

REVIEW

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Therapeutic methods of gut microbiota modification in colorectal cancer management – fecal microbiota transplantation, prebiotics, probiotics, and synbiotics

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ABSTRACT

The link between gut microbiota and the development of colorectal cancer has been investigated. An imbalance in the gut microbiota promotes the progress of colorectal carcinogenesis *via* multiple mechanisms, including inflammation, activation of carcinogens, and tumorigenic pathways as well as damaging host DNA. Several therapeutic methods are available with which to alter the composition and the activity of gut microbiota, such as administration of prebiotics, probiotics, and synbiotics; these can confer various benefits for colorectal cancer patients. Nowadays, fecal microbiota transplantation is the most modern way of modulating the gut microbiota. Even though data regarding fecal microbiota transplantation in colorectal cancer patients are still rather limited, it has been approved as a clinical method of treatment-recurrent *Clostridium difficile* infection, which may also occur in these patients. The major benefits of fecal microbiota transplantation include modulation of immunotherapy efficacy, amelioration of bile acid metabolism, and restoration of intestinal microbial diversity. Nonetheless, more studies are needed to assess the long-term effects of fecal microbiota transplantation. In this review, the impact of gut microbiota on the efficiency of anti-cancer therapy and colorectal cancer patients' overall survival is also discussed.

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Introduction

Currently, colorectal cancer (CRC) is the third most common cancer worldwide with more than 1.2 million new cases as well as 600,000 deaths occurring every year.¹ According to genomic mutation diversity, CRC can be classified into two typical types: colitis-associated colorectal cancer (associated with the presence of a mutation in the *TP53* gene) and sporadic colorectal cancer (caused by a mutation in the adenomatous polyposis coli gene – *APC*).² The etiology of CRC includes not only the genetic background but also environmental factors, such as high-fat diet, deficiency of fiber intake, red meat consumption, sedentary lifestyle, and many others.²

There seems to be an association between the composition of gut microbiota and the occurrence of colorectal cancer. Changes in the gut microbiota may contribute to the development of colorectal

cancer although they may also be attributable to the side effects of anti-cancer therapy. It should be emphasized that the human gut microbiota consists of not only bacteria but also viruses, fungi, and *Archaea*.³ The qualitative and quantitative alterations in gut microbiota may lead to an imbalance, also known as gut dysbiosis. There are many factors involved in the development of gut dysbiosis in colorectal cancer, mainly altered eating habits, low level of physical activity, infectious agents, surgery treatment, and the administration of antibiotics.⁴ In addition to the composition of the gut microbiota, metabolites produced by microbiota may play a crucial role as they can exert beneficial effects for the host, because of their antioxidant and anti-inflammatory properties, regulation of bowel barrier function, production of vitamins, as well as being a source of energy.⁵

Nowadays, there are several therapeutic approaches available with which to modify gut microbiota. This

paper summarizes the current knowledge about therapeutic methods of gut microbiota modifications in the management of colorectal cancer including the role and possibilities of fecal microbiota transplantation as well as the administration of probiotic strains, prebiotics, and synbiotics.

The role of microbiota in the colorectal carcinogenesis process

The link between human gut microbiota imbalance and colorectal carcinogenesis may involve several species-specific mechanisms.⁶ An abundance of *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Escherichia coli*, *Enterococcus faecalis*, *Helicobacter hepaticus*, *Peptostreptococcus anaerobius*, *Helicobacter pylori*, *Streptococcus bovis*, and *Porphyromonas gingivalis* has been related to CRC cancer development.⁷ Microbes are known to exert not only pathogenicity but also carcinogenicity.⁶ Certain bacterial species can trigger the development of colorectal cancer *via* multiple mechanisms. Some of the mechanisms through which bacteria can be implicated in CRC carcinogenesis are presented in Table 1.

The identification of CRC-associated pathogens is crucial for establishing gut microbiota as a potential screening tool for colorectal cancer.^{20,21} The fecal microbiome can be used as a tool toward developing targeted noninvasive biomarkers for colorectal cancer,

as shown by Yu *et al.* who investigated ethnically different cohorts (Danish, French, Austrian, and Chinese).²² It was confirmed that there was a link between the presence of *Fusobacterium nucleatum* and *Peptostreptococcus stomatis* and colorectal cancer. Furthermore, there was also an association found with other several species, such as *Parvimonas micra* and *Solobacterium moorei*. Twenty microbial gene markers were identified in their Chinese population that differentiated between the microbiome of colorectal cancer patients and healthy controls and four of those markers were also present in the Danish cohort. These four genes distinguished colorectal cancer metagenomes from controls with areas under the receiver-operating curve (AUC) of 0.72 and 0.77 in French and Austrian cohorts, respectively. Additionally, the co-occurrence of *Parvimonas micra* and *Fusobacterium nucleatum* suggests that there may be cooperation between those two species not only in colonization but also in the progression of CRC. It is possible that if an abundance of those bacteria is present in CRC samples as early as in stage II of CRC, these might be useful as noninvasive early diagnostic biomarkers for colorectal cancer when assayed from fecal samples.²²

Deoxycholic acid (secondary bile acid, DCA) is associated with intestinal carcinogenesis.¹⁶ Cao *et al.* investigated the effect of deoxycholic acid on the induction of intestinal dysbiosis as well as its role in intestinal carcinogenesis processes.²²

Table 1. Some selected potential microbiota-dependent mechanisms participating in colorectal carcinogenesis.

CRC-associated pathogens	Basic characteristics and/or properties	Functions/mechanisms	References
<i>Fusobacterium nucleatum</i>	● Opportunistic commensal, obligate, anaerobic, gram-negative bacterium	● It increases tumor multiplicity and selectively recruits tumor-infiltrating myeloid cells promoting tumor progression	8
<i>Bacteroides fragilis</i>	● Synthesis of the BFTs	● It activates STAT3 and stimulates the IL-17 production, consequently promoting NF-κB and Wnt pathway activation leading to tumor formation	9
<i>Escherichia coli</i>	● CDTs released from pathogenic <i>E. coli</i>	● It contributes to over-proliferation of normal epithelial cells ● It causes DNA damage and changes in the genome	10,11
<i>Enterococcus faecalis</i>	● Facultative, anaerobic, commensal bacterium of oral cavity and gastrointestinal tract	● It destroys DNA <i>via</i> free radicals (ROS, RNS)	12
<i>Helicobacter hepaticus</i>	● Pathogenic gram-negative bacterium	● It causes the secretion of proinflammatory mediators (IL-1β, IL-6, IL-8, TNF-α, IFN-γ)	13
<i>Peptostreptococcus anaerobius</i>	● Gram-positive anaerobic coccus bacterium	● It is involved in mechanism encompassing interaction with TLR2 and TLR4, activation of SREBP-2 signaling, and induction of oxidative stress	14
<i>Helicobacter pylori</i>	● Gram-negative bacterium	● Infection with <i>CagA+</i> <i>Helicobacter pylori</i> promotes the secretion of gastrin which may induce the proliferation of mucosal cells in the colon	15–17
<i>Streptococcus bovis</i>	● Gram-positive anaerobic bacterium	● The wall-extracted <i>Streptococcus bovis</i> antigen induces COX-2 expression promoting cell proliferation and angiogenesis as well as inhibiting apoptosis	18
<i>Porphyromonas gingivalis</i>	● Gram-negative oral anaerobe	● It can regulate NLRP3 inflammasome activity, therefore, plays a role in the progression of colorectal neoplasia	19

BFTs: *Bacteroides fragilis* toxins; CDTs: cell death toxins; ROS: reactive oxygen species; RNS: reactive nitrogen species; TLR: toll-like receptor; SREBP-2: sterol regulatory element-binding protein 2; STAT3: signal transducers and activators of transcription; NLRP3: Nod-like receptor 3.

Changes of the composition of intestinal microbiota were induced in DCA-treated *Apc^{min/+}* mice; it was observed that the transfer of fecal microbiota from DCA-treated mice to another group of mice increased tumor multiplicity, induced inflammation and activated tumor-associated Wnt/ β -catenin signaling pathway leading to changes in the cells' cycle, apoptosis, and proliferation. These results indicate that alterations in the microbiota induced by DCA may promote the process of intestinal carcinogenesis.²²

Wong *et al.* investigated whether stools from colorectal cancer patients could directly induce colorectal carcinogenesis in mice.²³ Conventional mice (male C57BL/6) with induced colon neoplasia were gavaged twice a week for 5 weeks with stool specimens from five patients with colorectal cancer or five healthy individuals (controls). Germ-free C57BL/6 mice were gavaged once with stool specimens from five patients with colorectal cancer or five controls. A higher proportion of Th-1 cells and Th-17 cells were detected in germ-free and conventional mice receiving stool from patients with colorectal cancer compared to mice fed with stool specimens from controls (2.25% vs. 0.44% and 2.08% vs. 0.31%, respectively, $p < .05$). There was an increased expression of the cytokines that modulate inflammation (C-X-C motif chemokine receptor 1, CXCR1, C-X-C motif chemokine receptor 2, CXCR2, IL-17A, IL-22, IL-23A) in conventional as well as in germ-free mice administered stool from colorectal cancer patients. This demonstrated that the fecal microbiota from patients with colorectal cancer can promote tumorigenesis in germ-free mice as well as in conventional mice administered a carcinogen.²³ Similar results were obtained by Li *et al.*; they examined *Apc^{min/+}* mice and noted that changes in gut microbiota enhanced the progression of intestinal adenoma.²⁴ It was shown that the administration of feces from colorectal cancer patients evoked an increase in tumor proliferation and a decrease in the apoptosis of tumor cells. There was also evidence of an impairment of gut barrier function, an upregulation of the proinflammatory cytokines profile as well as activation of the Wnt signaling pathway. Furthermore, the study of Rosshart *et al.* indicated that laboratory mice transplanted with the intestinal microbiome from wild mice displayed a better resistance to colorectal cancer.²⁵

Alterations in the intestinal microbiota in colorectal cancer and adenoma were also investigated by Ohigashi *et al.*²⁶ who examined 93 patients with colorectal cancer and 49 healthy individuals including 22 with adenoma and 27 without adenoma. A significant decrease in the concentration of short-chain fatty acids (SCFAs) in the colorectal cancer group was detected (compared to both adenoma and non-adenoma groups). At the same time, there was an increase in pH. Importantly, although the increase in pH was observed also in healthy individuals with adenoma, it was still only half that occurring in the CRC patients, strongly suggesting that it was not the development of CRC that was responsible for the changes, but instead, it was the cancer that had initiated and promoted the progression in the changed environment. Additionally, the counts of total bacteria were significantly lower in the colorectal cancer group as compared to healthy individuals (10.3 ± 0.7 vs. 10.8 ± 0.3 log₁₀ cells/g of feces; $p < .001$).²⁶

The alterations of the metabolome in colorectal cancer

The metabolome is described as the complete set of all small molecule (<1500 Da) metabolites found in a specific cell, organ, or organism. In addition to the changes in gut microbiota, there are also alterations in the fecal metabolome of patients associated with colorectal cancer.²⁷ An analysis of the colorectal cancer-associated metabolome has revealed differences in the biochemical composition of colorectal cancer patients' stools. For instance, significant alterations are evident in fatty acid metabolites and metabolites associated with bile acids in feces.^{28–31} It was shown that in stools taken from patients with colorectal cancer, there were higher levels of some amino acids as well as changes in the amounts of some short-chain fatty acids (SCFAs) in comparison with the values in healthy control subjects.³² The abundances of species of butyrate-producing bacteria, such as *Ruminococcus* spp. and *Pseudobutyrvibrio ruminis*, were lower in stool samples from CRC patients in comparison to healthy controls.³² Similarly, another study showed that colorectal cancer patients' stool samples were depleted of butyrate and several butyrate-producing bacteria.³³ It should be noted that SCFAs (mainly butyrate) are microbial metabolites

with anti-tumorigenic properties. Butyrate enhances the intestinal barrier by facilitating the assembly of tight junctions *via* the activation of AMPK (AMP-activated kinase protein) in Caco-2 cell monolayers (a human colonic epithelial cell line).³⁴ Furthermore, Chen et al. have reported that butyrate-activated T-regulatory cells block pro-inflammatory T cells and thus reduce the production of pro-inflammatory cytokines; consequently, butyrate may contribute to the prevention of colon cancer.³⁵

It was also reported that patients with colorectal cancer have an increased fecal bile acid concentration. Eating habits and consuming a high-fat diet lead to an increase in bile acid secretion and consequently may contribute to the occurrence of colorectal cancer.³¹ Furthermore, a higher level of H₂S is also detected in such cases.³⁶ Thus, the fecal metabolomics may be used for diagnostic purposes, and moreover, in the future, it may be exploited as a prognostic tool in the treatment of colorectal cancer.³⁷

Modification of gut microbiota in CRC – therapeutic methods

The composition and activity of gut microbiota are strongly associated with colorectal carcinogenesis

as well as with the efficiency of anti-cancer therapy.³⁸ Currently, there are several therapeutic methods used to alter the gut microbiota and as a consequence to improve the clinical outcome (Figure 1).

Fecal microbiota transplantation (FMT)

FMT is the most innovative method used to alter the gut microbiota, and it is defined as a transplantation of gut microbiota from healthy donors to sick patients *via* the upper or lower gastrointestinal route.³⁷ One of the aims of this therapy is to restore intestinal microbial diversity. There are banks of donated feces available for FMT. These specimens are prepared according to a well-established protocol to avoid potential risk factors (e.g., the presence of viruses or parasites). FMT has been approved as a clinical method to treat recurrent *Clostridium difficile* infection according to 2013 guidelines and it is currently the most common indication for FMT. There are also studies which have confirmed the beneficial effects of FMT in the treatment of other diseases, e.g., intractable functional constipation, inflammatory bowel diseases, and hematologic malignancies.³⁷ In a prospective, single-center trial,

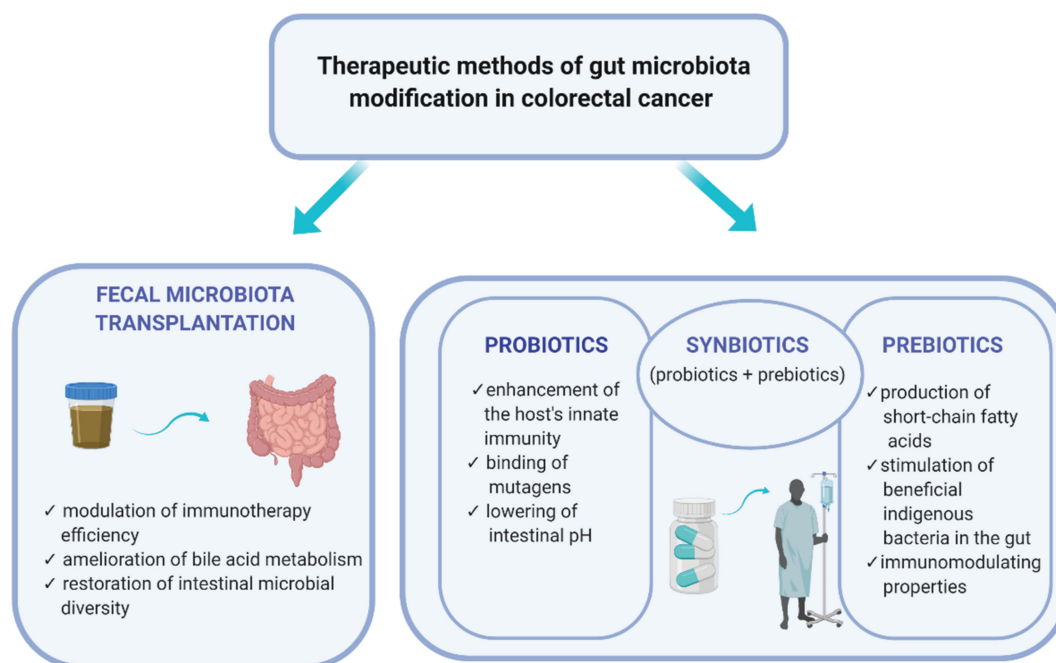


Figure 1. The selected effects of therapeutic methods of gut microbiota modification on colorectal cancer management – fecal microbiota transplantation and the administration of pre-, pro-, and synbiotics. The scheme is our own interpretation of results in the literature.^{37,38}

performed in the Department of Hematology, Oncology and Internal Diseases of the Medical University of Warsaw, Poland, it was demonstrated that FMT in patients with blood disorders (total $n = 20$: acute myeloblastic leukemia $n = 5$; acute graft-versus-host disease $n = 4$; chronic graft-versus-host disease $n = 2$; multiple myeloma $n = 3$; diffuse large B-cell lymphoma $n = 2$; myelodysplastic syndrome $n = 1$; lung cancer $n = 1$; thrombotic thrombocytopenic purpura $n = 1$; kidney transplant recipient $n = 1$) was able to inhibit gut colonization with antibiotic-resistant bacteria (ARB).³⁹ The gut-colonizing ARB included *Klebsiella pneumoniae* NDM1+ ($n = 14$), carbapenem-resistant *Klebsiella pneumoniae* ($n = 3$), *Klebsiella pneumoniae* extended-spectrum β -lactamase positive (ESBL+; $n = 2$), *Escherichia coli* ESBL+ ($n = 11$), *Pseudomonas aeruginosa* metallo- β -lactamase (MBL; $n = 2$), carbapenem-resistant *Pseudomonas aeruginosa* ($n = 2$), carbapenem-resistant *Enterobacter cloacae* ($n = 2$), vancomycin-resistant enterococci ($n = 2$), and other strains of ARB ($n = 3$). The complete decolonization of ARB was achieved in 75% of participants, while at least partial decolonization of ARB was noted in 80% of patients. It was shown that FMT was safe and efficient in these cases even though 40% of the patients had neutropenia at the time of FMT initiation.³⁹ Recently, the use of FMT in cancer management including colorectal cancer has been considered. The main aim of FMT is to reduce the activation of inflammatory, proliferative, and pro-carcinogenic pathways as well as microbiota-induced genotoxicity. Nonetheless, FMT has not been extensively studied in colorectal cancer.³⁷

Nowadays, immune checkpoint inhibitors (ICIs) are used in the treatment of cancer; they have exerted a significant impact on survival in patients with advanced diseases.⁴⁰ Nonetheless, treatment with ICIs can be associated with serious immune-related toxicities, such as ICI-associated colitis.⁴¹ Recently, Wang *et al.* reported the first case series of ICI-associated colitis (two patients with persistent symptoms despite receiving corticosteroids, infliximab – an anti-TNF- α agent, and vedolizumab – an anti-integrin agent) which was successfully treated with FMT, permitting the restoration of gut microbiota balance accompanied by a relative increase in the proportion of regulatory

T cells within the colonic mucosa.⁴¹ However, in this study, only two patients were treated and there were no control patients; therefore, it is plausible that the effect of FMT may have been attributable to some delayed efficacy of the prior immunosuppressive therapies. Routy *et al.* have shown that cancer patients treated with anti-PD-1/PDL-1 antibodies lived for a significantly shorter time if they had received oral treatment with antibiotics.⁴² Additionally, the response to therapy was associated with an abundance of *Akkermansia muciniphila*. Furthermore, both the transplantation of microbiota from patients responding to therapy and supplementation with *Akkermansia muciniphila* alone restored the sensitivity to immunotherapy.⁴² Nevertheless, additional studies will be necessary to confirm these results and define the mechanisms in more detail.

The safety of fecal microbiota transplantation is still controversial.³⁷ FMT is a rather innovative method used as a therapeutic approach to change gut microbiota, and since it has only recently been administered, there is a lack of long-time safety trials. Furthermore, there is a risk of adverse events (AEs) from FMT.³⁷ Youngster *et al.* have been reported that while frozen capsule FMT administered orally was effective in treating recurrent CDI, the therapy did evoke mild AEs, such as abdominal cramping and bloating.⁴³ Other authors have reported that patients have also experienced sore throat after FMT.⁴⁴ In the study of Kelly *et al.*, two deaths within 12 weeks after FMT were noted; however, only one death was related to FMT; due to aspiration during sedation when the FMT was being administered.⁴⁵ Additionally, the systematic review of Wang *et al.* evaluated the AEs associated with FMT. Initially, a total of 7562 articles were initially assessed but only 50 fulfilled the inclusion criteria. Although abdominal discomfort was assessed as mild and the most common AE after FMT (19 publications), more severe AEs, such as infection, relapse of inflammatory bowel disease, CDI, and death were also noted.⁴⁶ Overall, FMT is associated with AEs ranging in severity from mild to severe; but often it is not clear whether AEs are clearly or only possibly related to FMT. For instance, several papers have described fever or vomiting as common AEs after FMT, but only as possibly related to FMT.⁴⁶ There is a need to

conduct further randomized controlled trials assessing the AEs more precisely.

Probiotics

According to the Food and Agriculture Organization of the United Nations and World Health Organization, probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.”⁴⁷ Probiotics are used to manipulate gut microbiota. They are reputed to exert both anti-cancerous and anti-mutagenic activities.³⁸ Probiotics may have a possible molecular mechanism *via* microRNAs. In the study of Heydari *et al.* who used an animal colon cancer model, it was shown that the administration of *Lactobacillus acidophilus* DSM 13421 and *Bifidobacterium bifidum* at a dose 1×10^9 CFU/g for 5 months increased the expression of miR-26b, miR-18a, APC, PU.1 as well as PTEN while reducing the expression of miR135b, miR-155, KRAS.⁴⁸ Due to the increased expression of the tumor suppressor miRNAs and decreased level of the oncogenes after the treatment with probiotics, it was concluded that they may be considered as advantageous for colon cancer treatment.⁴⁸

Probiotics may also effectively protect the intestinal mucosa barrier in patients with colorectal cancer after surgical procedures; this was revealed in a meta-analysis of randomized, controlled trials (17 studies, $n = 1242$).⁴⁹ The ratio of lactulose/mannose and occludin levels was higher in experimental groups which were given probiotics in comparison with the value in the control group. The ratio of *Bifidobacterium/Escherichia* also changed, suggesting that the probiotics had been able to prevent the loss of balance in the microflora during the postoperative period. Bacterial translocation, as a measure of the biological barrier function, was also visibly lower in patients in the experimental group than in the control group. Alterations in the C-reactive protein (CRP) level did not give a clear result – changes in its level were not as unequivocal as in case of the above-mentioned factors, but in part of the studied cohorts, the lower level of CRP in the experimental groups suggested that there was a decreased level of inflammation after the supplementation with the probiotics. The level of IL-6, as

one of the proinflammatory cytokines, remained unchanged after treatment with probiotics.⁴⁹

The administration of probiotic strains can reduce the side effects of anti-cancer therapy, especially the adverse events after surgical procedures and chemotherapy as well as radiotherapy. Probiotics have significantly reduced the rate of all postoperative major complications (probiotics 28.6% vs. placebo 48.8%, $p = .010$).⁵⁰ Infection is one of the major risk factors associated with morbidity among patients with colorectal cancer after abdominal surgery. The administration of probiotics seems to be useful after surgical procedures. In a systemic review and meta-analysis of randomized trials, it was claimed that probiotics could prevent inflammation in the postoperative care of colorectal cancer patients, and they would be beneficial for surgical recovery.⁵¹ Another systemic review and meta-analysis confirmed that the administration of probiotics reduced by almost half the infection rate and the incidence of pneumonia.⁵² To sum up, probiotics may be used as a therapeutic method to prevent infections in patients with colorectal cancer in the postoperative period. Chemotherapy based on 5-fluorouracil is frequently associated with the risk of diarrhea. In a randomized trial, it was confirmed that the administration of *Lactobacillus rhamnosus* GG reduced abdominal discomfort as well as the frequency of severe diarrhea in patients with colorectal cancer being treated with 5-fluorouracil.⁵³ Moreover, it is important to mention that this probiotic strain was well tolerated by the patients.⁵¹ Radiotherapy is also associated with a risk of diarrhea; the condition is known as radiation-induced diarrhea. In a double-blind and placebo-controlled trial, it was demonstrated that the administration of VSL#3 (*Lactobacillus casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii*, *Bifidobacterium longum*, *B. breve*, *B. infantis*, *Streptococcus thermophilus*) could reduce the risk of diarrhea in patients who had received postoperative radiation therapy after surgery for sigmoid, rectal, or cervical cancers.⁵⁴

Next-generation probiotics (NGPs) are defined as “live microorganisms identified on the basis of comparative microbiota analyses that, when administered in adequate amounts, confer a health benefit on the host.”⁵⁵ The traditional probiotics were isolated from many sources such as gut and

traditional fermented foods and they have a long history of use. In contrast, NGPs have been recently isolated due to the new tools which make it possible to isolate, identify, and modify these commensal bacteria; therefore, the efficiency and safety of NGPs remain unclear.⁵⁵ Nevertheless, NGPs, such as *Bifidobacterium* spp., *Bacteroides fragilis*, *Akkermansia muciphila*, and *Faecalibacterium prausnitzii* have opened new therapeutic perspectives in CRC.⁵⁶ As Chang *et al.* have reported, some *Bifidobacterium* species strains may enhance the efficiency of cancer therapy with immune checkpoint inhibitors; therefore, the effects of anti-cancer therapy may be strain dependent.⁵⁶ Similarly, although *Bacteroides fragilis* may enhance the efficiency of immune checkpoint inhibitor cancer therapy, enterotoxin-containing *Bacteroides fragilis* has also been associated with CRC development.⁵⁶ *Akkermansia muciphila*, which is one of the most abundant single species in the human intestinal mucosa, may contribute to the efficiency of PD-1-based immunotherapy in mice; however, the mechanism is still unclear.^{57,58} *Faecalibacterium prausnitzii* takes part in butyrate production and thus it may contribute to the maintenance of intestinal homeostasis.⁵⁹ Currently, NGPs are considered as a novel potential therapeutic strategy to improve CRC treatment. Nonetheless, more studies will be needed to clarify the mechanisms and possibilities of NGPs.

Prebiotics

Prebiotics are selectively fermentable, non-digestible oligosaccharides or ingredients which cause alterations in the composition and activity of gut microbiota conferring health benefits.³⁸ Prebiotics are carbohydrates including fructooligosaccharides (FOS), xylooligosaccharides (XOS), galactooligosaccharides (GOS), inulin, and fructans. Fructooligosaccharides and galactooligosaccharides have been the compounds mainly investigated as prebiotics. These compounds possess many properties, such as the stimulation of beneficial indigenous gut bacteria, production of short-chain fatty acids, modulation of the immune response, modification of gene expression in bacterial cells in cecum, colon, and feces, enhancement of absorption of micronutrients in colon, and the

modulation of xenobiotic-metabolizing enzymes.³⁸ Prebiotic inulin enriched with oligofructose in combination with probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* was claimed to exert an antitumorigenic activity in azoxymethane-induced colon carcinogenesis in rats. There is also a report that the administration of inulin could reduce the cecal pH.⁶⁰

β (1–4) GOS is mainly produced by enzymatic transglycosylation by β -galactosidases or β -glucosidases.⁶¹ They have the generic formula of β (1–4) [D-Galactose]_n-D-Glucose where n ranges between 3 and 10 sugar moieties. Short-chain fatty acids, lactate, acetate, and gases are the products of GOS metabolism. β (1–4) GOS has several properties, mainly increasing the intestinal concentration of lactate and short-chain fatty acids, stool frequency, and weight. They also decrease the fecal concentration of a secondary bile acid – lithocholic acid, increasing fecal pH as well as reducing the activity of nitroreductase and β -glucuronidase. It should be emphasized that prebiotics such as β (1–4) GOS, lactulose, and fructooligosaccharides have a potential role in the prevention of colorectal cancer.⁶¹

Synbiotics

Synbiotics which are described as a combination of probiotic bacteria and growth-promoting prebiotic ingredients that achieve “synergism” have been used as a supporting treatment in several diseases including colorectal cancer.³⁸ In a randomized, double-blind clinical trial, it was shown that synbiotics significantly reduced the postoperative infection rates in patients with colorectal cancer.⁶¹ That study investigated 91 participants divided into two groups: one receiving synbiotic *Lactobacillus acidophilus* 10⁸ to 10⁹ CFU, *Lactobacillus rhamnosus* 10⁸ to 10⁹ CFU, *Lactobacillus casei* 10⁸ to 10⁹ CFU, *Bifidobacterium* 10⁸ to 10⁹ CFU and fructooligosaccharide (FOS) 6 g for 5 d prior to surgical procedure and 14 d after surgery with the other group treated with placebo. A surgical site infection occurred in 2% of the patients receiving the synbiotic product and 21.4% of the participants consuming placebo ($p = .002$).⁶² As Kuugbee *et al.* reported, the administration to rats of a cocktail consisting of oligofructose-maltodextrin-enriched *Lactobacillus*

acidophilus (6.4×10^{11} CFU), *Bifidobacteria bifidum*, *Bifidobacteria infantum* (1.9×10^{10} CFU) increased the expression of MUC2, ZO-1, occludin, and TRL2. All of these agents may participate in many beneficial processes such as decreased tumor growth, increase in mucin secretion, preservation of tight junctions, inhibition of inflammation, etc. Moreover, the expression levels of TRL4, caspase 3, COX-2, and β -catenin were decreased; these are proteins known to enhance the proinflammatory response, apoptosis, and tumor progression.⁶³ This pre- and probiotics cocktail modulated gut microbiota and reduced the development of colon cancer.⁶³

The association between the administration of synbiotics and surgical treatment has also been examined in a randomized, double-blind trial.⁶⁴ The effects of prebiotic and synbiotic treatment were investigated before colorectal surgery with 73 patients scheduled to undergo colorectal operations being recruited. They were divided into three groups: the first received prebiotics, the second consumed synbiotics, and the third underwent preoperative cleansing. No differences in the systemic inflammatory response, e.g., as measured *via* the levels of CRP, IL-6, fibrinogen, postoperative course as well as complication rate, were observed between all three groups. However, it was noted that more lactic acid bacteria were present in the colonic mucosa in the synbiotic group as compared to the rest of the participants.⁶⁴

To sum up, the administration of synbiotics in colorectal cancer seems to be useful perhaps due to their immunomodulatory properties and the ability to reduce the rates of postoperative infections.

Microbiota and surgical procedures

There are many factors contributing to the modifications of intestinal microbiota associated with surgical procedures. There is data showing that bacterial translocation during a surgical procedure can lead to septic complications. Consequently, preoperative mechanical bowel preparation (MBP) used to be a standard procedure to prevent perioperative infections.^{65,66} MBP, on the other hand, was proven to cause major changes in the intestinal microbiome and metabolome.^{67,68} Those observations were described during colonoscopy preparation but they had a short-

term effect.⁶⁹ Data about the long-lasting alterations of the microbiome are more controversial. Drago *et al.* detected changes at the family level, i.e., there was a reduction in *Lactobacillaceae*, an increase in *Enterobacteriaceae* abundance immediately after the colonoscopy. The abundance of *Rikenellaceae* and *Eubacteriaceae* was observed to be significantly higher as compared with samples collected before the procedure. The authors also observed a fourfold increase in the amounts of *Streptococcaceae* at the 1-month follow-up.⁶⁷ On the other hand, O'Brien *et al.* did not detect any significant changes in the microbiome three to 6 months after colonoscopy.⁷⁰ Large studies and meta-analysis have been unable to confirm the benefit of MBP alone in reducing infectious complications after colorectal surgery.⁷¹ However, recent data have highlighted the importance of specific bacterial species in anastomosis leakage and the potential role in a local recurrence of cancer.⁷² This has evoked interest in the selective eradication of certain bacterial species prior to the surgical procedure. Recent data from meta-analysis have revealed that MBP together with oral antibiotic prophylaxis can reduce anastomotic leak rates, 30-d mortality, overall morbidity, and the incidence of postoperative ileus, without increasing the risk of developing CDI.⁷³ The post-surgical pattern of the gut microbiota may also be considered as a potentially useful clinical marker. The microbiome analyzed after tumor removal differed in patients who developed new adenomas in follow-up colonoscopy in comparison with those without any pathological findings. This may lead to the exploration of noninvasive markers to predict cancer recurrence after curative treatment.⁷⁴

The impact of gut microbiota on the efficacy of anti-cancer therapy and overall survival

The composition of human gut microbiota has an impact on the process of colorectal carcinogenesis but there is also an association between the gut microbiota and the efficacy of anti-cancer therapy. The most recent studies have revealed an interaction between composition as well as the activity of gut microbiota and the efficacy and toxicity of chemotherapy (including oxaliplatin, irinotecan) and immunotherapy (ipilimumab). These relationships are also observed in the case of surgical treatment. Anastomotic leakage in colorectal surgery is

a serious complication since it is linked with increased morbidity and mortality.⁷⁵ The risk of anastomotic leaks after colorectal surgery depends on many factors, such as male gender, obesity, duration of operation, preoperative use of steroid and non-steroidal anti-inflammatory drugs, and the composition and activity of gut microbiota.⁷⁵ For instance, patients developing an anastomotic leak were observed to have a lower microbial diversity and an increased amount of mucin-degrading members of the *Lachnospiraceae* and *Bacteroidaceae* families.⁷⁵ For example, it is also known that *Enterococcus faecalis* takes part in the development of anastomotic leakage;⁷⁶ it contributes to anastomotic leak because it has high collagen-degrading activity.⁶⁶ *Fusobacterium nucleatum*, on the other hand, promotes chemoresistance to 5-fluorouracil by upregulating BIRC3 expression in colorectal cancer, as shown in the most recent study of Zhang *et al.*⁷⁷ As stated above, it is not only the composition of gut microbiota which plays an important role, its activity is also crucial. For instance, an increased frequency of constipation was observed in patients with colorectal cancer treated with 5-fluorouracil as assessed by methane colonic production.⁷⁸ Moreover, fecal pH was associated with symptoms experienced by patients receiving chemotherapy. It has been observed that a lower risk of diarrhea (OR, 0.56; 95% CI, 0.31 to 1.02; $p = .058$) and an increased risk of constipation (OR, 2.23; 95% CI, 1.35 to 3.68; $p = .002$) were associated with higher fecal pH.⁷⁸ In another study, the same authors have shown that breath methane excretors had higher fecal pH than non-excretors (7.05 vs. 6.57, $p < .001$) and less abdominal discomfort (30% vs. 54%, $p = .016$).⁷⁹ Furthermore, patients with resected right-sided cancer were less breath methane excretors than subjects with resected left-sided cancer (20% vs. 51%, $p = .029$; respectively). Additionally, patients with resected right-sided cancer had lower fecal pH in comparison to subjects with resected left-sided cancer (6.27 vs. 6.86, $p = .002$; respectively) and healthy subjects (6.80, $p = .010$).⁷⁹

Gut microbiota may be used as a prognostic biomarker to assess overall survival (OS), in patients with colorectal cancer as shown in a pilot study conducted by Wei *et al.*⁸⁰ A high abundance of *Fusobacterium*

nucleatum and *Bacteroides fragilis* was related to worse OS after the surgical procedure. On the contrary, a high abundance of *Faecalibacterium prausnitzii* was associated with a better OS. This study confirms the hypothesis that *Faecalibacterium prausnitzii* plays a protective role in this situation. In addition, clinical trials have revealed that there is a low abundance of *Faecalibacterium prausnitzii* in patients with ulcerative colitis.⁸⁰ The beneficial effects of *Faecalibacterium prausnitzii* are thought to be mediated mainly through its high capacity to induce IL-10 secretion in humans. Due to these anti-inflammatory properties, it may provide protection against colitis.⁸¹


Conclusion

In patients with colorectal cancer, not only are there changes in the composition of gut microbiota but there are also alterations in the metabolome. Gut dysbiosis has been convincingly associated with the process of colorectal carcinogenesis. Currently, there are several therapeutic methods available with which to alter the gut microbiota. The administration of prebiotics, probiotics, and synbiotics seems to be useful in these cases. For instance, the intake of synbiotics may replace the mechanical bowel cleansing prior to surgical procedures since not only does it modulate the gut microbiota but also it may well reduce the development of colorectal carcinogenesis. There are limited data regarding the use of fecal microbiota transplantation in colorectal cancer management. Nonetheless, there are studies which have confirmed the impact of fecal microbiota transplantation, e.g., on the immune response. Further trials should concentrate on evaluating the efficacy of fecal microbiota transplantation, for instance in reducing the severity of gastrointestinal side effects associated with anti-cancer treatment. Moreover, there is a clear need to evaluate the safety of fecal microbiota transplantation with respect to its long-term effects and the clinical outcome of patients with colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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