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Review Article

# Dysbiosis and the Chemopreventive Role of Prebiotics in Colorectal Cancer

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**Abstract**
Recent metagenomic evidence broadly supports the association and causality of gut dysbiosis with the development of colorectal cancer (CRC). Probiotics and prebiotics have been proposed as new preventive and therapeutic adjuncts for CRC management. While promoting the growth of ingested probiotics, an ideal prebiotic fibre should reach the colon intact to be fermented by a distinct group of beneficial commensal bacteria. Therefore, the selection of probiotic bacteria, the growth-promoting prebiotic fibre, and a combination of both probiotics and prebiotics (known as synbiotics) are critical in preventing carcinogenesis. Despite limited clinically measurable data, findings from preclinical animal studies have recognized the functional role of synbiotics of varying genera, strains, and doses in nourishing beneficial human gut microbiome to evade cancer. Nevertheless, translating such heterogeneous-based data from different study settings and measured outcomes into evidence-based recommendations is a very challenging task. The emerging concept is that an ideal synbiotic combination may effectively modify the intestinal microflora composition which helps imprint the immune systems for lasting chemoprevention effect. The present article reviews the roles of gut microflora in colorectal carcinogenesis followed by discussing the updated evidence of prebiotic chemoprotective effects in modulating gut microflora and host immunity against CRC. Besides, it also summarized the limitations of the clinical use of probiotics and prebiotics to achieve synergism in formulating ideal synbiotics.

**Keywords:** Colorectal Cancer, Dysbiosis, Microbiota, Prebiotics, Probiotics, Synbiotics
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## Introduction

Humans harbor trillions of widely diverse and immensely active microbial communities that are distributed along the oral GIT.[1] It comprises $10^{13}$ to $10^{14}$ microorganisms, primarily bacteria, that outnumber human cells by a factor of ten.[2] The bacterial population, which contains 300 to 500 different species of bacteria, plays an important role in the digestion of food, resistance to colonization from pathogenic microorganisms, production of short-chain fatty acids and essential vitamins, and modulation of the gut immune system.[3,4] It is crucial to maintain the homeostasis between gut microflora and intestinal function to acquire balanced immunity. Alteration of this symbiotic relationship may shift the microbial composition towards dysbiosis, which plays an essential role in the development of various gastrointestinal disorders including colorectal cancer (CRC), inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and celiac disease among others.[5-8]

CRC is the third most common form of cancer worldwide, mainly in industrialised countries. The incidence of CRC seems to grow gradually, leading to 694,000 deaths annually.[9] CRC, which originates from the epithelial cells lining the colon or rectum of the GIT, has its onset associated with multiple factors, and its prevalence is correlated to IBD.[10] Malignant forms of CRCs are characterised by the formation of polypoid adenomas leading up to intramucosal carcinoma, also known as a high-grade dysplastic adenoma. This phase develops through a multistep mechanism that involves specific gene mutations that may take years before it

becomes sporadic cancer. Accordingly, early cancer detection with subsequent complete endoscopic removal is currently the best strategy for cancer control. Besides, CRC patients who underwent chemotherapy often suffer from undesirable side effects. Cytotoxic agents are not selective, leading to collateral damage to healthy cells and tissues and an increased risk of cancer recurrence.

Hitherto, many studies have reported that diet could contribute 50-90% of the development of CRC.[11-13] Individuals who consume a diet high in red and processed meats, refined starches, and sugar while low in vitamins, fruits, vegetables, fibre, and whole grains tend to predispose themselves to CRC.[14,15] Meanwhile, there is an escalating research interest in the modulation of gut microbiota and alteration of host metabolism by consuming dietary fibres, specifically prebiotics in cancer prevention. The International Scientific Association for Probiotics and Prebiotics (ISAPP) defines prebiotics as "a substrate that is selectively utilized by host microorganisms conferring a health benefit".[16] Studies reported that prebiotics, especially, fructooligosaccharides (FOS), inulin, and galacto-oligosaccharides (GOS) can potentially reduce appetite, enhance insulin sensitivity, and lipid metabolism in both animals and humans.[17-19] Like probiotics, prebiotic supplementation has also been found to enhance the intestinal barrier by inducing the proliferation of commensal gut flora while reducing the levels of inflammatory cytokines.[15,17,19]

With the advent of metagenomics and meta-transcriptomic approaches using next-generation sequencing (NGS) tools and terminal restriction fragment length polymorphism (T-RFLP), the association of certain intestinal bacterial species with the consumption of prebiotics in the normal and intervention group can potentially be unraveled.[15,20-23] Remarkably, different individuals present unique "bacterial fingerprints" as the composition of gut microbiota is strongly dependent on a range of factors including host genetics, immunological factors, microbial species acquired at birth, diet, and antibiotic usage.[24] This technology has gained immense interest and has shifted the paradigm of our understanding and treatment of CRC towards the incorporation of gut resident microbes and their functions.

In light of the above-mentioned, this review highlights the association of gut dysbiosis and CRC with a special focus on using selected prebiotics to provoke both exploitative and interference competition, which is detailed in subsequent sections, leading to the establishment of benign beneficial microbiota in preventing cancer. Specifically, we discuss the updated evidence of prebiotic-induced chemo-protection and its mechanisms of action in the underlying prevention and inhibition of CRC carcinogenesis and progression.

## Dysbiosis of Gut Microbiota Promotes the Development of CRC

Dysbiosis or imbalanced gut microbiota composition is associated with increased susceptibility to colorectal cancer (CRC).[15,25] Different hypotheses for understanding the role of microbial unbalance in CRC carcinogenesis have been proposed. Some researches have agreed that the functional imbalance triggered by certain types of dysbiotic gut microbiota may cause pro-inflammatory responses and epithelial cell transformation that leads to cancer.[26-28] As proposed by Yang and Jobin[28] in their 'driver-passenger theory', the predominant clusters of mucosal bacteria (the driver) cause persistent DNA damage in human epithelial tissue, thus triggering tumor formation. The changes in the surrounding microenvironment during the tumorigenesis will then allow the colonization of opportunistic bacteria (the passengers) capable of facilitating tumor progression. Geng et al,[29] Vigneswaran and Shogan[30] and Sulzyc-Bielicka et al.,[31] supported such distinct roles of bacterial drivers and passengers in CRC pathogenesis.

In human health, the microbiota maintains intestinal homeostasis by regulating various biological functions, such as metabolic processes, immunity, and mucosal barrier. For instance, under anaerobic conditions, some species of microbiota, such as the *Bacteroides* genus, produce SCFAs that bind to GPR 41/43 receptors and stimulate peptide YY (PYY) production which suppresses gut motility and enhance nutrient absorption in the small intestine.[32-34] This is a key process in controlling mucosal proliferation, differentiation, and maintenance of mucosal integrity. The intestinal compartmentalization of commensal microbiota acts as a gut barrier for pathogens. The failure of this barrier results in an increased "intestinal permeability" that has been causally implicated in CRC. Other examples of bacteria-producing cancer-protective molecules are folate[35] and biotin,[36] which exert important metabolic functions and regulate the inflammatory response by stimulating the immune system essential for DNA synthesis and repair. A healthy gut microflora may potentially ward off the carcinogen-activating enzymes such as β-glucosidase, β-glucuronidase, 7-α-dehydrogenase, nitroreductase, and azoreductase thus attenuating colon carcinogenesis.[37-39]

Microbial dysbiosis, on the other hand, destroys the mutualistic relationships and potentially contributes to tumor development.[40-42] Increased production of microbial antigens and metabolites due to changes in microbial activity can majorly influence on the immune response and be a source of chronic inflammation.[28,43] Increased production of SCFA, like butyrate, might have a detrimental effect on the intestinal barrier, damaging epithelial cells and their junctions through activation of pro-inflammatory mediators such as cytokines, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6).[44,45] Some bacterial species produce toxins that breach the intestinal epithelial barrier and trigger a mucosal inflammatory response. Direct adherence and localization of *Fusobacterium nucleatum* via the FadA surface

**Table 1.** List of Microbiotas that are Most Likely Associated with the CRC (mucosa tissue samples/fecal samples)

| Phylum / Genus | Bacteria | Type of Tissue / Anatomical Site | Type of Study | CRC vs. Normal (*In vivo* / *In vitro*) | |
|---|---|---|---|---|---|
| | | | | Ref. (High in CRC) | Ref. (Low in CRC) |
| **Firmicutes** | | | | | |
| *Streptococcus* | *Streptococcus spp.* | proximal and distal tumors | *In vivo* (human study) | [22, 52, 53] | [52] |
| | *Streptococcus anginosus* | Gut | | | |
| *Ruminococcus* | *Ruminococcus spp.* | Gut, proximal and distal tumors | *In vivo* (animal study & human study) | [54-56] | [26] |
| | *Ruminococcus obeum* | Gut | | | |
| | *Ruminococcus bromii* | proximal and distal tumors | | | |
| *Blautia* | *Blautia spp.* | colonic mucosa in CRC | *In vivo* (human study) | - | [26, 53] |
| | *Clostridium coccoides* | Gut | | | |
| *Faecalibacterium* | *Faecalibacterium spp.* | colonic mucosa in CRC | *In vivo* (animal study) | - | [53, 57] |
| | *Faecalibacterium prausnitzii* | Gut | *In vivo* (human study) | | |
| Peptostreptococcus | Peptostreptococcus spp. | proximal colorectal tumors | In vivo (human study) | [22, 53] | - |
| | | colonic mucosa in CRC | | | |
| | | proximal and distal tumors | | | |
| *Enterococcus* | *Enterococcus spp.* | proximal and distal tumors | *In vivo* (human study) | [58] | - |
| *Gemella* | *Gemella spp.* | proximal and distal tumors | *In vivo* (human study) | [53] | - |
| *Granulicatella* | *Granulicatella spp.* | proximal and distal tumors | *In vivo* (human study) | [53] | - |
| *Holdemanella* | *Eubacterium biforme* | Gut | *In vivo* (human study) | [57] | - |
| *Lachnoclostridium* | *Lachnoclostridium spp.* | Gut | *In vivo* (human study) | - | [56] |
| *Lactobacillus* | *Lactobacillus spp.* | Gut | *In vivo* (animal study), *In vivo* (human study) | | [59] |
| *Mogibacterium* | *Mogibacterium spp.* | colonic mucosa in CRC | *In vivo* (human study) | [53] | |
| *Oscillibacter* | *Oscillibacter spp.* | Gut | *In vivo* (human study) | | [56] |
| *Parvimonas* | *Parvimonas micra* | Gut | *In vivo* (human study) | [60] | - |
| *Roseburia* | *Roseburia spp.* | Gut | *In vivo* (animal study), *In vivo* (human study) | - | [61] |
| *Selenomonas* | *Selenomonas spp.* | proximal colorectal tumors | *In vivo* (human study) | [22] | |
| *Subdoligranulum* | *Subdoligranulum spp.* | Gut | *In vivo* (human study) | [56, 58] | - |
| *Veillonella* | *Veillonella spp.* | proximal and distal tumors | *In vivo* (human study) | [53] | - |
| **Bacteroidetes** | | | | | |
| *Prevotella* | *Prevotella spp.* | proximal colorectal tumors | *In vivo* (human study) | [22, 53, 54] | - |
| | | proximal and distal tumors | | | |
| | | Gut | | | |
| | *Prevotella copri* | Gut | *In vivo* (human study) | - | [57] |
| | *Prevotella stercorea* | | | | |
| *Bacteroides* | *Bacteroides spp.* | proximal and distal tumors | *In vivo* (human study) | [53, 57] | - |
| | *Bacteroidetes fragilis* | Gut | | | |
| | *Bacteroides fragilis* | | | | |
| | *Bacteroides uniformis* | | | | |
| *Parabacteroides* | *Parabacteroides distasonis* | Gut | *In vivo* (human study) | [62] | [56] |
| | *Parabacteroides spp.* | | | | |
| *Porphyromonas* | *Porphyromonas spp.* | colonic mucosa in CRC | *In vivo* (human study) | - | [53] |
| *Prevotellaceae* | *Prevotellaceae spp.* | colonic lumen in CRC | *In vivo* (human study) | - | [53] |
| *Porphyromonadaceae* | *Porphyromonadaceae spp.* | Gut | *In vivo* (human study) | [52] | [52] |
| **Proteobacteria** | | | | | |
| *Gammaproteobacteria* | *Escherichia coli* | colonic mucosa in CRC | *In vivo* (human study) | [63] | |
| | *Escherichia-Shigella* | distal colorectal tumors | *In vivo* (human study) | [22] | [58] |
| | *Pseudomonas spp.* | proximal and distal tumors | | | |
| | *Pseudomonas aeruginosa* | HT-29 colorectal cancer cell line | *In vitro* (cell culture) | [53] | - |

| | | | | | |
|---|---|---|---|---|---|
| *Haemophilus* | *Haemophilus spp.* | proximal and distal tumors | *In vivo* (human study) | [53] | - |
| *Klebsiella* | *Klebsiella spp.* | proximal and distal tumors | *In vivo* (human study) | [64] | [58] |
| *Morganella* | *Morganella spp.* | proximal and distal tumors | *In vivo* (human study) | [53] | - |
| *Oxalobacter* | *Oxalobacter formigenes* | Gut | *In vivo* (human study) | [64] | - |
| **Actinobacteria** | | | | | |
| *Bifidobacterium* | *Bifidobacterium spp.* | colonic mucosa in CRC | *In vivo* (human study) | - | [53] |
| | *Bifidobacterium genus (Bb)* | Gut | *In vivo* (human study) | | |
| *Coriobacteriaceae* | *Coriobacteriaceae spp.* | colonic lumen in CRC | *In vivo* (human study) | [52, 53] | |
| | | Gut | | | |
| *Collinsella* | *Collinsella aerofaciens* | Gut | *In vivo* (human study) | [65] | - |
| **Fusobacteria** | | | | | |
| *Fusobacterium* | *Fusobacterium nucleatum* | Gut | *In vivo* (human study) | [22, 53, 66-68] | - |
| | | proximal and distal tumors | *In vivo* (Animal study) | | |
| | | colonic mucosa in CRC | *In vivo* (human study) | | |
| *Leptotrichia* | *Leptotrichia spp.* | distal colorectal tumors | *In vivo* (human study) | [22] | - |
| **Verrucomicrobia** | | | | | |
| *Akkermansia* | *Akkermansia muciniphila* | Gut | *In vivo* (human study) | [52, 65] | |
| | *Akkermansia spp.* | | | | |
| **Spirochaetes** | | | | | |
| *Treponema* | *Treponema spp.* | Gut | *In vivo* (human study) | [69] | |

protein and interaction with E-cadherin triggers the release of inflammatory factors (i.e. NF-κB, IL-6, IL-8, IL-10, and IL-18) and promotes activation of β-catenin and Wnt signalling which is a vital factor in inflammation related-CRC tumorigenesis.[41,46,47] On the other hand, enterotoxins (i.e. fragylisin) and reactive oxygen species produced by *Bacteroides fragilis and Enterococcus faecalis* are evidenced to induce oxidative DNA damage and epithelial barrier dysfunction in the inflammation-related CRC models.[44,48-50] Earlier, Wu et al,[51] reported that toxin from *B. fragilis* mediates changes in T-cell factor–dependent β-catenin nuclear signalling. These bacterial species demonstrated significantly different abundances between CRC and healthy samples.

Changes in diet style, such as increased consumption of sugars, refined starch, processed foods, food additives, unwashed fruit and vegetables, and trace amounts of harmful chemicals, primarily cause dysbiosis that potentially brings about CRC. A case in point, seminal analyses of the geographic variation in CRC demonstrated that more than two-thirds of the cases are attributed to dietary habits. A diet containing defensive micronutrients, counting β-carotene and other carotenoids, as well as carcinogens and mutagens has been associated with the risk of cancer development.[52] Furthermore, dietary factors like higher red and processed meat consumption and deficiency of fibre, calcium, vitamin D, and folate are well-recognized factors associated with higher CRC risk. Apart from that, smoking, obesity, excessive alcohol consumption, poor dental hygiene (allows bacteria to grow out of balance in the oral cavity), high levels of stress (i.e. anxiety or depression, which affect the immune system), and use/misuse antibiotics and antibacterial medications are among other well-known risk factors of dysbiosis and CRC.[53,54]

In addition to the cited factors, gene expression in host cells is another potentially important mechanism by which the microbiota impact host physiology. Yuan et al.,[55] recently conducted the first systems-level map of the association between microbes and host miRNAs in the context of CRC, providing targets for further experimental validation and potential interventions. They hypothesized that the host miRNAs likely regulate glycan production that could promote the recruitment of pathogenic microbial taxa to the tumor. These interactions might be a direct target for developing therapeutic strategies for CRC patients. Follow-up studies using model systems are recommended to assess the causal role of individual microbes and miRNAs in CRC.

A functional gene analysis conducted by O'Keefe et al.,[56] demonstrated that by switching the diet style between rural South Africans and African Americans, the native Americans who initially had greater expression of the *BcoA* gene encoding for butyryl-CoA:acetate CoA-transferase enzyme, which is responsible for the last step in butyrate synthesis, had a reduction in its gene expression. Furthermore, after switching the diet, it was reported that Americans who received the "African" diet reduced the *baiCD* gene abundance and the secondary bile acids, lithocholic and deoxycholic acid, while the elevation was observed in Africans who received the 'Western' diet. These reciprocal changes in aspects of the microbiome and metabolome may lead to

reciprocal changes in mucosal biomarkers of cancer risk.

## Functional Microbiome CRC Signatures as a Basis for Future Diagnostics

Alterations in the gut ecosystem and how it contributes to human health can now be assessed through various technologies. Fecal metagenomics, for instance, is a useful tool to determine microbial composition community (relative abundance of taxa) and has novel diagnostic value where it has been used to infer bacterial metabolic pathways in various GIT illnesses, particularly CRC. Hitherto, *F. nucleatum* was shown to be the most dominant phylotype in CRC and is a subject of intensive research.

The gut microbiota of healthy individuals is composed of permanent and transitory microbial species and subspecies. The majority of microbes forming the human gut microbiota could be divided into four major phyla: *Firmicutes* (<70%), *Bacteroidetes fragilis* (<30%), *Proteobacteria* (<5%), and *Actinobacteria* (<2%), and in smaller amounts of *Fusobacterium nucleatum* (*F. nucleatum*) and *Verrucomicrobia* (<1%), *Cyanobacteria* and *Escherichia coli*. Studies have indicated that these bacterial phyla play a critical role in CRC carcinogenesis.[57] A recent systematic review concluded that certain bacteria, such as *Staphylococcaceae*, *Alistipes*, *Fusobacteria*, *Porphyromonadaceae*, *Akkermansia spp*., *Coriobacteridae*, and *Methanobacteriales* were consistently augmented in CRC. While some others (such as *Bifidobacterium* (*Bb*), *Lactobacillus*, *Ruminococcus*, *Faecalibacterium* (*Fp*), *Roseburia*, and *Treponema*) were underrepresented.[58,59] In contrast, Liang et al., (2014) reported that *Lactobacillus* increased significantly in hamster models during CRC formation.[60] Table 1 shows a list of microbiotas most likely associated with CRC.

A simple fecal microbial biomarker for detecting CRC has been reported, whereby an increase in *F. nucleatum* and a decrease in *Fp* and *Bb* could be a sign of CRC.[61] It is worth noting that such gut microbiome changes are linked to a multistep process in CRC tumorigenesis. A fecal metagenomic and metabolomic study by Yachida et al.,[62] was conducted on 616 samples from CRC patients to unravel taxonomic differences of gut microbiota in cases of multiple polypoid adenomas and intramucosal carcinomas. Interestingly, it was discovered that the relative abundance of *F. nucleatum* spp. was significantly and proportionately higher from intramucosal carcinoma to more advanced stages. Meanwhile, *Atopobium parvulum* and *Actinomyces odontolyticus* were significantly elevated only in multiple polypoid adenomas and/or intramucosal carcinomas. This finding suggests that the shifts in the microbiome are triggered from the very early stages of tumor development.

Human population-based studies indicated that augmentation of *F. nucleatum* is associated with a low level of T-cell infiltration, high-level microsatellite instability, and advanced disease stage in CRC tissue.[63] In agreement with this study, Liang et al.,[64] reported that quantifying *F. nucleatum* in faeces by quantitative polymerase chain reaction could discriminate CRC patients from healthy controls with a high sensitivity of 78% and specificity of 80%. In addition, *Fp* and *Bb* have gained much attention in CRC prevention. Shah et al., recently conducted the first microbiome-based meta-analysis for CRC to identify a common microbial marker in fecal samples of 195 CRC patients.[65] They emphasized a marked increase in *Parabacteroides distasonis*, *Parvimonas micra* ATCC 33270, *Streptococcus anginosus*, and other strains of *Proteobacteria* along with the previously reported taxa, such as *Fusobacterium* species. Using linear discriminant analysis coupled with the effect size measurements, Gao et al.,[22] found that *Fusobacterium*, *Prevotella*, and *Peptostreptococcus* are the key phylotypes contributing to the dysbiosis of mucosa-associated microbiota in CRC patients.[66]

Despite recent interesting findings, having quantitative real-time PCR and 16S rRNA NGS methods in dysbiosis and CRC could untangle the complexity of microbial gut microbial ecosystem in CRC to help us define the microbial dysbiosis role in CRC. If these mechanisms are confirmed, restoration of healthy microbiota may be a potential strategy to alleviate the progression of CRC.[67] The impacts of several factors, in particular, probiotics, prebiotics, faecal microbiota transplantation (FMT), dietary alteration, calorie restriction, and administration of antibiotics, have been studied to find out a suitable way to restore the healthy gut microbial structure. Furthermore, the bacterium that can be used as a CRC detection marker or a therapeutic target and treatment will soon be unraveled for better intervention in tumor progression.

## Definition and Scope of Prebiotics

In the last 20 years, the definition of prebiotics has varied greatly, highlighting the importance of reaching a consensus on the precise nature, mechanisms of action, health benefits, and applicability of prebiotic substances. In 1995, Gibson and Roberfroid[68] established the initial definition of prebiotics which were described as "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health". Nine years later, the authors redefined the term prebiotics as "A Selectively fermented ingredient that allows specific changes in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host health".[69] The authors reviewed the concept in terms of three criteria: (a) Resistance to digestion and gastrointestinal absorption; (b) Fermentation by intestinal microflora; (c) Selective stimulation of the growth and/or activity of gut bacteria associated with health. Although this definition was broadly embraced, the prebiotic concept has since been revised to include its usage in extra intestinal locations.

While the majority of prebiotics are typically taken orally, they can also be administered to other areas of the body that are inhabited by microorganisms, such as the skin and vaginal tract. To redefine the concept of prebiotics, a revised definition of the term prebiotics was proposed by the authors again and approved by the ISAPP in 2017. Currently accepted definition of prebiotics is "A substrate that is selectively utilized by host microorganisms conferring a health benefit".[16] Dietary fibers can also be categorized into three groups based on their structure: prebiotic dietary fibers (such as FOS and GOS), dietary fibers that are considered candidates for prebiotics (including polydextrose, resistant starch, and pectin), and dietary fibers that are not recognized as prebiotics (such as cellulose and lignin).[70]

Prebiotics could confer a health benefit due to their nature as fermentable dietary fibre, which can nourish and conserve beneficial gut microbiota in our gut.[71] Dietary fibre is a complex carbohydrate, such as galactooligosaccharides (GOS), fructooligosaccharides (FOS), inulin, and resistant starches, that resist digestion in the small intestine and reach the colon where they are fermented by the gut bacteria.[16-19] Prebiotics such as GOS, FOS, and inulin have been granted "Generally Regarded as Safe" (GRAS) status by the FDA. Thus they are considered safe for use in food and supplements.[71] Whereas other types of prebiotics such as xylooligosaccharides (XOS), isomaltooligosaccharides (IMOS), glucooligosaccharides, pectin oligosaccharides (POS), mannanooligosacharides (MOS), gentiooligosaccharides (GTO), chitooligosaccharides (CHOS), soybean oligosaccharides (SOS), and polydextrose that are not commercially available in high purity and the safety of these oligosaccharides are yet to be evaluated.

As a dietary fibre, prebiotics serves as a nutrient for beneficial bacteria in the gut, promoting their growth and metabolic activity such as fermentation. The fermentation of prebiotic fibres in the gut produces short-chain fatty acids (SCFAs), which have been shown to have anti-cancer properties. This can help to increase the diversity of the gut microbiota, which has been linked to improved gut health and a reduced risk of various diseases such as CRC.[16-19,72] The ability of prebiotics to alleviate gut dysbiosis associated with CRC could be clarified on its effects to modulate the diversity of gut microbiota, increase the integrity of the intestinal barrier, and secretion and presence of beneficial compounds that could stimulate an immune response.[72] Thus, any intervention for CRC using dietary fibres that could stimulate beneficial indigenous gut microbiota, decrease the number of enteropathogens, reduce intestinal permeability and enhance the immune response potentially enhance patient outcomes.

## Potential Chemopreventive Roles of Prebiotics against CRC

Given that, the majority of CRC is sporadic rather than familial, dietary interventions that could improve gut microbiota have recently emerged as potential preventive strategies against the development of CRC. There is evidence that specific dietary fibres confer chemo-preventive effects by maintaining the integrity of the epithelial layer of the intestines and enhancing the resistance against pathogenic colonization. A meta-analysis by Friedenreich, Brant[73] concluded that consumption of over 27 g of fibres per day could lead to a 50% reduction in CRC risk as compared to the consumption of less than 11 g. Interestingly, it is particularly elicited by prebiotics. Several prebiotics are found in natural foods, such as chicory, cereals, agave and milk. However, most of the natural sources have prebiotics in trace amounts that necessitate refining them from foods or producing them by enzymatic, chemical or thermal processes to yield enough prebiotics to get their effects.[74] Disaccharide lactulose, oligosaccharides, resistant dextrin, polydextrose, arabinoxylans and resistant starches, polyols (lactitol and isomalt) are being considered as emerging prebiotics.[75] Inulin and fructo-oligosaccharides produced by the fermentation of the dietary fibres were reported to induce the proliferation of beneficial gut microbes, namely *Bifidobacterium spp.* and *Lactobacillus spp.*[76] These metabolites may cause changes in the gut microbial composition and encode distinct functional capacities to prevent CRC development in humans.

In fact, many epidemiologic and animal studies have demonstrated a chemo-preventive effect of prebiotics, and it is postulated to happen mainly through the restoration of the composition or activity of the healthy microflora.[77] Isomalto-oligosaccharides (IMO), for example, are partially indigestible prebiotics, composed of 125 glucose monomers linked by a (1→6) glycosidic linkages with a mixture of isomaltose (Glu α 1→6 Glu), isomaltotriose (Glu α1→6 Glu α1→6 Glu), and panose (Glu α1→6 Glu α1→4 Glu).[78,79] Many studies have indicated that IMO alters the gut microflora by increasing the level of *Bifidobacteria* and *Lactobacilli* as well as reducing the levels of harmful bacteria like *Clostridia* and *Bacteroides.*[80,81] CRC study on the protective role of IMO using an *in vivo* model is scarce. However, but a two-month human trial suggested that daily supplementation of 10 g of IMO could effectively reduce the colon cancer risk markers and improve colonic ecology.[82] Meanwhile, inulin and oligofructose have been shown to reduce the incidence of chemically induced CRC in rodents. Both prebiotics significantly increased the number of apoptotic cells per crypt and reduced the numbers of aberrant crypt foci (ACF) in Azoxymethane (AOM)-induced rats.[83] These findings indicate their protective role at an early stage of CRC development. Another study on resistant starch also demonstrated a reduced number of ACFs and aberrant crypts in 4-week-old AOM-induced A/J mice, which was further linked to the regulation of apoptosis-associated gene expression levels in tumor samples.[84] This is supported by

Wang et al.,[85] who revealed that resistant starch in the diet of dimethylhydrazine-induced C57BL/6 mice prevent tumor carcinogenesis of colon epithelial cells and promotes apoptosis through endoplasmic reticulum (ER) stress-mediated mitochondrial apoptosis pathway. A recent study conducted on 140 CRC patients revealed that prebiotic intake could improve serum immunologic indicators as well as the abundance of four commensal microbiota containing opportunistic pathogens in them, potentially reducing surgical stress.[86] However, conflicting results are reported in several recently published human studies measuring the direct physical indices of CRC risk after prebiotic consumption.[87] A literature search on CRC and prebiotic consumption in the human study revealed at least four articles on resistant starch. None of the studies demonstrated any effects of resistant starch on CRC development. Although there were relative improvements in crypt mitotic location, no changes in the gene expression and DNA methylation after resistant starch consumption were observed in cell proliferation and apoptosis, crypt morphology, or ACF. Table 2 summarizes the findings from recent literature on the chemo-protective effect of prebiotics on CRC.

**Table 2.** The Findings of Recent Literatures on the Protective Effects of Prebiotic in Fighting CRC

| Prebiotic | Parameters | Reference |
|---|---|---|
| Galactooligosaccharides | Modulate the intestinal microbiota<br>Break into SCFA (acetate, propionate and butyrate)<br>Increase intestinal lactate<br>Reduce fecal concentration of lithocholic acid<br>Reduce fecal pH<br>Inhibit nitroreductase and B-glucuronides activities | [98]<br>[99] |
| Resistant starch | Regulation of apoptosis<br>Reduce fecal pH | [97]<br>[96] |
| Inulin | Increase levels of SCFAs<br>Increase fecal levels of *Bifidobacterium* spp | [100] |
| Fructooligosaccharide | Break into SCFA,<br>Improved mineral absorption<br>*In vitro* cytotoxic and anti-proliferative activities<br>In vivo antioxidant enzyme activities | [101]<br>[102] |
| Isomalto-oligosaccharides | Decrease the fecal secondary bile acid<br>Reduce fecal glucuronidase and mucinase activity | [94] |
| Arabinogalactan | Immunomodulation against cancer in *in vitro* and *in vivo* | [103] |
| Chitooligosaccharides | Enhance the colonic concentrations of SCFAs<br>Stimulate probiotic growth<br>Inhibit the growth of potential pathogen<br>Anti-inflammatory<br>Immunostimulation | [104, 105] |
| Arabinoxylooligosaccharides | Reduce gut infections<br>Better absorption of minerals<br>Stimulate the growth of probiotics | [106] |
| Gentiooligosaccharides | Stimulate probiotic growth | [107] |
| Pectinoligosaccharides | Stimulate apoptosis process *in vitro*<br>Anti-inflammatory effect | [108] |
| Beta-glucan | Immunostimulation | [109] |
| Xylooligosaccharide | Increase the population of probiotics<br>Alleviate the incidence and multiplicity of ACF Ameliorate the level of lipid peroxidation<br>Improve the activities of glutathione-S-transferase and catalase in colonic mucosa and liver | [110] |

## Mechanisms of Prebiosis in Fighting CRC

While more papers are published based on the effects of prebiotics in reducing infection and enhancing recovery in patients following perioperative CRC, studies on its anticancer properties against CRC in humans are very limited. With the inclusion of the animal CRC model related to anticancer research, only 21 articles were found based on the PubMed search from 2009 to 2019, whereas only 5 articles were published from 1999 to 2008. The initiation and progression of CRC result from the accumulation of genetic mutations and epigenetic alterations of the human genome due to dietary and environmental factors.[88] Despite a growing amount of evidence elucidating the possible mechanisms of action or prebiosis in modulating CRC carcinogenesis, the precise mechanisms remain unclear. Such knowledge is vital to provide new perspectives for early diagnosis, particularly identifying high-risk populations and treatment for CRC. Figure 1 exhibits several possible mechanisms of prebiosis in CRC studies.

## Simulation of Beneficial Indigenous Gut Microbes

Although the precise mechanisms by which prebiotics inhibit colon tumours are not completely elucidated, it is likely that the modulation of gut microbiota mainly contributes to the effects of these dietary fibres. Intestinal microbes convert host-consumed nutrients, such as vitamins, amino acids or dietary fibres, into other metabolites. The converted metabolites, such as SCFAs, biogenic amines and other amino-acid-derived metabolites (e.g., serotonin or gamma-aminobutyric acid) play an important role in regulating health and recovery.[74,93] A specific type of dietary prebiotic fibres produces a distinctive proportion and distribution of SCFAs in the gut. SCFAs can decrease intestinal pH to prevent the outgrowth of pathogenic microflora, shape host

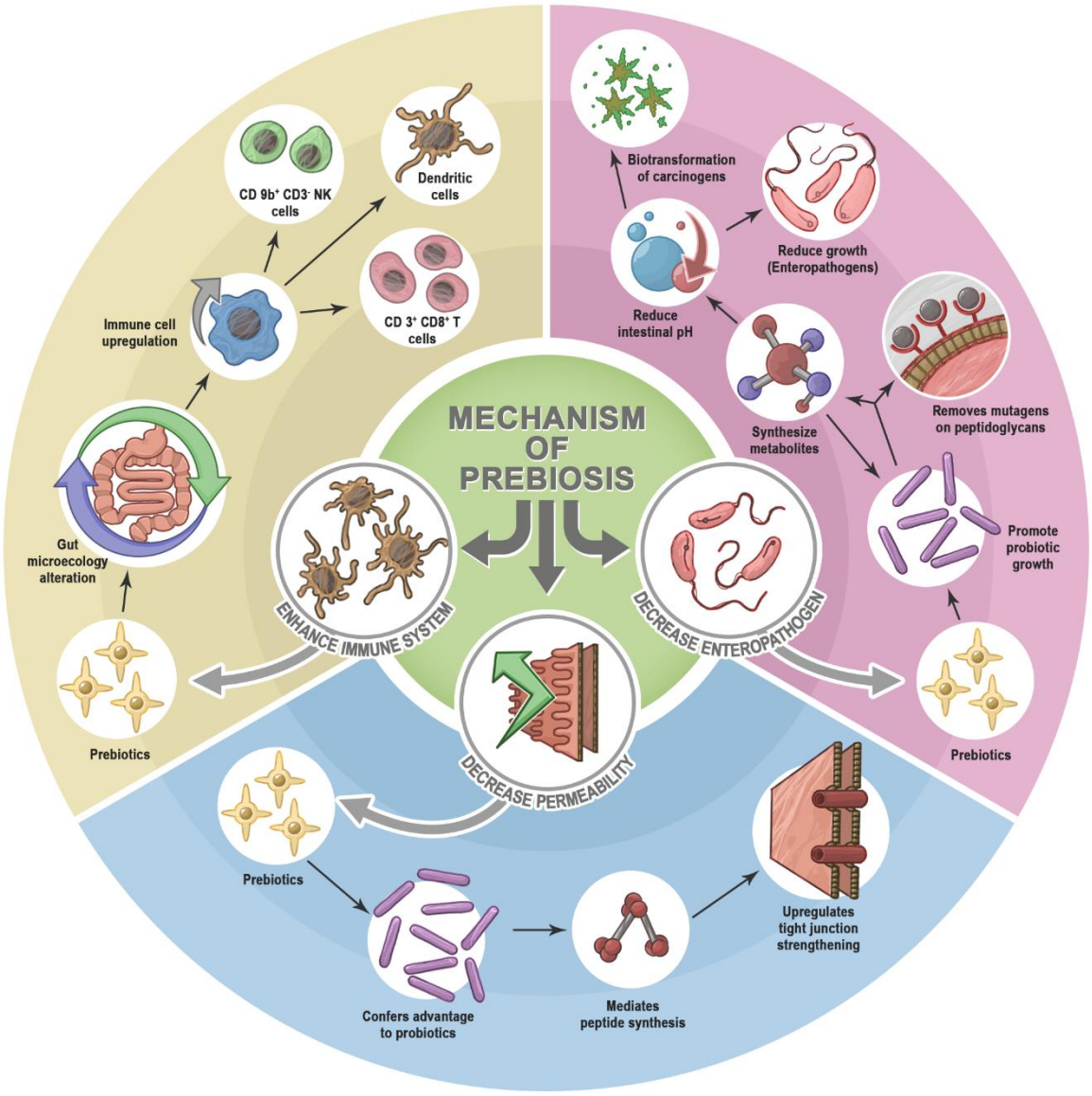


**Figure 1.** Summary of the Proposed Mechanisms of Prebiosis Action in Inhibition of CRC. The ingestion of prebiotics: i) provides nutrients to improve probiotic growth in the gut. Subsequently, some species of probiotics will synthesize metabolites that reduce the host's intestinal pH, effectively reducing enteropathogen growth and mediating the biotransformation of dangerous carcinogens.[89] The probiotics also facilitate the removal of ultimate mutagens on the pathogen peptidoglycans,[90] ii) result in the production of probiotic peptides that upregulates tight junction strengthening,[91] and iii) alter gut microecology, which upregulates immune cell activity, followed by an increased expression of dendritic cells, CD3+CD8+ T cells, CD49b+CD3− NK cells.[92]

intestinal anatomy and gut mucosal immune system, as well as protect against oxidative DNA damage and reduce altered cell proliferation.[94] A higher concentration of SCFAs, particularly butyrate, has been detected in the proximal colon, where rapidly fermentable dietary fibre is fermented. Alternatively, modulated SCFAs produced by slow fermentable dietary fibre are found in the distal colon.[95] Many researches have shown that butyrate induces the proliferation of normal colonocytes while suppressing cells with colorectal tumour phenotype.[96] Due to its accumulation in the cytoplasm during cancer progression, butyrate plays a role as a histone deacetylase inhibitor (HDACi), enabling it to regulate the transforming growth factor β (TGF-β) signalling pathway during cell sensitization and exert its pro-apoptotic effects in cancerous colonocytes. Butyrate's HDACi activity also helps to prevent macrophage-derived inflammation by suppressing the activity of pro-inflammatory cytokines in the gut.[97,98]

In diet intervention studies, Drabinska et al,[99] demonstrated that prolonged dietary administration of oligofructose-enriched inulin significantly increased *Bifidobacterium* count and increased faecal acetate and butyrate levels. Furthermore, researchers have shown that mixtures of multiple dietary fibre types confer added advantages over single dietary fibre. For example, a combination of raftilose (oligofructose) and guar gum in an *in vitro* fermentation

study produces higher total SCFAs than guar gum alone.[100] Meanwhile, Ghaffarzadegan et al.,[101] demonstrated that consumption of both guar gum and pectin modulate high proportions of butyrate in comparison to rats fed with individual fiber. Additionally, in an *in vivo* study by Qamar et al.,[102] revealed a significant inhibition of ACF formation in mice fed with a combined diet of GOS and inulin when compared to the individual prebiotic treatments. This effect is associated with bacterial enzymatic activities and SCFAs at dose-dependent effects.

### Decreased Number of Enteropathogens

Many researches have also indicated that *in vitro* incubation of faecal bacterial cultures with oligofructose and/or inulin have shown to promote the growth of beneficial commensal bacteria, namely *Bifidobacteria* and *Lactobacilli*, whilst enteropathogens, such as *E. coli* or *Clostridia*, are maintained at low levels.[83,103] Among other prebiotics that have a similar growth-promoting effect on these bacteria are GOS, arabinoxylooligosaccharides, gentiooligosaccharides, xylooligosaccharides, and chitooligosaccharides (Table 2). Moreover, the abundance of *Bifidobacteria* and *Lactobacilli* in the gut facilitates the removal of the ultimate mutagens bound on the cell surface and peptidoglycans of the bacterial cell.[104] Selective fermentation by *Bifidobacteria* and *Lactobacilli* produces lactic acid bacteria and other SCFAs as metabolic products from non-digestible carbohydrate fermentation in the gut, reducing intestinal pH, thus catering for a favourable microenvironment for the probiotic growth while providing a bactericidal environment for putative enteropathogens. This favourable microenvironment stimulates the production of β-glucuronidase, a bacterial enzyme responsible for the biotransformation of procarcinogens and carcinogens into less toxic metabolites.[105]

### Reduced Intestinal Permeability

The strengthening of tight junctions is another possible mechanism by which prebiotics promote the growth of beneficial bacteria to convey their anticancer properties via the normalisation of increased intestinal permeability. However, the evidence for the mechanisms involved still remains elusive. In an *in vitro* study using the trans-epithelial electrical resistance (TER) assay and Caco-2 cell line as a model, Mundi et al.,[106] demonstrated that prebiotics (Raftilose) confer added advantage to probiotic supplementation (*Bifidobacterium* Bb 12) by increasing the integrity of Caco-2 intestinal monolayers treated with the tumor promoter, deoxycholic acid (DCA). Metabolites or bioactive factors (e.g. some forms of peptides) mediated by these probiotics are known to be a factor in the upregulation of various cell signaling pathways (i.e. integrin-p38 MAP kinase-dependent mechanism) that lead to the strengthening of tight junctions and the intestinal barrier function.[107]

### Enhancement of the Immune Response

Another recently proposed mechanism of prebiosis in exerting anticancer properties is by activation of the immune response following alteration of gut microecology. Yang et al.,[108] has revealed that arabinogalactan significantly induced the expression of cytokines, chemokines, and co-stimulatory receptors in an *in vitro* microarray study. The study also noted an increase in the percentage of DC, $CD3^+CD8^+$ T cells, $CD49b^+CD3^-$ NK cells among splenocytes, and cytotoxicity activity in tumor-bearing mice. More recent data showed differences in the immunomodulatory effects of candidate probiotic bacteria against the stages of CRC carcinogenesis. Regarding the chemoresistance of tumor tissues, novel immune checkpoint therapy may not confer any effects on patients with stage III or IV CRC.[109] Therefore, developing a better understanding of the chemosensitivity of prebiotic therapy and its underlying mechanisms in CRC should be a priority.

### The Synbiotics and the Selectivity as a Challenge to Achieve Synergism in Combating CRC

Although scientific evidence demonstrating the health-promoting potential of prebiotics continues to accumulate, not all dietary prebiotics are good fermentative substrates for the growth of certain probiotics. For example, *Lactobacillus* strains with low starch-degrading activity cannot ferment resistant starch, thus exhibiting low pro-apoptotic activity towards CRC cells.[110] Some controversial experimental findings also prebiotics or synbiotics may not have protective roles against CRC. Rats fed with an inulin diet showed a similar number of polyps as those fed with a high-beef diet, and the number of rats bearing CRC was 100% in the inulin group while 89% in the high-beef group.[111] Differential synbiotics treatment outcome against CRC was observed between the beneficial probiotic *Bifidobacterium* and *Lactobacillus* strains with germinated brown rice in forming ACF, whereby *B. animalis* did not reduce the formation of ACF significantly as compared with *L. acidophilus*.[112] This finding could be due to the selectivity of gut microbiota to exert the synergism effect.

It is worthwhile to note that prebiosis is indirect, and its selectivity may or may not increase the host's resident gastrointestinal beneficial microbiota. Since the terms allude to synergism, synbiotics should be the key to solving this problem, specifically by using prebiotic compounds that selectively favor the survival, growth, and activity of the selected indigenous probiotic strain(s). An ideal synergistic synbiotics product should incorporate appropriate single or multi-strain probiotics with a suitable mixture of prebiotics that confer an added or synergistic effect by promoting the multiplication of the endogenous beneficial microbes and reducing the number of cancer-promoting bacteria. Recently, a synbiotics blend of *L. acidophilus*, *B. animalis* subsp.

*lactis*, and germinated brown rice, as a candidate prebiotic, have been shown to inhibit CRC carcinogenesis by enhancing antioxidative capacity and inducing apoptosis in rats treated with 1,2-dimethylhydrazine (DMH) and dextran sulfate sodium (DSS).[112] In another similar animal model, a combination of *B. lactis* and resistant starch enhanced the acute apoptotic response to a genotoxic carcinogen (AARGC) and colonic fermentative events. It indicates that resistant starch has shown to serve as a metabolic substrate for *B. lactis* to exhibit its proapoptotic action.[110] Meanwhile, an *in vivo* study conducted by the same group validated the protective effect of *B. lactis* in their synbiotic combination with resistant starch in AOM-induced CRC in rodent models.[113] This finding corroborates the hypothesis that the synbiotic effect confers additional advantages in CRC prevention strategy as compared to prebiotic or probiotic alone.

It should be mentioned, however, that the beneficial effects of synbiotics against CRC are mostly postulated based on animal studies. To date, very few human trials on this subject have been published, and these reports often give inconclusive results. One of the earliest clinical findings is from a 12-week randomized, double-blinded, placebo-controlled human study. It was reported that a mixture of oligofructose-enriched inulin in conjunction with *L. rhamnosus* GG (LGG) and *B. lactis* Bb12 (BB12) reduced the risk of CRC mainly by alleviation of genotoxin exposure. This intervention also stimulated the growth of *Bifidobacteria* and *Lactobacilli* and reduced *Clostridium perfringens*.[114] Meanwhile, in another 4-week crossover, randomized, double-blind, placebo-controlled trial of synbiotic combination of resistant starch and *B. lactis*, a significant change in the fecal stream bacterial community was observed. However, there were no significant alterations in the faecal chemistry and epithelial kinetics.[115] In a recent study, Krebs[116] showed no statistical differences in the systemic inflammatory response in both prebiotic and synbiotic groups, although there was a considerably higher abundance of lactic acid bacteria (LAB) present in the mucosa of the CRC patients. A meta-analysis conducted on 21 RCT (1776 participants), also showed that probiotic and synbiotic supplementation resulted in a reduction of time to first flatus, time to first defecation, days to first solid diet, days to first fluid diet, and days to postoperative hospital stay after gastrointestinal cancer surgery when compared to the control group. Furthermore, probiotic and synbiotic supplementation decreased the incidence of abdominal distension and postoperative ileus.

The challenge to produce an ideal synergistic combination of prebiotics and probiotics has spurred researchers to gather empirical evidence for its use in the prevention and treatment of CRC. Due to the huge diversity of the gut microbiome, the introduction of any new probiotics and prebiotics may lead to homeostasis dysregulation in the intestinal microflora or commensal dysbiosis associated with the pathogenesis of various diseases. This occurs when some prebiotics (other than inulin or trans-galactooligosaccharides) or dietary fibers provide a source of energy for the growth of opportunistic pathogenic bacteria. This overgrowth of the pathogenic bacteria is always related to the competitive removal of the supplemented probiotic bacteria in the gut of the host.

The administration of this synbiotic treatment also poses a challenge. Certain strains and species of probiotics are vulnerable to extreme gastric conditions where they could be digested before they can reach the small intestine in order to colonize and exert the intended benefits. Therefore, probiotics require a protective shield that can also act as a transport to safely released in the intestines.[117] In this case, the matched prebiotics can coat the probiotics, allowing them for gastric digestion. Optimization of the tailored supplements is, however, largely dependent on the selectivity of the prebiotics, the strain of probiotics, and several other environmental factors, such as temperature and pH.[118] It should also be noted that due to the unique gut microbiome in each individual, the viability and effectiveness of the prebiotics and probiotics may vary.[119] Therefore, finding a perfect combination of prebiotics and probiotics to enhance the prophylactic and chemo-protective properties of synbiotics may only be feasible with a comprehensive interpretation of the microbiome in individual human and animal studies.

## Conclusion and Future Remarks

Prebiotics have been widely reported to be beneficial in combating CRC by increasing the colonization and proliferation of commensal microbes in the colon and reducing carcinogen production. Most marketed prebiotics exert protective effects against CRC by increasing the abundance of *Bifidobacterium* and *Lactobacillus* while decreasing the abundance of pathogenic microbes, such as *Clostridia* and *Bacteroides*. A combination of multiple dietary prebiotics demonstrates better therapeutic outcomes for CRC development than a single prebiotic treatment. Many have proposed the mechanisms of action that underlie their protective activity. However, the exact processes that are responsible for these actions remain elusive. Nonetheless, the possible central mechanisms may involve the stimulation of beneficial indigenous microbes via selective colonic fermentation of prebiotics, leading to decreased number of enteropathogens via SCFA production, enhanced immune response, enhanced micro-nutrition absorption in the colon, modified gene expression in the cecum, colon, and faeces, elimination of exogenous carcinogens and enhanced activity of xenobiotic metabolizing enzymes.

Apart from these, using prebiotics with probiotics to form symbiotics for combating CRC has provided synergistic and

additive protective effects as compared to individual treatment alone. However, current reports on these benefits are conflicting and merit further research to determine the exact mechanisms involved, which are necessary to facilitate unbiased preclinical and clinical trials. To encourage using prebiotics in combating CRC, future investigations should focus on the pre- and post-treatment outcome against CRC carcinogenesis, patients in different stages of CRC and tumor location. Besides, molecular analytical tools, such as metagenomics, meta-transcriptomic, functional gene analysis or NGS approaches, should be used to precisely identify the association of certain intestinal microbes with the matched CRC and healthy tissues as well as the compositional shift in response to prebiotic treatment. In the context of symbiosis, it is pivotal to determine its optimal formulation and working dose to enhance the efficacy in treating CRC with minimal side effects. Furthermore, the compatibility between prebiotics and different probiotic strains to achieve optimal therapeutic effects should also be explored.

## Authors' Contributions

Conceptualization by IMF; Writing and original draft preparation by IMF, NH, NMAN, and WYS; Writing, review, and editing by IMF, AS, IM, KR, and LSM; Preparation of figures by IMI and IMF. The final version of the manuscript has been examined and endorsed by all the authors involved.

## Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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