprehensive overview on the published literature between April 2021 and March 2022 in the area of CRC and the microbiota with potential highest impact in basic and translational research. This work includes studies on characterization of microbial alterations in CRC, functional models of microbiota in CRC, gut-immune system interaction and provides some summary on preventive and therapeutic knowledge.

Keywords: Colorectal cancer, Microbiome, Gut-immune axis, *Fusobacterium nucleatum*.

INTRODUCTION

Colorectal cancer (CRC) remains one of the most common gastrointestinal cancers with enormous impact on health care systems. Besides screening programs to prevent and identify early CRC and implementation of molecular understanding of CRC biology, microbiome research is considered the next level of scientific progress that could lead to improvement of diagnosis, prediction, prevention and treatment of CRC patients. The amount of published data on the gut-microbiome axis is overwhelming. This review summarizes recent advances in the field of microbiota and CRC published between April 2021 and March 2022 (Table 1).

MICROBIOTA IN CRC FROM THE CLINICAL PERSPECTIVE

Detailed characterization of the gut microbiome in cancer patients specifically in cancer tissue and preneoplastic lesions remains one of the crucial partially elucidated topics. The whole-genome sequencing approaches were used to characterise CRC primary tumours, corresponding metastases and matched normal tissue for gut microbiota including viral,



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phage and bacterial species¹. The findings showed an enrichment of certain bacteria and viruses in colorectal cancer and associated metastases, compared to healthy controls. The main bacteria in colorectal cancer were *Fusobacterium nucleatum* (*F. nucleatum*), *Streptococcus sanguinis*, *F. hwasookii* and *Angerococcus mediterraneensis*. Virome analysis revealed phages with 46% belonging to Myoviridae, Siphoviridae and Podoviridae. The most abundant viruses were cytomegalovirus, human herpesvirus 6B and 7, human endogenous retrovirus K113, Epstein-Barr virus and Megavirus chilensis. The colonic microbiota also harbors several different kinds of fungi. Analysis of fecal mycobiome between healthy controls, patients with adenoma and CRC revealed *Phanerochaete chrysosporium*, *Lachancea waltii*, and *Aspergillus rambellii* as enriched in CRC whereas, surprisingly, *Candida spp.* and *Pseudocercospora pini densiflorae* were more abundant in healthy tissue².

TABLE 1. SUMMARY OF THE PUBLICATIONS WITH THE KEY POINTS RELATED TO THE ROLE OF MICROBIOTA IN CRC INCLUDED IN THE REVIEW.

Topic	Authors	Ref.	Key points
Microbiome of CRC in Humans	Marongiu et al	1	<ul style="list-style-type: none"> In CRC, <i>F. nucleatum</i>, <i>S. sanguinis</i>, <i>F. hwasookii</i> and <i>A. mediterraneensis</i> are the most abundant bacteria, the most common viruses are phages, CMV, HHV 6B and 7, human endogenous retrovirus K113, EBV and Megavirus chilensis
	Gao et al	2	<ul style="list-style-type: none"> <i>Phanerochaete chrysosporium</i>, <i>Lachancea waltii</i>, <i>Aspergillus rambellii</i> are enriched in CRC
	Zhao et al	3	<ul style="list-style-type: none"> The microbiome differs between CRC-tissue and adjacent, healthy tissue
	Loftus et al	4	<ul style="list-style-type: none"> Oral microbes are linked to the carcinogenesis of CRC
	Lui et al	5	<ul style="list-style-type: none"> Differences in microbiome along the adenoma-carcinoma cascade
	Yang et al	6	<ul style="list-style-type: none"> <i>F. plautii</i> and dysbiosis is associated with young-onset CRC and metabolism
	Phipps et al	7	<ul style="list-style-type: none"> The microbiota differs between left- and right-sided colon in non-tumour tissue but not CRC
	Vilchez-Vargas et al	9	<ul style="list-style-type: none"> Mapping of microbiome along the mice GI-tract
	Wang et al	10	<ul style="list-style-type: none"> The oral microbiota are different in CRC patients
	Microbiome and molecular aspects of CRC	Salvucci et al	11
Wu et al		12	<ul style="list-style-type: none"> Virulence of <i>F. nucleatum</i> is influenced by CarRS, Lysine metabolism and RadD
Hong et al		13	<ul style="list-style-type: none"> <i>F. nucleatum</i> induces glycolysis in CRC and promotes it via lncRNA ENO1-IT1
Zhang et al		14	<ul style="list-style-type: none"> <i>F. nucleatum</i> induces ICAM1 via ALPK1 and promotes migration of CRC-cells
Chen et al		15	<ul style="list-style-type: none"> <i>F. nucleatum</i> downregulates METTL3 resulting in a more aggressive CRC biology
Bertocchi et al		16	<ul style="list-style-type: none"> <i>E. coli</i> promotes liver metastasis niches, disrupts the gut-vascular barrier and increases PV-1
Okumura et al		17	<ul style="list-style-type: none"> Butyrate producing <i>P. gingivalis</i> and <i>P. asaccharolytica</i> promote CRC
Li et al		18	<ul style="list-style-type: none"> A higher Squalene-epoxidase causes a pro-carcinogenic dysbiosis
Sugimura et al		19	<ul style="list-style-type: none"> <i>L. gallinatum</i> induces indol-3-lactic acid to act anti-cancerogenic
Bell et al		20	<ul style="list-style-type: none"> <i>L. reuteri</i> reduces tumor cell survival via reuterin and alteration of Redox-balance
Benito et al		21	<ul style="list-style-type: none"> <i>L. gasseri</i>, <i>B. bifidum</i> inhibited adenoma formation via suppression of Wnt
Liu et al		22	<ul style="list-style-type: none"> <i>L. rhamnosus</i> GG also inhibits Wnt-pathway and CRC when colonized early
Pieters et al		23	<ul style="list-style-type: none"> Mutations in Lynch Syndrome patient may be depend on the microbiota

CONTINUED

TABLE 1 CONTINUED. SUMMARY OF THE PUBLICATIONS WITH THE KEY POINTS RELATED TO THE ROLE OF MICROBIOTA IN CRC INCLUDED IN THE STUDY.

Topic	Authors	Ref.	Key points
Microbiome and immune system in CRC	Borowsky et al	24	• <i>F. nucleatum</i> decreases stromal memory T-helper cells in CRC tissue
	Sakamoto et al	25	• <i>F. nucleatum</i> increases MDSC and decreases CD8 ⁺ T-cells in liver metastasis
	Yin et al	26	• <i>F. nucleatum</i> also decreases NK-cells and increases T _{regs} in liver metastasis
	Zhang et al	27	• The expression of Chemokine receptors depends on the microbiota
	Peucker et al	28	• Depending on the microbiota, myeloid cells inhibit CD8 ⁺ T-cell activity
	Zhang et al	29	• <i>L. paracasei sh2020</i> increases CXCL10 and recruits CD8 ⁺ T-cells
	Xing et al	30	• <i>O. splanchnicus</i> induces T _{H17} -cells and protects from CRC in mice
	Oh et al	31	• <i>O. splanchnicus</i> supernatant induces apoptosis in CRC
	Fan et al	32	• <i>A. mucinophila</i> activates M1-Cells via TLR2 and acts anti-tumorigenic
	Shao et al	33	• <i>B. fragilis</i> inhibits M1-cells and acts anti-tumorigenic in a various CRC-models
Zhu et al	34	• <i>C. albicans</i> induces ILC3 and thus IL-22 in CRC which correlates with fungal burden	
Microbiome and CRC prevention	Illescas et al	35	• Mediterranean diet causes a shift to anti-inflammatory microbes
	Yang et al	36	• A high-fat diet causes a shift to pro-carcinogenic taxa
	Kordahi et al	37	• <i>B. fragilis</i> in pre-neoplastic polyps are pro-inflammatory and the microbiome differs depending on the presence of polyps
	Ngyuen et al	38	• A higher sulfur microbial diet score increases risk for early-onset adenomas
	Montalban-Arques et al	39	• <i>Clostridiales</i> are associated with lower tumor burden and activate CD8 ⁺ cytotoxic T-cells with <i>Roseburia intestinalis</i> and <i>Anaerostipes caccae</i>
	Shaw et al	40	• A high BMI increases pro-carcinogenic <i>Fusobacteria</i> and <i>Prevotella</i>
	Brennan et al	41	• Acetylsalicylic acid and metabolites kills pro-carcinogenic <i>F. nucleatum</i>
	Hiraishi et al	42	• Lactulose restores protective physiological microflora

A study by Zhao et al³ analyzed six independent CRC cohorts (n=353 patients) and compared the structure of microbiota between cancerous and adjacent noncancerous tissues. *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria* and *Actinobacteria* were the major phyla in the CRC gut microbiota. After analyzing the microbiota datasets, depletion of normal microbiota (*Clostridia* and *Bacteroidia* phyla) and significant enrichment of oral-originated pathogens (such as *F. nucleatum* and *Parvimonas micra*) were observed in CRC samples compared to normal tissues. The four main bacterial species that were altered in multiple datasets were *F. nucleatum* (mostly enriched in CRC patients), *F. prausnitzii* (mostly depleted in CRC patients), *P. micra* and *S. sputigena*. After analyzing the microbiota subtypes in greater detail, two microbiome-based CRC subtypes were identified. However, no significant microbial diversity/ differences were noted between tumour and normal samples.

Another study⁴ analyzed the taxonomic, functional and structural gut microbiota changes in CRC patients by comparing the whole-genome shorted sequenced fecal samples from total 252 healthy and late-stage CRC subjects. As expected, the bacterial species diversity was significantly higher in the CRC microbiota with the relative elevation in the abundance of oral microbiota species, such as *F. nucleatum*, *P. stomatis*, *G. morbillorum*, *P. micra*, *S. anginosus* and *D. pneumosintes*. Those oral disease-associated species seem to be positively associated with an increased risk for CRC

development. Based on the bacterial-association network analysis the authors concluded that oral-disease associated *P. stomatis* and *S. parasanguinis* may be considered as the influential bacterial species (bacterium which serve as the main point of connection between other species) in CRC subjects, while *Bacteroides fluxus* and *B. pectinophilus* were shared between the healthy and CRC subjects. The research also measured the abundance of protein families (TIGRFAMs) and protein domains (Pfam) and concluded that the only significantly elevated TIGRFAM in CRC subjects was linked to proline iminopeptidase while the elevated Pfams were linked to bacterial invasins and adhesins.

To understand the microbial community architecture along the colorectal carcinogenesis cascade, Liu et al⁵ analyzed 436 tissues biopsies from patients with CRC, pre-malignant adenomas and adjacent normal tissue and correlated the data with genetic alterations such as KRAS mutation and microsatellite instability (MSI). They found substantial variation of microbial communities within tumour tissues as well as along the adenoma-carcinoma sequences. Interestingly, intratumoral microbial heterogeneity was associated with genetic alterations of KRAS mutation or MSI. The results of this study strongly suggest that certain groups of microbes are associated with certain mutations and thus an interaction in the adenoma-carcinoma sequence could be postulated. Considering the potential role of microbes in cancer progression, another study⁶ had a look at young-onset colorectal cancer patients. *Flavoifractor plautii* was observed as one of the bacterial species linked to young-onset CRC, while *Streptococcus* was the key phylotype in the old-onset CRC. These conclusions were supported by the results from 728 patients from initial cohort, independent validation in 310 samples and by a special functional metagenomic analysis.

An study⁷ observed a difference in microbiota between the left- and right-sided colon in non-tumourous tissue, whereas the microbiota in colorectal cancer did not differ greatly between the two sides. Although previous study⁸ reported on comparable mucosal microbiome pattern in colon for various regions, the results may be partially affected by the sample collection procedure through the endoscopy channel. Bacterial profiling of mucosal microbiome in murine model indeed identified a continuous shift of relative bacterial abundance non only between different GI regions, but also specifically in colon⁹.

Another interesting clinical study by Wang et al¹⁰ analyzed the oral and gut microbiota of CRC patients and healthy controls (HC) and their association with host clinical factors. Matching saliva, cancerous tissue/healthy biopsies and stool samples were collected, analyzed and compared. The salivary and mucosal but not stool microbiota diversity in CRC patients was statistically significantly different compared to healthy controls. Interestingly, the salivary microbiota α -diversity was higher whereas the mucosal diversity was lower in CRC patients compared to healthy individuals. The relative abundance of *Bacteroides*, *Streptococcus* and *Desulfovibrio* genera was increased in the saliva of CRC patients. As expected, *Firmicutes* and *Bacteroides* in the mucosal microbiota were more abundant in CRC patients. What is more, oral and mucosal microbiota were clustered into different types. Higher oral abundance of *Streptococcus*, *Veillonella*, *Neisseria* and *Fusobacterium* genera and higher mucosal abundance of *Fusobacterium*, *Bacteroides*, *Streptococcus* and *Peptostreptococcus* genera was observed in CRC patients.

MOLECULAR INSIGHT OF MICROBIOME IN CRC

F. nucleatum has received the broadest attention in CRC for its potential clinical and translation relevance. The relationship between *F. nucleatum* and different molecular subtypes and CRC intrinsic subtypes has been studied by Salvucci et al¹¹. Using The Cancer Genome Atlas (TCGA) data the authors identified two distinct subpopulations of CRC patients dependent on mesenchymal traits and *F. nucleatum*. Mesenchymal tumors and high *Fusobacteriales* were associated with approximately two-fold higher risk of worse outcome which was not the case in non-mesenchymal patients.

Fusobacterium nucleatum is known to colonise not only oral cavity but also CRC tissue, however, the molecular mechanism and subsequent consequences of this colonization remain poorly understood. Wu et al¹² used a genome-wide transposon mutagenesis to understand coaggregation factors related to *F. nucleatum*. They identified a two-component signal trans-

duction system named CarRS and a lysine metabolic pathway relevant for enhanced modulation of RadD, an adhesin protein of *F. nucleatum*, which is involved in interspecies interaction, virulence, nutrient acquisition to create a respective environment and participate in biofilm formation.

Molecular analysis highlighted involvement of *F. nucleatum* in colon carcinogenesis. A positive influence of *F. nucleatum* on the glucose metabolism was observed in CRC cells *in vitro*¹³. This effect was mediated by lncRNA (long non-coding RNA) ENO1-IT1 (enolase1-intronic transcript 1), which further promoted carcinogenesis and was associated with worse outcome. Another study further elaborated on the influence of *F. nucleatum* in development of metastasis in CRC. *F. nucleatum* enhanced the adhesion of CRC cells to endothelial cells as well as promoting extravasation and metastasis by inducing a new pattern recognition receptor ALPK1 and upregulation of ICAM1 expression. High expression of both proteins (ALPK1 and ICAM1) was associated with shorter overall survival time in CRC subjects. This study¹⁴ was one of a few which confirmed the role of gut microbiota in the distant spread of CRC. In addition, *F. nucleatum* was shown to induce a decline in m6A modifications in CRC cells and also in patient-derived xenograft (PDX) tissues through downregulation of an m6A methyltransferase (METTL3)¹⁵. Reduction of METTL3 led to upregulation of kinesin family member 26B (KIF26B) which is linked to a more aggressive tumor biology and a worse survival.

Besides *F. nucleatum*, other species are considered to play function role in formation of metastasis. Bertocchi et al¹⁶ investigated the mechanism in modulation of premetastatic niche in the liver. *Escherichia coli* was shown to disrupt the gut vascular barrier along the gut-liver axis which led to an increased level of endothelial marker PV-1. PV-1 expression was dependent on virulence factor VirF and linked to premetastatic niche development in the liver. Furthermore, PV-1 associated with liver-bacteria dissemination and metachronous distant metastases.

A study by Okumura et al¹⁷ found an association between increased butyrate secretion and colonic carcinogenesis. Among 12 bacterial taxa enriched in feces of CRC patients, 2 *Porphyromonas* species (*P. gingivalis* and *P. asaccharolytica*) were found to induce an oncogenic stress-response by increased secretion of the bacterial metabolite butyrate. Increased levels of these bacterial taxa observed in the CRC subjects were associated with the simultaneous increase in butyrate levels and accordingly inflammatory responses, suggesting that butyrate-producing bacteria may accelerate the carcinogenesis. However, not only the bacteria-related metabolism may be important, but also the host own metabolism is believed to impact the composition of gut microbiota which furthermore can contribute to carcinogenesis. For instance, higher expression of squalene epoxidase led to a dysbiosis with a higher density of bacteria associated with carcinogenesis in a mouse model¹⁸. The transfer of the altered microbiota to healthy mice was sufficient to accelerate cell proliferation and impair the barrier function of the gut.

Besides procarcinogenic bacterial species, there was increasing evidence that certain bacterial species may be associated with beneficial anti-tumorigenic effects. Abundance of *Lactobacillus gallinarum* is reported to be lower in patients with colorectal cancer. Sugimura et al¹⁹ showed using *in vivo* murine tumorigenesis model that *L. gallinarum* reduced tumour numbers compared to placebo or *E. coli*. *In vitro* experiments using culture-supernatant confirmed antiproliferative and proapoptotic effect in CRC cells and organoids, which led to identification of indole-3-lactic acid as potential protective metabolite. *L. reuteri* also has an anti-tumorigenic effect *via* secretion of its metabolite reuterin. Reuterin was observed to alter the redox balance and thus reduce proliferation and survival of colorectal cancer cells *via* selective protein oxidation²⁰. Another study²¹ found, that supplementation of *L. gasseri*, *Bifidobacterium bifidum* and quercetin inhibited the formation of adenomas *via* suppressing the pro-carcinogenic Wnt-pathway in APC-min Mice. A similar pattern has been shown for *L. rhamnosus* GG which was also associated with anti-inflammatory and antiproliferative effect related to Wnt-pathway²².

Besides sporadic tumours, tumours with hereditary background provide an excellent avenue for assessing the impact of the microbiome on carcinogenesis. Therefore, understanding of microbiome-carcinogenesis links in subjects with Lynch-Syndrome is exceptionally relevant, even though at present still poorly elucidated. Pieters et al²³ investigated the influence of microbiota on the tumor formation in mode *Msh2*-Lynch mice model. Under specific-pathogen-free conditions the authors observed a lower mutational rate and almost complete loss of intestinal tumor development which was linked to lower intestinal inflammation as in conventional mice. This effect

could be reversed following fecal microbiota-transplantation of stool from conventional mice and was linked to particular increase of the abundance of mucus-degrading taxa such as *Desulfovibrio* and *Akkermansia* and increase in MSI.

GUT-IMMUNE AXIS IN CRC

The involvement of gut microbiome in various steps of carcinogenesis may be linked to direct interaction with epithelial from one side or be involved in very complex interaction with immune system through so called gut-immune axis from another side. *F. nucleatum* is suggested to suppress antitumor T-cell activity and thus promote CRC (Figure 1). A recent study²⁴ analyzed the tumour stromal T-cell subsets in well characterized CRC cases using multiplex immunofluorescence combined with digital image analysis and machine learning algorithms. They found an inverse association between *F. nucleatum* and stromal CD3+ lymphocytes and particular decrease in CD3+CD4+CD45RO+ T-cells (stromal memory helper T-cells) in *F. nucleatum* positive cancers. On the other hand, tumour-associated macrophages and intra-epithelial T-cells did not differ significantly. A different group²⁵ analyzed the composition of T-cells, tumour-associated macrophages and myeloid-derived suppressor cells (MDSC) in liver metastases of CRC in relation to *F. nucleatum*. Whereas tumour associated macrophages also did not differ, there was a higher abundance of MDSC and, interestingly, a lower density of cytotoxic CD8+ T-cells.

In a CRC metastasis model, *F. nucleatum* promoted metastases growth via regulation of the immune system and the influx of immune cells²⁶. Next to a higher abundance of myeloid-derived suppressor cells, an increase of regulatory T-cells was also observed *in vivo*. Furthermore, there was not only a lower density of cytotoxic T-cells, but also of natural killer cells, which are known to induce anti-tumour activity. A possible explanation could be an influence of chemokine expression for the attraction of immune cells like CD8+ T-cells depending on the microbiota. However, more studies are needed to clarify the whole role of the interaction between microbiota and migration of immune cells into the tumour tissue²⁷.

In respect to the anti-tumor activity of CD8+ T-cells another study²⁸ found a myeloid cell dependent sensing of microbes resulting in an inhibition of cytotoxic T cells. In this study, microbial sensing by myeloid cells promoted IL-6 release and expression of co-inhibitory B7H3 and B7H4 molecules in tumor cells, which led to inhibition of CD8+ T-cell-dependent anti-tumour

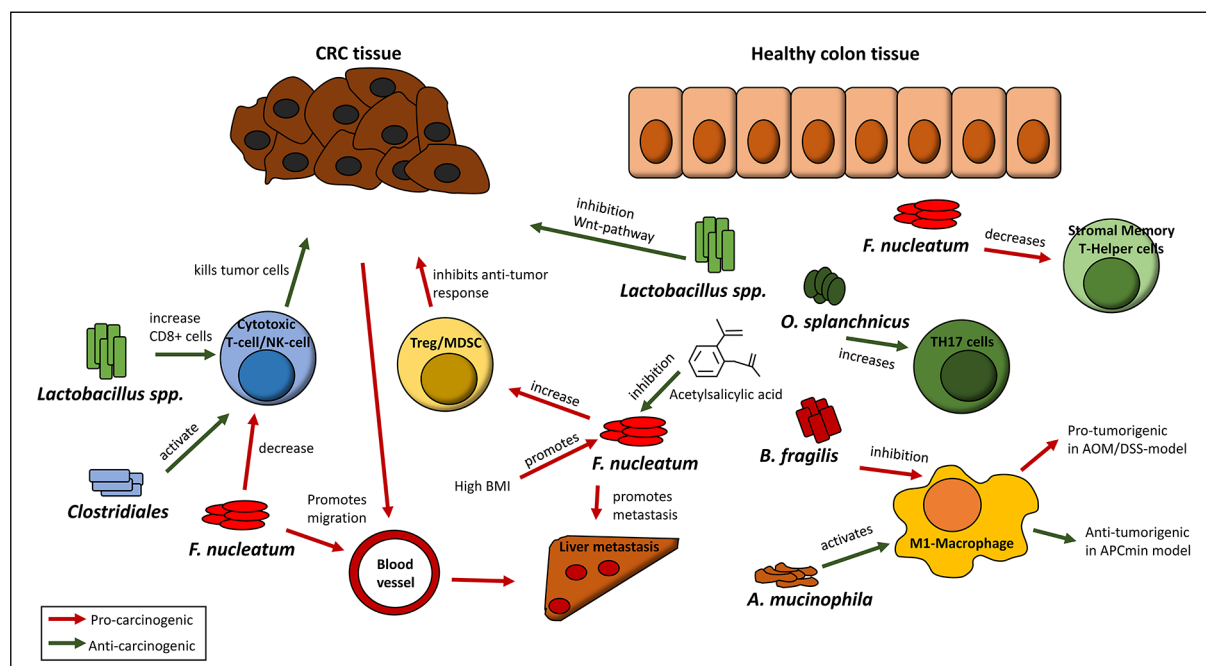


Figure 1. An overview of results related to gut-immune system interaction related in CRC.

immunity. While targeting the pathways led to activation of CD8⁺ T-cells and CRC growth, suggesting that targeting the pathway including microbiota may provide an additional target for immune-targeted therapy. For instance, a study²⁹ showed that a novel microbe *Lacticaseibacillus paracasei* sh2020 induced an upregulation of CXCL10 and the recruitment of CD8⁺ cytotoxic T-cells which was led to anti-tumor activity. A higher abundance of *Lacticaseibacillus paracasei* sh2020 was associated with a better response to anti-PD-1 treatment.

Odoribacter splanchnicus seems to be also involved in regulation of innate immune signaling and anti-tumoural effect. The interaction between the innate and adaptive immune systems and the microbiota were analyzed in a mouse model lacking a pro survival molecule in myeloid cells³⁰. Those mice exhibited altered microbiota with resistance to colitis and CRC. Further analysis revealed a higher abundance of *Odoribacter splanchnicus* (*O. splanchnicus*) which induced Th17 cells and was crucial for protection to colitis and CRC in wild-type mice. Another study³¹ analyzed the effect of the cell free supernatant from healthy people with *O. splanchnicus* *in vitro* and *in vivo* mouse models. In both models, *O. splanchnicus* induced anti-proliferative activity through apoptosis in CRC cells leading to a protective effect most likely mediated through malic acid.

The number of mechanistic studies on gut-immune system interaction is evolving continuously. In APC^{min/+} mouse, bacterium *Akkermansia mucinophila* (*A. mucinophila*) was shown to interact with the host immune system in a TLR2 and NLRP3-dependent manner³². *A. mucinophila* activated M1-like macrophages and promoted tumor suppressive effects. In a AOM/DSS-mouse model of colorectal carcinogenesis *Bacteroides fragilis* exerted a negative regulation of M1 macrophages *via* secretion of short-chain fatty acids like butyrate and a negative influence on NLRP3 signaling³³. This effect leads to a less inflammation and colitis-associated carcinogenesis. Therefore, likely there is complex interaction between various co-factors in microbiota-host interaction and different models of carcinogenesis may lead to variable results.

Besides bacteria, fungi interact with the host immune system and can promote colorectal carcinogenesis. Zhu et al³⁴ found, that *Candida albicans* induced glycolysis and IL-7 secretion in macrophages. This leads to an activation of IL-22 producing group 3 innate lymphoid cells (ILC3). The amount of IL-22 correlates with the fungal burden in colorectal cancer.

MICROBIOME AND CRC PREVENTION

Diet is among the most important factor affecting the microbial niche and has been associated with CRC. Mediterranean diet has been linked to a positive impact on gastrointestinal diseases. A meta-analysis³⁵ showed a shift between anti- and pro-inflammatory bacteria in people under Mediterranean diet that favors anti-inflammatory taxa. Since pro-inflammatory bacteria may favor inflammation and subsequently the development of colorectal cancer, a preventive effect for Mediterranean diet may be considered and generally recommended. On the contrary³⁶, a high-fat diet resulted in a pro carcinogenic shift of microbiota in a mouse model, which could be reversed *via* antibiotic depletion of the microbiota. The transfer of the microbiota from mice under high-fat diet was also sufficient to support carcinogenesis. Modulated metabolic products are most likely at least partially responsible for the observed changes. Recent study³⁷ reported on distinct microbial pattern in patients with and without polyps. There was a correlation between *Bacteroides fragilis* and increased inflammatory cytokines in mucosa adjacent to the polyps, suggesting bacterial involvement in modulation of mucosal microenvironment. High-fat-diet in AOM-treated and APC^{min/+} mice compared to control diet promoted carcinogenesis. Fecal microbiota transplantation from high-fat-diet feed mice promoted oncogenic genes, promoted proliferation and impaired gut barrier.

Nguyen et al³⁸ evaluated the impact of sulfur microbial diet and the risk of colon adenoma development in a Nurses Health Study II. According to the data, the higher sulfur microbial diet scores were associated with increased risk for early-onset adenomas in young and adolescence women. But also, the vice versa, *Clostridiales* bacteria were associated with lower tumor burden in colon cancer models, while oral application of *Clostridiales* strain prevented and even treated CRC in mice. Besides *Clostridiales* strains, *Roseburia intestinalis* and *Anaerostipes caccae* were linked to activation of CD8⁺ T cells suggesting microbiome as potential immune-modulating therapy³⁹.

Diet may be associated with weight and a high body-mass index (BMI), which are known risk factors for CRC. Shaw et al⁴⁰ compared the microbiota from patients with colorectal cancer and found a difference depending on the patient's BMI. Especially *Fusobacteria* and *Prevotella* were increased in patients with a BMI over 25 kg/m². These findings suggest that BMI-dependent changes in the microbiota may be a risk factor for the development of CRC.

While it may be rather challenging to comply with long-term diet changes, there is a great hope that diet-based treatment could solve this gap and contribute to reliable protection in colorectal carcinogenesis. Acetylsalicylic acid, which is deacetylated to salicylic acid, has been reported to effectively kill the pro-carcinogenic *F. nucleatum* in vitro⁴¹. In an APC^{min/+} mouse model effectiveness of aspirin-supplementation in inhibition of *F. nucleatum*-potentiated colonic tumorigenesis was confirmed. Even in human adenoma tissue from patients with daily intake of acetylsalicylic acid the lower abundance of *F. nucleatum* and some other pro-carcinogenic bacteria like *Bacteroides fragilis* has been reliably shown.

Another study⁴² investigated the effect of the commonly used laxative lactulose. In the AOM/DSS mouse model alterations in the composition of microbiota contributed to inflammation and carcinogenesis. This effect could be counteracted by lactulose, which was associated with a more physiological microbiome with higher density of *Muribaculum* and *Lachnospiraceae*, which was subsequently associated with a decrease in inflammation and tumorigenesis.

CONCLUSIONS

The overview of the past year on the advances in understanding of microbiota in CRC provides not only additional puzzles in characterization of microbial alterations, but also high-quality functional studies that deliver potential goals on prevention and targeted-therapy. Further excellent studies with a scope on multi-omic analysis including multilevel approach involving microbiome and immune system will be necessary to uncover the missing gaps in the field of microbiota-host interactions.

Conflict of Interest

The authors declare no conflict of interest.

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Robert Jaensch: acquisition of the data, drafting of the article, Paulius Jonaitis: acquisition of the data, drafting of the article, Juozas Kupcinskas: conception and design of the study, acquisition of the data, drafting of the article, critical revision, supervision, Alexander Link: conception and design of the study, acquisition of the data, drafting of the article, critical revision, supervision, final approval of the version.

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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