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Probiotics and Postbiotics in Colorectal Cancer: Prevention and Complementary Therapy

Kvakova M *et al.* Probiotics and postbiotics in CRC prevention and therapy.

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Abstract

Colorectal cancer (CRC) is a leading cause of human mortality worldwide. With conventional anti-cancer therapy not always being effective, there is growing interest in innovative "drug-free" cancer treatments or interventions that improve the efficacy of established therapy. CRC is associated with microbiome alterations- a process known as dysbiosis that involves depletion and/or enrichment of particular gut bacterial species and their metabolic functions. Supplementing patient treatment with traditional probiotics (with or without prebiotics), next-generation probiotics (NGP) or postbiotics represents a potentially effective and accessible complementary anti-cancer strategy by restoring gut microbiota composition and/or by signaling to the host. In this capacity, restoration of the gut microbiota in cancer patients can stabilize and enhance intestinal barrier function, as well as promote anti-carcinogenic, anti-inflammatory, anti-mutagenic or other biologically important biochemical pathways that show high specificity towards tumor cells. Potential benefits of traditional probiotics, NGP and postbiotics include modulating gut microbiota composition and function, as well as the host inflammatory response. Their application in CRC prevention is highlighted in this review, where we consider supportive *in vitro*, animal and clinical studies. Based on emerging research, NGP and postbiotics hold promise in establishing innovative treatments for CRC through conferring physiological functions *via* the production of dominant natural products and metabolites that provide new host-microbiota signals to combat CRC. Although favorable results are reported, further investigations that focus on strain and dose specificity are required to ensure efficacy and safety of traditional probiotics, NGP and postbiotics in the CRC prevention and treatment.

Key Words: Colorectal cancer; Traditional probiotics; Next-generation probiotics; Postbiotics; Gut microbiota.

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Core Tip: The effect of traditional probiotics, next-generation probiotics (NGP) or postbiotics in colorectal cancer (CRC) prevention and complementary therapy can be associated independently or in mutual cooperation with several mechanisms, including suppression of inflammation, enhancing apoptosis of tumor cells, restoring intestinal barrier function, competition with pathogens and by promoting healthy gut microbiota composition and function. Traditional probiotics, NGP or postbiotics supplementation is also a potential strategy to boost the effectiveness of chemotherapy and immunotherapy, reduce the rate of postoperative complications and improve quality of CRC patients' lives.

INTRODUCTION

Colorectal cancer (CRC) is the third most diagnosed cancer in males and the second in females worldwide, thus s significant change in lifestyle is required to facilitate effective CRC prevention[1]. CRC is a heterogeneous disease of the intestinal epithelium, comprising colon, rectum and anus. It is characterized by a dysregulated immune response, accumulation of stem cells mutations, intestinal barrier disruption and dysbiosis, which is often regarded as an unfavorable alteration in gut microbiota composition and function. Up to 90% of CRC risk is thought to be lifestyle dependent, primarily due to dietary or environmental factors including feeding patterns that modulate consumption of fiber, red and processed meat or alcohol consumption, low omega-3 fatty acids and vitamin D intake. Obesity, lack of physical activity and smoking are also significant risk factors that are reported to promote CRC-associated microbiota changes[2]. CRC is linked with microbiome alterations, which include depletion and/or enrichment in particular bacterial species that are present in CRC

patients (extensively reviewed by Torres-Maravilla *et al*[3], Ternes *et al*[4], Janney *et al*[2], Fong *et al*[5] and Wirbel *et al*[6]).

The human microbiota is a complex ecosystem of bacteria, viruses, eukaryotes and archea, which can regulate a variety of host physiological functions, including digestion, immune response, metabolism, disease pathogenesis, elimination of toxins, biosynthesis of key compounds such as essential vitamins and cofactors. Microbiota can even modulate gut-brain axis function to alter, for example anxiety and mood. Symbiotic bacteria that colonize the human gut can be classified into several phyla, comprising Bacteroidetes and Firmicutes, followed by Proteobacteria, Fusobacteria, Actinobacteria, Verrucomicrobia, and Spirochaetes. Microbiome composition varies between healthy individuals, as well as in CRC patients[7]. CRC-associated bacteria that have been identified so far include enrichment of *Fusobacterium nucleatum*, *Enterococcus faecalis*, *Streptococcus gallolyticus*, entero-toxigenic *Bacteroides fragilis*, *Escherichia coli*, *Peptostreptococcus* spp., and *Ruminococcus* spp. By contrast, *Lactobacillus* spp., *Bifidobacterium* spp., *Faecalibacterium* spp., *Roseburia* spp., *Clostridium* spp., *Granulicatella* spp., *Streptococcus thermophilus* and other species of *Lachnospiraceae* family are depleted in CRC[2-5]. These altered microbiota signatures can potentially be used to provide future diagnostics, and their presence/absence may contribute to the pathogenesis or prevention/treatment of CRC. However, the pathophysiological role of dysbiosis in CRC still remains unclear, since microbiota changes may reflect changes in host health status and some bacteria may even confer protection as a compensatory response to disease progression. This complexity is clearly evident by the report of specific bacteria associated with tumor initiation phase (driver bacteria) whereas other bacteria are associated with tumor development during progressive stages of CRC (passenger bacteria). Driver bacteria reportedly contribute to the formation of a tumor microenvironment that is comprised of normal epithelial cells and cancer cells. In this milieu secreted microbial metabolites trigger damage to normal host cells, thus reprograming their metabolism to change the intestinal microenvironment and microbiome profile towards a more "CRC supportive" composition[8-10]. Potential driver

bacteria include *Bacteroides fragilis*, *Escherichia coli*, *Enterococcus faecalis*, *Bacillus*, *Bradyrhizobium*, *Methylobacterium*, *Streptomyces*, *Shigella*, *Citrobacter*, *Salmonella*, *Intrasporangiaceae* and *Sinobacteraceae*. On the other hand, the passenger bacteria, occupy an existing tumor microenvironment where they are thought to either promote or inhibit CRC progression. Reported passenger bacteria include species *Fusobacterium*, *Parvimonas*, *Peptostreptococcus*, *Campylobacter*, *Streptococcus*, *Schwartzia*, *Burkholderiales*, *Caulobacteraceae*, *Delftia*, *Oxalobacteraceae*, *Faecalibacterium* and *Sutterella*[8-11]. The host gut microbiota and immune system play important role in CRC prevention and development. Therefore, probiotics, next-generation probiotics (NGP) or postbiotics could be used as weapons to prevent CRC, to support the treatment and to improve the clinical outcomes in CRC patients. This minireview summarizes recent CRC findings from clinical, animal and *in vitro* studies, and discusses the efficiency of probiotics, NGP and postbiotics in CRC prevention and therapy.

TRADITIONAL PROBIOTICS

Probiotics are defined as "live, non-pathogenic microorganisms that, when administered in adequate amounts, may confer a health benefit on the host"[15]. Probiotics have a centuries-long history of safe use as prevention and adjuvant therapy in combating human disease. They are also promising candidates in modulating human gut microbiota composition and function in CRC patients. Traditional widely used probiotics mainly belong to *Bifidobacterium* spp., *Lactobacillus* spp. and other lactic-acid-producing bacteria, including species belonging to *Streptococcus, Enterococcus* and *Lactococcus*, complemented by yeasts of the genus *Saccharomyces*. The beneficial effects of probiotics, functioning in a species and/or strain-specific manner, include sustaining a healthy microbiome, reversing dysbiosis, preventing pathogenic infections and mucosal adhesion of pathogens, stabilizing and enhancing intestinal barrier function. Probiotic bacteria may achieve these beneficial function in part by producing anti-carcinogenic, anti-inflammatory, anti-mutagenic and other biologically important

compounds such as short-chain fatty acids (SCFA), vitamin K or B-group vitamins[5,7,16,17].

Current research builds on a foundation of work demonstrating that gut microbiota modulation through administration of probiotics and/or prebiotics plays an important role in CRC prevention and therapy. In a randomized, double-blinded, placebo-controlled trial, 60 patients underwent surgical CRC resection, of whom 29 received the probiotic powder (*Bifidobacterium animalis* subsp. *lactis* HY8002 ($1x10^8$ CFU), *Lactobacillus casei* HY2782 ($5x10^7$ CFU), and *Lactobacillus plantarum* HY7712 ($5x10^7$ CFU)) and 31 placebo, for 4 wk, starting at one week preoperatively. The treatment group receiving probiotic powder showed an increase in abundance of *Bifidobacterium*, *Akkermansia*, *Parabacteroides*, *Veillonella*, *Lactobacillus*, *Erysipelatoclostridium* and a reduction in bacteria associated with CRC, such as *Prevotella*, *Alloprevotella*, *Fusobacterium*, *Porphyromonas*. Lower serum zonulin, improved postoperative bowel function and postoperative recovery was evident in the probiotic group compared with placebo[18]. In another randomized clinical trial, a group of 31 CRC patients received probiotic supplement *Bifidobacterium longum* BB536 ($5×10^{10}$ CFU/ 2 g/ daily) preoperatively for 7–14 days and postoperatively for 2 wk. Attenuated postoperative inflammatory responses (high-sensitivity C-Reactive Protein), reduced risk of postoperative infectious complications and accelerated health recovery after colorectal resection were evident in the treatment group. Hospital stay was significantly shortened and correlated significantly with increased Actinobacteria and decreased Firmicutes after probiotic intervention[19]. Aisu *et al*[20] administered BIO THREE® 2 mg- *Enterococcus faecalis* T110, 10 mg- *Clostridium butyricum* TO-A and 10 mg- *Bacillus mesentericus* TO-A to 75 CRC patients 15 days prior to the surgery. Incidence of postoperative complications and superficial incisional infections were lower, and these health effects were as shown to associate with an increased mean proportion of beneficial *Bifidobacterium*, postoperatively, even though this organism was not administered as part of the probiotic regime. The change in microbial diversity and improved integrity of the mucosal barrier were also observed by Liu *et al*[21] after *Lactobacillus plantarum* CGMCC 1258, *Lactobacillus acidophilus* LA-11,

Bifidobacterium longum BL-88 (2.6x10^{14} CFU/ 2 g/ daily) administration 6 days preoperatively and 10 days postoperatively to CRC patients. The numbers of beneficial bacteria, including *Bifidobacteria* and *Lactobacilli*, increased in the probiotic group after surgery, whereas they decreased in the placebo group. In contrast, Enterobacteriales and *Pseudomonas*, were decreased in the probiotic group whereas they increased in the placebo group. Based on a number of clinical trials, the preoperative oral intake of probiotics combined with the postoperative treatment in patients who need gastrointestinal surgery is potentially recommended. Larger rigorously controlled clinical trials are required to endorse these preliminary positive outcome studies since avoidance of probiotic use has also been recommended in patients with immunodeficiency and dysbiosis. More studies and the key outcomes are listed in Table 2.

NEXT-GENERATION PROBIOTICS

One potential approach to achieve CRC prevention and treatment is through NGP administration. As we describe above, the most frequently used probiotics belong to *Bifidobacterium* spp. and *Lactobacillus* spp. However, recent studies using metagenomic approaches have revealed the importance of further identification and characterization of commensal species, mainly anaerobic ones, residing in the gastrointestinal tract that play an important role in regulating the immune system and maintaining overall gut health. Growing evidence suggests that dysbiosis may contribute to CRC progression as well as several other diseases[32-35]. Although there is no official definition of NGP, it is generally defined as live microorganisms identified on the basis of comparative microbiota analyses between healthy and sick individuals/animals that, when administered with strain-specificity and in dose dependent manner, confer health benefits on the host[36,37]. Compared with healthy individuals, patients with CRC possess a different compositional structure and physiological activity of the gut microbiota with SCFA-producing bacteria being depleted. This suggests that SCFA-producing bacteria might potentially exhibit anti-inflammatory and anti-carcinogenic

properties, as well as being NGP candidates in CRC prevention and therapy. SCFA, primarily acetate, propionate, and butyrate, are the key physiological metabolites of the microbial fermentation of dietary fiber in the colon. Butyrate is the major energy source for colonocyte homeostasis, promoting growth stimulation and production of protective cytokines that maintain gut barrier integrity and function[17,38-40]. Furthermore, increasing levels of SCFA in the gut helps to create a favorable microenvironment for beneficial bacteria by inhibiting the growth and adhesion of pathogens, and by enhancing vitamin bioavailability, mineral absorption and promoting mucosal integrity. Most butyrate-producing bacteria in the human colon belong to the Firmicutes phylum, clostridial clusters IV and XIVa, the most dominant species being *Faecalibacterium prausnitzii* and *Eubacterium rectale,* followed by *Eubacterium* spp. as well as *Anaerostipes* spp. and *Roseburia* spp. In addition to butyrate-producing bacteria, other NGP candidates with important regulatory effects on gut homeostasis include *Akkermansia muciniphila*, non-toxigenic *Bacteroides fragilis*, *Propionibacterium freudenreichii* and some strains of *Bacillus* spp. and *Clostridium* spp., which belong to Generally Recognized As Safe microorganisms[7,41,42].

Chronic oral administration of *Butyricicoccus pullicaecorum* BCRC 81109 (butyrate producing bacteria) to BALB/cByJNarl male mice decreased colon tumor progression over 9 wk. This protection against CRC clinical outcome was linked to activation of the SCFA transporter solute carrier family 5 member 8 and/or G-protein-coupled receptor (GPR) 43[43]. Chen *et al*[38] also observed in an *in vivo* animal study that application of butyrate producing bacteria *Clostridium butyricum* ATCC 19398 (2×10^9 CFU/0.2 mL 3 times a week for 12 wk) inhibited intestinal tumor development by an increasing apoptosis of CRC cells, by modulating the Wnt/β-catenin signaling pathway. There was also a reduction in pathogenic bacteria and bile acid-biotransforming bacteria, whereas an increase in beneficial *Lactobacillus* spp. and SCFA-producing *Rumincoccaceae* and *Eubacterium* spp. was evident. Thus, reduction in colonic secondary bile acids, increased cecal SCFA levels and activated G-protein coupled receptors- GPR43 and GPR109A were mechanistically implicated. Growth of CRC cell lines (HCT-116 and SW1116) was

significantly inhibited by strains *Bacillus subtilis* ATCC 23857 and *Clostridium butyricum* ATCC 19398, and by their main metabolites bacitracin and butyrate. mRNA levels of important receptors and transcriptional factors related to inflammation for example, TLR4, MYD88, nuclear factor kappa B (NF-KB), IL-22, Survivin were decreased and expression of $P21^{WAF1}$ was increased after treatment of SW1116 cells with *Bacillus subtilis* and *Clostridium butyricum* NGP[44]. Purified components produced by NGP cells were also studied and inhibition of human cancer cell proliferation by controlling the cell cycle was detected. Polysaccharide A purified from *Bacteroides fragilis* NCTC9343 (non-toxigenic) induced the production of the pro-inflammatory cytokine IL-8[31] and aspartic protease Amuc_1434 (recombinant enzyme) from *Akkermansia muciniphila* upregulated the expression of tumor protein 53, increased mitochondrial reactive oxygen species (ROS) levels and promoted apoptosis of LS174T cells[45]. Pahle *et al*[46] employed *Clostridium perfringens* enterotoxin (CPE) in CPE-gene therapy to selectively target claudin-3 and -4 expressing colon carcinomas *in vitro* and *in vivo* by using a translation optimized CPE expressing vector. Elevated toxicity of the optimized CPE expressing vector was evident in claudin-positive cells 48 h after the transfection, with toxicity rates of 76–92% and rapid cytotoxic effects such as membrane disruption and necrosis. Further *in vivo* studies focused on the efficiency of NGP application in CRC are listed in Table 3 and postbiotics derived from NGP are considered below.

POSTBIOTICS

Postbiotics is an intensely researched subject that remains a largely understudied topic in CRC. Owing to the phenomenal number and variety of metabolites produced by bacteria, it has been an enormous challenge to isolate and characterize the specific compound/s responsible for the therapeutic efficacy. Moreover, defining safety profiles and appropriate application doses of particular postbiotics in the preclinical and clinical settings may require regulatory guidelines and approvals[5]. The International Scientific Association for Probiotics and Prebiotics (ISAPP) offers expertise in microbiology, microbial physiology, gastroenterology, nutrition, food science and pediatrics. ISAPP

recently provided the clear definition and scope of postbiotics to include "preparation of inanimate microorganisms and/or their components that confer a health benefit on the host"[50]. Postbiotics, which exert desired physiological effects to the host, include inactivated microbial cells or cell components (cell surface proteins, endo- or exo-polysaccharides, peptidoglycan-derived muropeptides and teichoic acids) or important metabolites secreted by gut microbiota through a fermentation process or released under certain conditions such as a change in intestinal environment or after lysis (SCFA including acetate, propionate and butyrate; enzymes; bacteriocins; reuterin; acetoin; organic acids, *etc.*)[5,50,51]. Therefore, the isolation and characterization of new postbiotics is a growing field and requires careful biochemical characterization of beneficial mechanisms. Supplementation with postbiotics, can in some cases be an effective and safer strategy to prevent and/or treat diseases, compared with ingestion of viable probiotic bacteria[5].

Microbial metabolites undoubtedly play an important role in CRC pathogenesis. Many studies have shown that certain postbiotics exert anti-tumor activity, including selective cytotoxicity against tumor cells suggesting their therapeutic potential (Figure 1)[5]. For example, SCFA are well-known inhibitors of epigenetic enzymes histone deacetylases, that play a central role in gene regulation, thus SCFA have the ability to induce cell cycle arrest, and/or apoptosis in many cancer cell lines[52]. Cell-free supernatants (CFS) of different *Lactobacillus* and *Bifidobacterium* strains have been shown to induce apoptosis or inhibit proliferation of CRC cell lines[53-55]. Chen *et al*[56] demonstrated that supernatants of *Lactobacillus johnsonii* BCRC17010 and *Lactobacillus reuteri* BCRC14625 strains in high concentrations were able to damage HT-29 cell membranes causing elevated lactate dehydrogenase release. A recent study has reported a potent selective cytotoxicity effect of postbiotic metabolites from *Lactobacillus plantarum* strains *via* anti-proliferative effects and induction of apoptosis in HT-29 cells whilst sparing the normal cells[57]. Cousin *et al*[58] showed that metabolites from *Propionibacterium freudenreichii* ITG P9, namely propionate and acetate, had induced intrinsic apoptosis of CRC cells, *via* the production and release of SCFA acting on mitochondria. Moreover, CFS or SCFA in

combination with Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), increased the pro-apoptotic gene expression (TRAIL-R2/DR5), decreased the anti-apoptotic gene expression of FLIP and XIAP in HT-29 cancer cells and enhanced the cytotoxicity in CRC cells compared to human healthy intestinal epithelial cells. Further control studies are required to delineate specific molecular targets in these models since enhanced toxicity to fermentation induced acidic pH shifts remains a potential protective mechanism.

As inflammation is undeniably linked to carcinogenesis, any postbiotic that inhibits inflammation is also an important candidate acting as anti-tumor agent. It was shown that *Lactobacillus rhamnosus* GG- derived protein p40 can play a role in the prevention of CRC by suppressing intestinal epithelial inflammation, inhibiting epithelial cells apoptosis and by promoting IgA production[59-61]. CFS derived from several other probiotic strains, such as, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus* GG and *Bifidobacterium breve*, were able to downregulate inflammation, exhibit antioxidant activity or maintained intestinal barrier integrity[62-64].

To date, only a few animal studies have been performed to evaluate the effectiveness of postbiotics in CRC prevention and therapy *in vivo*. The stage is now set to expand this work with the use of translational *in vivo* models and clinical trials, which are essential to demonstrate efficacy. Sharma and Shukla[65] observed that CFS from *Lactobacillus rhamnosus* MD 14 MH656799 containing acetamide, acetate, propionate, butyrate, thiocyanic acid and oxalic acid attenuated early colon carcinogenesis in Sprague–Dawley rats (n = 36). The protective mechanism was linked to reduced fecal procarcinogenic enzymes, oxidants, aberrant crypt foci, vis-à-vis downregulating oncogenes [β-catenin, K-ras, Cox-2, nuclear factor kappa B] and upregulating tumor suppressor p53 gene leading to an almost healthy colon histology. De Moreno de LeBlanc *et al*[66] evaluated the effect of the enzyme catalase as a postbiotic from catalase-producing *Lactococcus lactis* htrA-NZ9000 on the prevention/regression of 1,2-dimethylhydrazine (DMH) induced CRC in BALB/c mice (n = 180-210). Catalase-producing *Lactococcus lactis* increased catalase activity in DMH-treated mice and

reduced H_2O_2 levels compared with the control group. Using the histopathological grading scale of chemically induced CRC, mice that received catalase-producing *Lactococcus lactis* had significantly less colonic damage and inflammation (2.0±0.4) compared to control animals that received non-catalase-producing *Lactococcus lactis* (4.0±0.3) or placebo-treated animals (4.7±0.5). Increased antioxidant activity reduced levels of H_2O_2 and ROS involved in CRC onset and progression.

There are also promising results from studies of postbiotics derived from NGP. Recently, numerous *in vitro* studies showed that supernatant from SCFA-producing bacteria, such as *Butyricicoccus pullicaecorum* BCRC 81109[43], *Clostridium butyricum* ATCC 19398[38], *Propionibacterium freudenreichii* TL142[67], *Propionibacterium acidipropionici* CNRZ80, *Propionibacterium freudenreichii* subsp. *freudenreichii* ITG18, *Propionibacterium freudenreichii* subsp. *shermanii* SI41[68] suppressed CRC cells proliferation and induced apoptosis. The same results were documented by Zhao *et al*[69], where single strain CFS from human *Bacillus* strains BY38, BY40, BY43, BY45 exhibited inhibitory effects on the proliferation of CRC cells in a dose-dependent manner through the induction of cell apoptosis. These results suggest that NGP could represent novel and promising anti-tumor agents against CRC. Further *in vitro* studies focused on the activity of postbiotics derived from different probiotic strains in CRC cell lines are listed in Table 4.

CONCLUSION

Traditional probiotics have utility in the management of CRC as adjuvant treatment, mainly to reduce postoperative complications and to alleviate the side effects of chemotherapy. Anti-tumorigenic mechanisms of probiotics include the modification of intestinal microbiome, improvement of intestinal barrier integrity, immune potentiation and maintaining gut homeostasis. However, it is well-known that the efficiency of probiotics is strain specific. The available clinical data indicate that CRC patients most often benefit from combined administration of strains *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium lactis* and *Bifidobacterium longum*. Use of their combination or in combination with other species is more effective than individual

supplementation. Nevertheless, consideration of each CRC patient's health status is still strictly recommended before administering viable probiotics. The gut microbiota is emerging as a contributing factor in the etiopathology of CRC. It is necessary to consider gut microbiota-drug interactions, including composition and metabolic activity of gut microbiota, which can both positively and negatively affect the outcome of CRC therapy. And even though research in this area is still in its infancy, it can be assumed that future clinical treatment and prevention of CRC will focus on supplementing the microbiome with commensal species (NGP candidates) that are predominantly anaerobic. Recent studies indicate that SCFA-producing bacteria, especially butyrate producers, such as *Akkermansia muciniphila*, *Propionibacterium freudenreichi* and *Butyricicoccus pullicaecorum* belong to beneficial NGP that may have applicability in CRC therapy. Furthermore, it was discovered that strains previously defined as potential pathogens appear to possess probiotic properties when these lack key virulence factors, for example non-toxigenic *Bacteroides fragilis NCTC9343* has positive effects on patient's health. A significant disadvantage of NGP is, above all, their safety as this has not yet been sufficiently confirmed in animal and clinical studies. Safety validation is of particular importance before administering NGP to oncology patients. Although NGP research is experimentally demanding, emerging data shows great potential. Therefore, it is necessary to continue and explore new possibilities of NGP use in the therapy or prevention of diseases, including CRC, especially through clinical trials. Supplementation with postbiotics should be favorable in CRC therapy, because postbiotics have the ability to stimulate immune responses, inhibit cancer cell proliferation, induce apoptosis and necrosis, and they can shape microbiome composition in CRC patients. The advantage of postbiotics is that they do not pose a risk of unwanted infection to the patient, although screening for product contamination will be important. Moreover, it is possible to accurately determine and verify administered doses of a particular postbiotic. However, this emerging research area currently lack *in vivo* or clinical data to assess feasibility. In conclusion, the administration of traditional probiotics, NGP or postbiotics, supported by various

experimental studies, is an efficient complementary therapeutic approach to combat CRC. A protective effect of probiotics and postbiotics against CRC onset is also indicated, however, lifestyle changes are recommended as a first line of defense in CRC prevention.

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