Gut Microbiota and Probiotics in Colon Tumorigenesis

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Abstract

The human gastrointestinal tract harbors a complex and abundant microbial community reaching as high as 10^{13}-10^{14} microorganisms in the colon. This endogenous microbiota forms a symbiotic relationship with their eukaryotic host and this close partnership helps maintain homeostasis by performing essential and non-redundant tasks (e.g. nutrition/energy and, immune system balance, pathogen exclusion). Although this relationship is essential and beneficial to the host, various events (e.g. infection, diet, stress, inflammation) may impact microbial composition, leading to the formation of a dysbiotic microbiota, further impacting on health and disease states. For example, Crohn's disease and ulcerative colitis, collectively termed inflammatory bowel diseases (IBD), have been associated with the establishment of a dysbiotic microbiota. In addition, extra-intestinal disorders such as obesity and metabolic syndrome are also associated with the development of a dysbiotic microbiota. Consequently, there is an increasing interest in harnessing the power of the microbiome and modulating its composition as a means to alleviate intestinal pathologies/disorders and maintain health status. In this review we will discuss the emerging relationship between the microbiota and development of colorectal cancer as well as present evidence that microbial manipulation (probiotic, prebiotic) impacts disease development.

Key Words: microbiota, inflammation, probiotics, colorectal cancer

1. **Introduction**

Higher vertebrates have intimately co-existed with a myriad of microorganisms throughout evolution and this relationship has proven mutually beneficial for both entities. Although numerous external and internal surfaces are colonized by microorganisms, the human gastrointestinal (GI) tract represents the most abundant reservoir of microbes. It is estimated that the intestinal tract harbors over 100 trillion bacteria regrouped in about 1000 species. The collective prokaryote community outnumbers eukaryote cells in the human body by a factor of 10 and their collective genome is 100 times more abundant than the host genome [1]. We are now just beginning to understand the genomic composition and contribution of the microbiota to intestinal homeostasis. This vast microbial community is rapidly acquired through close contact with the mother microbiota including passage through the birth canal, breast feeding and skin contact [2]. These various modes of microbial exposures clearly impact on the intestinal microbiota composition since naturally delivered infants have a larger number of *bifidobacteria* species than did babies delivered by caesarean section [3]. In addition, *Bifidobacterium* are the prevalent genus in breast-fed infants whereas *Enterococci* prevail in formula-fed infants [4]. The functional implication of early differential colonization is unclear but tantalizing observations suggest a relationship with disease susceptibility [5-7]. For example, the intestinal microbiota in children from Europe and rural Africa who are exposed to a modern western diet and a rural diet respectively, have significant differences in microbial composition. The major difference was that rural Africa children showed a significant enrichment in *Bacteroidetes* and depletion in *Firmicutes* when compared to European children [7]. These differences may well explain the higher incidence of IBD in Europe than Africa [8]. In addition, microbial composition is modulated by numerous extrinsic factors such as diet, age, medication (antibiotics, NSAIDs), treatment (radiation, surgery), stress and disease [9]. However, our current lack of knowledge about microbiome core (microbial community) plasticity prevents the establishment of a health/disease microbial threshold. Nevertheless, it is clear that various biological and physical situations impact microbial composition, with some of

these changes linked to disease development. It is not surprising that diseases of GI origin such as IBD and colorectal cancer have been subjected to increasingly intense investigation of the microbiota.

2. Endogenous microbiota, risk factors and development of colorectal cancer

Colorectal cancer (CRC) is among the most common worldwide cancers, accounting for over 1 million cases and about half a million deaths annually [10]. The incidence rates of CRC are higher in the Western world but are rapidly increasing in developing countries. The main two contributing factors to the occurrence of CRC are genetic (15%) and environmental factors [11]. In addition, inflammatory conditions have been shown to favor the development of CRC as patients with IBD have an annual risk increase of 1%/year (10 years post-diagnosis) [12]. Another emerging factor involved in CRC susceptibility is the intestinal microbial composition. Since the microbiota impacts numerous physiological functions related to cancer risk including control of epithelial cell proliferation/differentiation, production of essential nutrients and/or bioactive food components, prevention of overgrowth of pathogenic organisms and stimulation of intestinal immunity, recent efforts have been directed at defining the microbiota of CRC. Although few studies have tackled microbial composition in human CRC, recent reports have indicated differences between the microbiome of patients and healthy subjects. For example, the stool of CRC patients harbored increased *Bacteroides-Prevotella* populations compared to normal controls [13]. Another study investigated bacterial communities present at the intestinal mucosal surface of patients with adenoma. Interestingly, at the genus level, patients with adenomas showed increased abundance of *Dorea spp.*, *Faecalibacterium spp.* and lower proportions of *Bacteroides spp.* and *Coprococcus spp.* than non-adenoma subjects [14]. This finding revealed that alterations in bacterial community composition is associated with CRC; although at this point, it is unclear whether these changes preceded disease onset or simply reflect a consequence of the disease.

Interestingly, factors influencing CRC development (age, NSAID, diet) were also demonstrated to impact on microbial composition. For example, Mäkivuokko et al. [15] reported that there was a significant reduction in overall numbers of microbes in elderly subjects compared with young adults. Moreover, lower numbers of *Firmicutes* and an increased proportion of *Bacteroidetes* in the elderly were observed in this study. Furthermore, the total number of microbes was higher and lower numbers of *Collinsella spp.* were evident in the elderly subjects who take NSAID compared both with young adults and the elderly who do not take NSAID on a regular basis. Enck et al. [16] reported that individual bacterial species consistently and significantly increased with age (*E. coli, Enterococci spp.*), decreased at an older age (*Bacteroides spp.*), or were stable throughout the life span (*Lactobacilli, Bifidobacteria*). These results suggest that the use of NSAIDs, combined with age, may influence the process of tumorigenesis by changing the composition of the intestinal microbiota.

High animal protein intake and a high fat diet are additional risk factors associated with colon cancer. Americans who have a high colon cancer risk typically consume a high-animal protein and fat diet, whereas Africans, who have the lowest colon cancer risk, consume a staple diet of maize meal, rich in resistant starch. O'Keefe et al. [17] analyzed the contents of colon in native Africans and African Americans and Caucasian Americans. Butyrate is produced by specific colonic bacteria, predominantly *Clostridia clusters XIVa* and *IV of Firmicutes*, from food residues such as dietary fiber or resistant starch. Their results demonstrated that the colonic content of butyrate, and all of the chief short chain fat acids (SCFA) produced by bacterial fermentation of undigested carbohydrates, were significantly higher in native Africans compared with African Americans and Caucasian Americans. In another study [18], fecal colony counts of 7-adehydroxylating bacteria were higher and *Lactobacilli* were lower in African Americans compared to Caucasians and colonic crypt cell proliferation rates were also higher in African Americans. These studies provide evidence that higher dietary intakes of animal products may alter the gut microbiota and therefore play a key role in carcinogenesis.

Obesity is a closely related risk factor of human CRC. Numerous studies [19-23] have verified the association between an alteration of the dominant phyla of bacteria in the gut and body weight, both in humans and animal models. The gut microbes have been shown to impact insulin resistance, inflammation, and adiposity via interactions with epithelial and endocrine cells. The intestinal epithelia cells (IECs) have a complex and mutually beneficial relationship with the gut flora. The bacteria metabolize some nutrition components in the gut, e.g. carbohydrates; in turn, the IECs metabolize the short-chain fatty acids and use them as a source of energy. In fact, the gut microbial community is essential for processing dietary polysaccharides. Bäckhed et al. [24] found that adult germ-free (GF) C57BL/6 mice given a normal microbiota harvested from the cecum of conventionally raised animals results in a 60% increase in body fat content and insulin resistance within 14 days, despite reduced food intake. Furthermore, colonization of GF mice with an "obese microbiota" results in a significantly greater increase in total body fat than colonization with a "lean microbiota". These results identify the gut microbiota as an additional contributing factor to the pathophysiology of obesity.

The increasing understanding of GI barriers, and the adjacent GI microbiota provide evidence that these two functional entities are key to achieving and maintaining gut health [25,26]. Gut microbiota contribute to the maintenance of an intact GI barrier and disruption of this barrier can impact inflammatory and allergic diseases. The GI barrier refers to a functional entity consisting of epithelial defense and metabolic functions, the mucosal immune system and the enteric nervous system (ENS) [25]. The formation of an intestinal mucus layer is an important physical barrier protecting the intestinal epithelium against invasive micro-organisms. The mucus layer is formed by various mucin proteins which are a family of heavily glycosylated proteins including mucin1, mucin 2, mucin 3a and others. Mucins are produced mostly by Goblet cells. Mucin 2 (MUC2), the principal component of intestinal mucus, along with small amounts of related-mucin proteins, polymerizes into a gel that provides an insoluble mucous

barrier that serves to protect the intestinal epithelium, Another important epithelial cell involved in bacteria-host interactions are the Paneth cells, which are critical for the maintenance of the intestinal barrier by producing zinc and antimicrobial peptides. The combined action of Goblet and Paneth cells has a significant impact on microbial composition and GI barrier function [27, 28]. Altered MUC2 expression and/or glycosylation leads to accompanying intestinal pathologies, including IBD and colon cancer [29]. The generation of a MUC2 mutant mouse model, alone or in combination with an *Apc* mutation, had unique physiopathologic features useful for dissecting complex relationships between tumor development and chronic inflammation [30]. Paneth cells directly sense enteric bacteria through cell-autonomous MyD88-dependent toll-like receptor (TLR) activation, triggering expression of multiple antimicrobial factors [31]. E-cadherin is a major component of adherens junctions, which play a key role in intestinal homeostasis and barrier function. Impaired expression of E-cadherin was linked to maturation and positioning of Goblet cells and Paneth cells in the murine small intestine and colon [32].

Cheese whey protein is rich in the amino acids of threonine and cysteine and promotes synthesis of mucin. In a model of dextran sulfate sodium (DSS), which is an irritant that causes colonic inflammation (colitis) by eroding the mucosal barrier -, feeding rats with a diet containing cheese whey protein reduced gene expression of inflammation markers and increased fecal mucin secretion and fecal *lactobacilli* and *bifidobacteria* counts [33]. Furthermore, the number of Goblet cells in the small intestine increased by the simultaneous incubation of *Bifidobacterium bifidum* IATA-ES2 with wheat gluten proteins, IFN-γ and enterobacteria using the rat intestinal loop model [34]. Thus, dysbiosis could be involved in the damage of the GI barrier and it is clear that further research is needed to fully elucidate the complex interactions between the microbiota and the GI barrier.

3. Gut microbiota, innate signaling, inflammation and colon carcinogenesis

The inter-relation between bacteria and inflammation is complex as bacteria and inflammation could mutually impact upon each other. This interaction can, in turn, modulate development of CRC (see Fig.1). As diagrammed in the figure, the types and level of activation of the various innate sensors can influence the gene expression pathways, level of inflammation and the consequences of these changes on DNA damage and chromatin alterations, which , when combined with host genetic factors can lead to tumorigenesis.   Swidsinski et al [35] showed that intracellular *E. coli,* is highly colonized in the colonic mucosa of patients with colorectal carcinoma and adenoma but not normal colonic mucosa, leading to hypothesis that *E. coli* colonization in the colon may indicate that a specific microorganism can be responsible for malignant pathology.   However, tumorigenesis is complicated and a linear association between dysbiosis-inflammation-tumorigenesis is not fully supported by other studies [36,37], suggesting that there are other regulatory pathway between dysbiosis and tumorigenesis, other than inflammation.
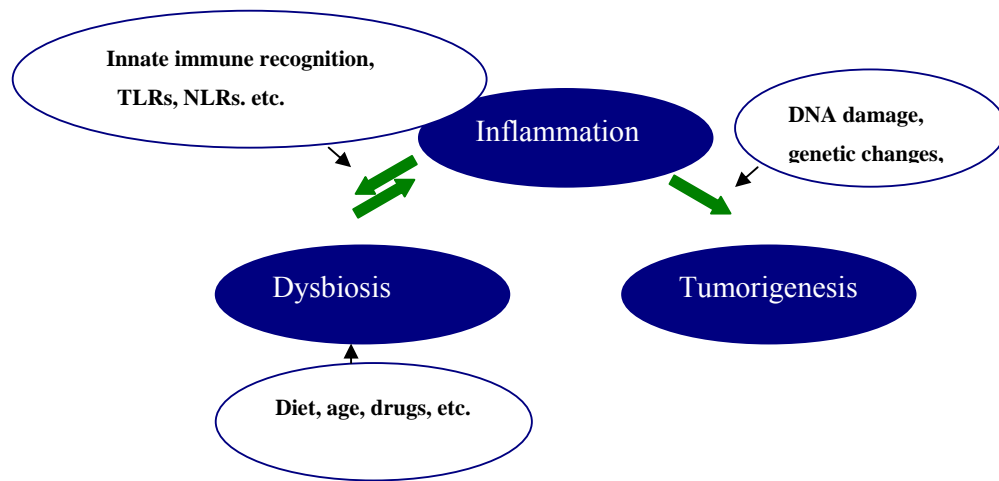

Figure 1: The evolving relationship between colon dysbiosis, inflammation and tumorigenesis. Experimental models have addressed the individual and collective role of microorganisms in the development of inflammation and CRC. Dysbiosis-inflammation-tumorigenesis provides an important model of carcinogenesis in the human colon. Please refer to the text for a more detailed discussion.

Experimental models have addressed the individual and collective role of microorganisms in the development of inflammation and CRC. For example, *Enterotoxigenic Bacteroides fragilis* (ETBF) secretes *B. fragilis* toxin (BFT) and causes human inflammatory diarrhea but also asymptomatically colonizes a proportion of the human population. In another animal study [38], GF IL-10$^{-/-}$ or WT mice were monoassociated with *Bifidobacterium animalis subsp.* The bacteria caused mild colitis in monoassociated IL-10$^{-/-}$ mice, whereas the intestinal tracts of WT animals remained free of inflammation. The result suggested a potential pathogenic role for this commensal bacterial species in a susceptible host. Wu et al. [39] indicated that mice chronically colonized with ETBF developed colitis and induced colonic tumors in multiple intestinal neoplasia (MIN) mice. Uronis et al. [40], showed that conventionalized IL10$^{-/-}$ mice exposed to the procarcinogenic compound azoxymethane (AOM) developed spontaneous colitis and colorectal carcinomas while AOM-WT mice were colitis-free and mostly developed low grade dysplasia. Interestingly, the bacterium *Bacteroides vulgatus* caused low level inflammation in IL10$^{-/-}$ mice, resulting in reduced colorectal tumors as compared to conventionalized IL10$^{-/-}$ mice. Moreover, GF AOM-treated IL10$^{-/-}$ mice showed no intestinal inflammation or CRC. The results support the concept that specific microbial entities differently impact on the development of intestinal inflammation and CRC. The molecular mechanism by which bacteria affect the development of intestinal inflammation and cancer is unclear but may be related to the triggering of a series of innate sensors responsible for microbial detection.

A number of studies have addressed the role of the host innate immune system in regulating carcinogenesis. The most studied innate sensors relating to colitis and CRC are the Nod-like receptors (NLR) and the Toll-like receptors (TLR). The interaction of gut microbiota and epithelial cells is an active process, in which NLRs and TLRs are likely directly involved. Colitis-associated cancer (CAC) is a colorectal disease where cancer arises in patients suffering from long bout of chronic intestinal

inflammation. CAC can be modeled in mice by injection of the AOM and by repeated exposure to DSS,. Using a mouse model system of CAC (AOM-DSS), Chen et al. [41] demonstrated that tumors increased in Nod1-deficient mice compared to wild type mice. In addition, $Apc^{MIN/+};Nod1^{-/-}$ mice, which harbor a mutation in Apc and are also deficient in Nod1, developed more tumors. This result suggests that Nod1 pathway could enhance the tumor-promoting effect of attenuated Wnt signaling. Additionally, depletion of the gut microbiota using antibiotic treatment suppressed tumor development in Nod1-deficient mice. Furthermore, NLRP3-signaling from immune cells was recently shown to play a protective role against colitis and CAC. [42] Additionally, NLRP6 has recently been shown to prevent development of CAC (Normand, S et al PNAS 2011). All together, these data support a role of the host innate immune signaling pathways in the regulation of inflammation-mediated colon cancer development.

Toll-like receptors (TLRs) belong to the Toll-like receptor/interleukin-1 receptor (TLR/IL-1R) superfamily which is defined by a common cytoplasmic Toll/interleukin-1 receptor (TIR) domain. The single immunoglobulin IL-1 receptor-related molecule (SIGIRR), a negative regulator for Toll-IL-1R signaling, plays a critical role in gut homeostasis, intestinal inflammation, and colitis-associated tumorigenesis by maintaining the microbial tolerance of the colonic epithelium [43-46]. Cells from SIGIRR-deficient mice showed enhanced activation in response to either IL-1 or certain Toll ligands [43]. In human colonic samples, SIGIRR was expressed mainly in IECs at levels significantly higher in inactive compared to active mucosa. In mice, colonic SIGIRR expression decreased rapidly after colitis development and returned gradually to basal levels [45]. SIGIRR may exert its inhibitory effect through blocking the molecular interface of TLR4, TLR7 and the MyD88 adaptor mainly via its BB-loop region [46]. Moreover, the innate adapter protein MyD88 prevents the development of CAC by transmitting IL-18 receptor signaling [47]. In contrast, TLR4 promotes the development of CRC in the AOM/DSS model [48]. TLR4 is expressed on CD4+ T cells and TLR4 triggering in

10

CD4+ T cells affects their phenotype and their ability to provoke intestinal inflammation. In a model of spontaneous colitis [49], $IL10^{-/-}TLR4^{-/-}$ mice displayed accelerated development of disease, with signs of overt colitis as early as 8 week of age, when compared with $IL10^{-/-}$ and $IL10^{-/-}TLR9^{-/-}$ mice which did not develop colitis by 8 months. Mechanistically, Lipopolysaccharide（LPS）stimulation of TLR4-bearing CD4+ T cells inhibited ERK1/2 activation upon subsequent cell stimulation. These data highlighted the complex relationship between the microbiota and the status of inflammation and CRC in the host and supported the model where susceptibility to develop CRC is modulated by the microbiota and by the repertoire of host innate sensors. Consequently, modulation of the intestinal microbiota using probiotics or prebiotics could influence the development of tumors.

4. Probiotics and colon cancer

The term "probiotics" has been in use for several decades and the Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation defined the term in 2001 as "live micro-organisms which confer a health benefit on the host when administered in adequate amounts"[50]. *Lactic acid bacteria* and *Bifidobacteria* are the most common types of microbes used as probiotics, while certain yeasts and bacilli may also be beneficial to the host. The immunomodulatory effect of probiotic bacteria has been postulated by Metchnikoff over 100 years ago [51]. There has been an increased interest in the scientific community on the protective roles of probiotics on intestinal diseases, especially IBD and colon carcinogenesis. A meta study [52] in 2006 showed that most of the studies (epidemiological and interventional) evaluating the effect of probiotics administration on the incidence of CRC and/or of precursor lesions had positive results although the authors of this publication argue that "There are no positive data from interventional studies so far."

4.1. In *vitro* studies

The precise mechanisms by which probiotics inhibit colon cancer may involve multiple pathways, including cell cycle, reactive oxygen species (ROS), apoptosis, production of specific bacterial enzymes and effects on the host metabolome. Polyamine synthesis is an early event during the G1 phase of the cell cycle and is necessary for cells to initiate their proliferative processes. Orlando et al. [53] found that *Lactobacillus GG* administration induced a significant reduction in polyamine biosynthesis in both the HGC-27 and DLD-1 cancer cell lines. The antiproliferative effect of the probiotic microorganisms was observed after 24 hours of the treatment. The anti-proliferative capabilities of probiotics may also relate to their ability to adhere to cells. Lee et al. [54] found that *Bacillus polyfermenticus SCD* was strongly adherent to Caco-2 cells and inhibited the growth of colon cancer cells in a dose dependent manner. Kim et al. [55] assessed the anticancer activity and bacterial enzyme inhibition of *Bifidobacterium adolescentis SPM0212*. The strain inhibited the proliferation of three human colon cancer cell lines: HT-29, SW 480, and Caco-2 and also dose-dependently inhibited TNF-α production and changes in cellular morphology. This specific bacterial strain could inhibit harmful fecal enzymes, including β-glucuronidase, β-glucosidase, tryptophanase, and urease.

The interaction of gut microbiota and epithelial cells is an active process, in which some proinflammatory cytokines are induced by probiotics from epithelial cells. TLRs are likely directly involved in this process. Paolillo et al. [56] reported Caco-2 cells exposed to *L. plantarum* bacteria significantly induced human beta-defensin 2 (HBD-2) mRNA expression and HBD-2 secretion in a time dependent manner compared to controls. The *L. plantarum*-induced increase in HBD-2 expression was inhibited by anti-TLR-2 neutralizing antibodies. Some strains of *Lactobacilli* can stimulate macrophages and dendritic cells to secrete IL-12, a cytokine that plays a key role in activating innate immunity. Shida et al [57] examined the IL-12-inducing ability in mouse peritoneal macrophages of 47 *Lactobacillus* strains belonging to 10

species. Almost all strains belonging to the *Lactobacillus casei* group or to *Lactobacillus fermentum* induced high levels of IL-12 and phagocytosis of the *Lactobacilli* was necessary for the IL-12 induction. Their results also demonstrated that *Lactobacillus* strains having a rigid cell wall resistant to intracellular digestion effectively stimulate macrophages to induce IL-12.

4.2. In *vivo* experiments

Probiotics contribute to the development of the mucosal immune system by influencing the innate inflammatory response and reducing mucosal inflammation. Probiotics also act through effects on dendritic, epithelial cells, native T cells in the lamina propria of the gut and can thus influence adaptive immunity. Previous studies have demonstrated the anti-inflammatory effect of probiotics on intestinal inflammation. *Lactic acid* bacteria are present in many foods, such as yogurt, and are frequently used as probiotics to improve some biological functions of the host. Perdigon et al. [58] found that yogurt induced a great reduction in the inflammatory immune response and inhibited tumor growth in 1,2-dimethylhydrazine(DMH) treated BALB/c mice. In addition, they observed an increase in the IgA-secreting cells and in CD4+ T lymphocytes. A yogurt based formulation containing microencapsulated live probiotic bacterial cells has been used in colon cancer prevention and therapy studies. Urbanska et al. [59] studied the properties of microencapsulated probiotic bacterial cells in a yoghurt formulation in MIN mice carrying a germline APC mutation. Daily oral administration of the microencapsulated *Lactobacillus acidophilus* resulted in significant suppression of colon tumor incidence, tumor multiplicity, and reduced tumor size. Furthermore, treated animals exhibited fewer GI intra-epithelial neoplasia with a lower grade of dysplasia in tumors. The immunomodulating and immunostimulating properties of yogurt and fermented milks have also been well documented. Yogurt feeding itself was correlated with increased or decreased levels of some cytokines, such as TNFα, IFNγ and interleukins [58-59]. Interestingly, Pagnini et al. [60] reported that probiotic*s* may promote gut health through stimulation, rather than suppression, of the innate immune system. They reported that

the multiple probiotic formulation VSL#3 (*Streptococcus thermophilus*, *Bifidobacterium infantis,* and *Lactobacillus acidophilus*) could prevent the onset of ileitis in the SAMP1/YitFc murine model by local stimulation of epithelial innate immune responses, i.e. increased production of epithelial-derived TNF-α and restoration of the epithelial barrier function in vivo. Leblanc et al. [61] reported that a yogurt diet given to BALB/c mice before and after the carcinogen 1, 2-dimethylhydrazine (DMH) treatment, inhibited tumor formation. In the DMH-yogurt group, cellular apoptosis increased during the treatment. Yogurt feeding induced TNF-α and IFN-γ expression in cells isolated from large intestine nodules and the expression of these cytokines also increased in cells obtained from Peyer's Patches of the yogurt control group.

Another key player in colon inflammation is the presence of reactive oxygen species (ROS) and the antioxidant properties of probiotics may contribute to their broad effects [62]. Likewise, polyphenols from tea and other beverages such as red wine can play a role as possible chemopreventive agents by reducing oxidative damage, and result in an increase in gut colonization by some probiotics strains [63]. Park et al. [64] assessed the effects of *Bacillus polyfermenticus* on the antioxidant system and the process of colon carcinogenesis in male F344 rats. The rats fed with *Bacillus polyfermenticus* exhibited significantly lower numbers of aberrant crypt foci than were observed in the DMH treated group. Leukocytic DNA damage and plasma lipid peroxidation levels, as well as a lower plasma total antioxidant potential, recovered in response to supplementation with *Bacillus polyfermenticus*. These results indicated that *Bacillus polyfermenticus* could exert a protective effect on the antioxidant system and the process of colon carcinogenesis.

An underlying mechanism that might be involved in the activity of probiotics may be the induction of apoptosis. Apoptosis induction by commensal bacteria could possibly represent a physiologic ''oncologic surveillance'' mechanism for colonic proliferative disease prevention [65], but this hypothesis awaits further testing for confirmation.

5. Prebiotic and synbiotics

Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to health. Synbiotics refer to nutritional supplements combining probiotics and prebiotics that are thought to act together; i.e. synergism. It has been suggested that a combination of a probiotic and a prebiotic, i.e. synbiotics, might be more active than either a probiotic or prebiotic alone in preventing CRC. A high olive oil-containing diet supplemented with a freeze-dried fruit and vegetable extract (OFV), increased the family *Lachnospiraceae* in Apc (MIN) mice and reduced the development of intestinal adenomas in this model [66]. As another example, Le Leu et al. [67] evaluated the effect of bacteria *Bifidobacterium lactis* and "resistant starch" (RS) and their combination (synbiotic) on their ability to protect against CRC. *Bifidobacterium lactis* utilizes the carbohydrate "resistant starch" (RS) as a substrate and up-regulates the acute apoptotic response to a carcinogen in the colon. Rats fed RS in combination with *Bifidobacterium lactis* showed a significantly lowered incidence and multiplicity of colonic neoplasms compared with the control group. No protection against cancer was seen in the group supplemented with only *Bifidobacterium lactis*. Interestingly, fermented milk product containing Bifidobacterium animalis subsp. lactis DN-173 010 strain protect against development of colitis in the T-bet$^{-/-}$; Rag2$^{-/-}$ mice through reduction of colitogenic microorganisms (Veiga P, et al Proc Natl Acad Sci U S A 107: 18132-18137). In addition, mice exposed to sialyl(alpha2,3)lactose-deficient milk were more resistant to DSS-induced colitis, which again associated with the reduction of potentially colitogenic microorganisms (Fuhrer A et al J Exp Med 207: 2843-2854. In another diet based study, Lara-Villoslada et al. [68] reported short-chain fructooligosaccharides(SC-FOS) increased cecal *Lactobacilli* and *Bifidobacteria* counts as well as short-chain fatty acid (SCFA) production in healthy rats. In colitic rats, SC-FOS feeding caused a decrease of MPO activity, leukotriene B4 (LTB4) production and iNOS expression. This anti-inflammatory effect was evidenced by a significant reduction in the extent of colonic damage. However, in

15

contrast, Burn et al. reported that the use of aspirin and/or resistant starch had no effect in hereditary nonpolyposis colon cancer (36). Thus there is a growing body of data indicating that specific bacterial species, in combination with dietary changes/additions, can modulate overall gut inflammation. Long term studies are now needed to assess the impact of these mechanisms of reducing gut inflammation on CRC.

6. Clinical trials

Despite the experimental evidence in rodent models showing a beneficial effect of probiotics that inhibits the development of CRC, a systemic approach for the evaluation of probiotics leading to the substantiation of health claims in humans is very limited. A FAO/WHO Working Group has generated guidelines for the criteria and methodology for evaluation of probiotics in clinical trials [69]. Since then, several studies have been reported, generally in the form of randomized, double- blinded, measurement of efficacy and side effect compared with placebo. In one study the administration of *Lactobacillus casei* was tested as a method to prevent the occurrence of colorectal tumors [70]; the occurrence of tumors with a grade of moderate atypia or higher was significantly lower in the patient group after 2-4 years treated with *Lactobacillus casei* as compared to the control group. In a 12-week clinical trial [71] completed in 2007, polypectomized patients were treated with *Lactobacillus rhamnosus GG (LGG)* and *Bifidobacterium lactis Bb12 (BB12)* and oligofructose-enriched inulin. The treatment resulted significant changes in fecal flora of the patients as *Bifidobacterium* and *Lactobacillus* increased and *Clostridium perfringens* decreased. The intervention also significantly reduced colorectal proliferation in the patients. A two-period crossover study on 38 healthy men was reported with treatment periods of 4 weeks of *Lactobacillus rhamnosus LC705 (LC705)* together with *Propionibacterium freudenreichii ssp shermanii JS (PJS)* [72]. The administration of *LC705* and *PJS* was followed by an increase in the fecal counts of *Lactobacilli* and *Propionibacteria* and a decrease in the activity of β-glucosidase with increasing counts of *Propionibacteria*. Recently, Gianotti et., al [73], reported in

a prospective trial that in 31 CRC patients, *Lactobacilli johnsonii (La1),* but not *Bifidobacterium longum (BB536),* affects intestinal microbiota by reducing the concentration of pathogen and modulating the intestinal immune response. Thus, in clinical trials, probiotics are suggested to play a protection role in the initial process of carcinogenesis. However, it remains to be determined if long term administration of probiotics can result in significant changes in the incidence of CRC in humans. Some of the challenges faced in these studies will be the selection and standardization of the appropriate microorganisms, control for dietary intake, time and frequency of microorganism dosing and development of biomarkers that can be followed over the long term course of any such clinical trials.

7. Conclusions

There is a growing body of evidence that the gut microbiota contributes to colon tumorigenesis. Animal experiments have revealed that microorganisms can directly impact gut immunity and inflammation but statistically and biologically significant evidence of such effects is still needed in human trials. Probiotics can inhibit the inflammatory process by enhancing host immune responses, altering the bacterial phylotypes in the colon and impacting the gut metabolome. They may also have anti-tumor properties through direct anti-proliferative activity on tumor cells. However, further experimental models are needed to better understand the exact mechanisms involved in the effects of probiotics on the host. The results of these studies will hopefully lead to more rational and standardized approaches for the use of probiotics in the prevention and treatment of human colorectal cancer.

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