Probiotic metabolites as epigenetic targets in the prevention of colon cancer

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Dietary interventions for preventing colon cancer have recently attracted increased attention from researchers and clinicians. The probiotics have emerged as potential therapeutic agents but are also regarded as healthy dietary supplements for nutrition and health applications. The probiotic metabolome may interfere with various cellular and molecular processes, including the onset and progression of colon cancer. Probiotic metabolites may lead to the modulation of diverse cellular signal transduction and metabolic pathways. The gut microbial metabolites (organic acids, bacteriocins, peptides, etc.) have been noted to interact with multiple key targets in various metabolic pathways that regulate cellular proliferation, differentiation, apoptosis, inflammation, angiogenesis, and metastasis. Progress in this field suggests that epigenetic alterations will be widely used in the near future to manage colon cancer. The present review provides insights into the molecular basis of the therapeutic applications and the chemopreventive activities of certain probiotic metabolites, with emphasis on the interaction between these metabolites and the molecular signaling cascades that are considered to be epigenetic targets in preventing colon cancer.

INTRODUCTION

Probiotics, the live microbial food supplements with the ability to beneficially affect the gut microbiome, have long been known to augment a variety of immunological and metabolic parameters through diverse mechanisms. The following mechanisms are among those that are well defined: altering composition of already existing normal microbial ecosystem, maintaining gut lumen epithelial barrier functions, and modulating mucosal and systemic immune responses of the host (Figure 1). The probiotics recommended for human applications are primarily the two classes of lactic-acid-producing microorganisms, the bifidobacteria (e.g., Bifidobacterium longum, Bifidobacterium infantis, and Bifidobacterium adolescentis) and the low-GC-content lactic acid bacteria such as Enterococcus spp., Lactobacillus spp., Lactococcus spp., Leuconostoc spp., Pediococcus spp., and Streptococcus spp. Many of these bacteria are also normal symbionts of mammalian (human and animal) and other vertebrates’ intestine and are already in use as probiotics or direct-fed microbial agents and starter cultures. In particular, the Lactobacillus spp. are ubiquitously present in fermented foods, vegetation, the oral cavity, the gastrointestinal (GI) tract, and the intestinal tract of various warm-blooded animals. The use of probiotics as dietary sources, a proposed mode of administration, and a new potential therapeutic strategy is based on the following evidence: (a) the ability of probiotics to modify the gut microbiota in a host beneficial manner, (b) the evidence that this modification may have health benefits for the host, and (c) the evidence that probiotics may have health benefits for the host.

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ecosystem, and the urogenital tract and are found to confer multiple health-promoting attributes.5,8,10 Traditionally, the natural ecological niches serve as important sources of new leads for pharmaceutical industries, but with the invention of novel structural classes in decline, the need to search for alternative sources of biocatalysts, therapeutic agents, and chemical diversity is increasing.11 The distal gut in humans and monogastric animals, in particular, is metabolically a highly active organ and is regarded as a versatile digester, playing an important role in the generation and utilization of metabolic energy.12 This largely unexplored area has attracted microbial ecologists from around the globe who have initiated culture-independent metagenomic studies targeting the genome of the gut microbiota, collectively called the “microbiome,” and exploring its association with the host.13 The gut microbial metabolome, which consists primarily of biomolecules, such as antagonistic proteins or peptides, organic acids, and hydrolytic enzymes, may have several therapeutic and industrial biotechnological applications.2,14,15

**Figure 1** Role of probiotics, prebiotics, and gut microbial short-chain fatty acids in gut health. Probiotics prevent colonization of microbes that can possibly lead to genotoxicity in the colonocytes; in addition, they enhance mineral absorption and prevent epigenetic alterations in colon cells. It has been noted that diet affects the composition of gut microbiome2,3 and that dysbiosis contributes to compromised epithelial integrity and impaired immune tolerance. Recent studies have identified various metabolites and regulatory pathways that connect diet, the gut microbiome, the gut metabolome, and immune responses.4

**Abbreviations:** IFNγ, interferon gamma; LPS, lipopolysaccharide; NOD2, nucleotide-binding oligomerization domain-containing protein 2; NF-κB, nuclear factor kappa beta; SCFAs, short-chain fatty acids; TNF, tumor necrosis factor.

Cancer is an emerging and major common public health problem in developing countries. Colorectal cancer (CRC) is a complex disease associated with advancing age, and evidence has shown that its risk can be managed to a large extent by lifestyle and environmental factors.16,17 Results of screening and surveillance for primary prevention of CRC have shown this disease to be a common cancer, with approximately 1,437,180 new cancer cases and 565,650 deaths reported in the United States in 2008.18,19 Jass20 has brought attention to several contributing factors worthy of consideration while...
evaluating colorectal adenomas as surrogate endpoints for CRC, though the yearly conversion rates of the adenomas to CRC may vary from 0.1% to 0.25%. Furthermore, despite appreciable understanding of the molecular basis of the pathogenesis of CRC, the reliable and robust markers that would be used for screening, surveillance, and primary preventive measures for CRC are still lacking. This is primarily because CRC is a complex and multifactorial disease in which interaction of host genetics with the environment is highly complex, and environment plays a vital role in progression of the disease. Diet is a major factor that has multiple effects, including alteration of both the transcriptome and the metabolome of the host, and thus it may reduce CRC incidence by as much as 80%. Metabolites from dietary sources may affect the intestinal mucosa directly from the luminal side, or indirectly by affecting the metabolism of the whole body.

Some dietary components that are toxic when ingested in excess are also subject to microbial biotransformation in the GI tract, and the metabolites (Figure 2) that are produced are assimilated into the circulatory stream, where they may shift the cellular balance towards undesirable situations such as susceptibility to, or induction of, genetic and epigenetic modifications in the host’s cellular genome. Mounting evidence suggests that some of the gut microbes and their metabolites may, either independently or in conjunction with each other, influence the risk of developing atopic disease.

The impact of nutrients on epigenetic alterations in the intestinal mucosa and colonocytes is of paramount interest, as aberrantly methylated nucleotides may serve as prognostic markers for CRC. Since epigenetic changes are potentially reversible, they provide promising targets for preventive as well as therapeutic interventions. Information is increasing on eating habits, nutrients, and dietary components and their impact on cellular mechanisms and epigenetics in preventing CRC. Probiotics and prebiotics alter metabolic processes within the lumen of the gut and within the colonic epithelium, which may prevent carcinogenesis. Such processes include the biotransformation of certain organic compounds to carcinogens, the subsequent hepatic detoxification of these compounds, DNA damage and repair in the epithelium, and apoptosis of damaged cells. Evidence from experimental models shows that aberrant crypt foci are reduced and apoptosis of damaged cells is increased after the administration of probiotics. Butyrivibrio fibrisolvens, a hemicellulolytic butyrate-producing gut anaerobe, was shown to reduce aberrant crypt foci in experimental CRC. This organism is found widely in domestic ruminants and wild animals and has also been detected in human intestines in several populations in rural southern India, where lower rates of CRC have been observed.
The anticarcinogenic attributes of probiotics may lead to a resurgence of interest in the utilization of probiotics or probiotic-containing foods.\(^{31,32}\) The vital functions that can minimize or prevent CRC include control of epithelial cell proliferation and differentiation, production of essential bioactive components, suppression of pathogens, and stimulation of gut immunity.\(^{33}\) Except for a few studies (reviewed by Ishikawa et al.\(^ {34}\)), however, information on clinical trials using probiotics for the prevention and cure of colon cancer is scarce.

**PROBIOTICS AS MULTIFACETED AGENTS**

Probiotics,\(^ {35,36}\) prebiotics, and dietary phytometabolites (such as polyphenols, phytoestrogens, nonprotein amino acids and saponins, etc.) can alter the gut microbial population and influence the incidence of CRC.\(^ {37}\) Among the beneficial effects of probiotics observed in humans are stimulation of gut immunity through various mechanisms, prevention of intensity and duration of diarrhea, and enhanced tolerance to ingested lactose.\(^ {38}\) In addition, the lactobacilli have other pro-health attributes such as synthesis of vitamin B, improvement of mineral and nutrient absorption, and decreased the expression of proinflammatory cytokines, thus promoting the gut immunological barriers.\(^ {41}\) In addition, probiotics have been found to stimulate nonspecific resistance to invading pathogens,\(^ {42}\) thereby aiding in the modulation of host immunity to harmful antigens with a potential to downregulate hypersensitivity reactions.\(^ {43}\)

**GUT MICROBIAL SHORT-CHAIN FATTY ACIDS**

The expression and regulation of genes is highly dependent on, and coordinated by, nutrients, micronutrients, and microbial metabolites.\(^ {23,44,45}\) The fiber and some fermentable oligosaccharides are subjected to gut microbial breakdown, resulting primarily in the production of short-chain fatty acids (SCFAs), viz., acetate, propionate, and butyrate in the general ratio of 60:25:15.\(^ {46}\) In addition, formate, valerate, caproate, and the branched-chain fatty acids such as isobutyrate, 2-methylvalerate, and isovalerate are also produced in low quantities from the catabolism of some branched-chain amino acids.\(^ {46}\) The SCFAs are bioactive molecules. They possess anti-inflammatory activities that play a vital role in the regulation of immune functions at the intestinal mucosal cell surface\(^ {1}\) and are postulated to be important effector molecules with multiple roles. Evidence shows that the ability of SCFAs to activate the apoptosis cascade and reduce the growth of certain tumors through histone hyperacetylation may reduce the risk of cancer.\(^ {47}\) Studies have shown that polyunsaturated fatty acids and volatile fatty acids mutually interact and can protect against colon cancer.\(^ {48-50}\)

The volatile fatty acids are the predominating metabolites in most of the herbivorous ungulates. The concentration of butyrate is highest in the cecum of monogastric animals and humans, though it is progressively reduced from the cecal to the distal colon, owing to the rapid absorption of butyrate across mucosa and the subsequent lack of fermentable carbohydrates in the distal colon.\(^ {51}\) SCFAs have been found to induce apoptosis, presumably related to epigenetic modification, cell cycle arrest, and activation of proapoptotic cellular genes. Although the incorporation of fatty acids into CRC chemotherapy regimens is still in its infancy, evidence is accumulating to allow identification of the length of fatty acid chains capable of exerting the most effective antineoplastic activity.

The data on the effect of butyrate on colon cancer is extensive, but it cannot be considered conclusive. Based on studies on the absorption and metabolism of SCFAs, it is estimated that the daily production of butyrate in the human large bowel is more than 200 mmol, which is readily absorbed across the mucosa.\(^ {52}\) It was inferred, based on observations in other species studied, that humans had a larger capacity for absorption and metabolism of SCFAs in the GI tract.\(^ {52}\) Three major pathways are involved in the uptake of butyrate in the GI tract: diffusion of the undissociated form through lipid membranes of the distal colon, counter-transportation mediated by bicarbonate ions, and paracellular diffusion of the anionic form in the proximal colon.\(^ {53}\) It is believed that butyrate can minimize the incidence of CRC. Clustering of SCFAs within the colonic lumen aids in preserving a suitable pH, which is vital for the effectiveness of numerous enzymes and for inhibiting the metabolism of carcinogenic agents in the gut.\(^ {54}\)

SCFAs exert several other important health-promoting actions, such as lowering intestinal pH, acting as energy sources for colonocytes, stimulation of colonic blood flow, contraction of smooth muscle cells, transepithelial chloride secretion, and proliferation of colonic epithelial cells through various proliferative stimuli.\(^ {55}\) Evidence shows that dietary fibers and SCFAs lower the incidence of inflammatory bowel disease (IBD) by decreasing the expression of proinflammatory cytokines induced by nuclear factor kappa B (NF-κB) and by stimulating absorption of sodium and water.\(^ {56-58}\)

Notably, the proportions of SCFAs in the intestine are determined by the microbial consortia that ferment dietary carbohydrates; the consortia, in turn, are influ-
enced by the diet itself. For instance, certain prebiotics augment the growth of bifidobacteria and thereby influence the proportion of SCFAs. The studies on Jurkat cells and on primary T cells demonstrate that T-cell activation in the presence of n-butyrate is characterized by nuclear factor of activated T-cells (NF-AT) plays an important role in the activation of various early immune response genes. The study provides evidence that n-butyrate interferes with NF-AT regulation, a mechanism that might represent a bacterial strategy to subvert host defense, which could be of clinical significance with reference to the GI tract, which is a natural site of metabolic synthesis and abundance of n-butyrate. Luhrs et al. have inferred that butyrate reduced the expression of proinflammatory cytokines by inhibiting activation of NF-κB and degradation of IκBα. An increase in SCFAs in the ceca of germ-free rats was observed, without a concomitant change in lactate concentration, after microorganisms derived from human intestine were inoculated into the ceca and the rats were fed fermented milks.

**Health-promoting effects of gut microbial butyrate**

n-Butyrate is most widely studied as an energy source for colonocytes and as a chemopreventive agent. In addition, n-butyrate is also known to influence cell-specific gene expression in intestinal cells, thereby influencing immune responses and oxidative and metabolic stress. Several lines of evidence indicate that SCFAs may serve as epigenetic drugs or histone deacetylase (HDAC) inhibitors that play an important role as anticancer biomolecules with antiproliferative effects against tumor cells. Sodium butyrate, used as an HDAC inhibitor, inhibits most HDAC enzymes except class III and class II HDAC6 and HDAC10. Fermented fecal supernatants were found to be rich in butyrate and propionate and were found to exhibit strong anti-HDAC activity in colon cancer cell lines. The anti-HDAC activity was attributed to the disruption of processes involved in the generation of dendritic cells from bone marrow stem cells, primarily through associated sodium-coupled monocarboxylate transporter (Slc5a8)-dependent inhibition of histone deacetylases. Slc5a8 is a key transporter that facilitates the transportation of butyrate and propionate into cells, and it is likely that these acids block the development of dendritic cells by interfering in the expression of some transcription factors in dendritic cell precursor cells. Cohort studies have been unable to detect significant effects, but most case-control investigations advocate a protective role of probiotics or fermented dairy foods against colon cancer. Intervventional studies have also revealed a shift of intermediate markers of CRC risk in human subjects from a high-risk to a low-risk profile following intake or consumption of probiotics and fermented dairy foods. Table 1 summarizes representative publications demonstrating the anticarcinogenic attributes of probiotic metabolites and some common dietary phytochemicals.

There is convincing evidence that optimal production of SCFAs is an indicator of healthy gut microbial fermentation, and that the pre- and probiotics have multiple beneficial impacts on the host. In view of the role of gut microorganisms in the production of SCFAs, probiotic therapy may be considered as an alternative approach for managing chronic IBD. *Faecalibacterium prausnitzii* is one of the most abundant bacteria in human GI tract and is an important producer of butyrate for utilization by colonocytes. When the response to conventional drug therapy is suboptimal in IBD, prebiotics and probiotics such as lactic-acid-producing bacteria and *F. prausnitzii* are recommended for treating IBD. In addition, butyrate-independent anti-inflammatory effects of *F. prausnitzii* in IBD models have been observed. Treatment with *F. prausnitzii* was found to lead to reduced interleukin (IL)-12 levels and increased IL-10 levels, and thus a reduced frequency of colitis, suggesting that butyrate might be useful in protecting the host from IBD.

A probiotic bacterium identified in humans, *Propionibacterium freudenreichii*, has been reported to destroy colorectal adenocarcinoma cells through SCFA-mediated apoptosis. Lan et al. demonstrated a major impact of a shift in extracellular pH on the mechanisms of propionibacterial SCFA-mediated HT-29 cell apoptosis, in the pH range of 5.5 to 7.5, the milieu that also prevails in the colon.

The majority of the anticarcinogenic effects of butyrate have been observed in vitro by using cancer cell lines. In these models, the addition of butyrate has been found to inhibit cellular proliferation and to induce apoptosis, necrosis, and differentiation of carcinoma cell lines. Butyrate has also been shown to stimulate a physiological pattern of cellular proliferation in the basal crypts in the colon as well as a reduction in the number and size of aberrant cryptic foci, which serve as earliest detectable neoplastic lesions in colon carcinoma. Maier et al. have shown that butyrate reduces expression of the genes cyclin D1 and c-myc, which are vital for the development of CRC.

An optimal anticancer drug would be one that destroys tumoral cells but not healthy somatic cells. Butyrate is reported to induce cell cycle arrest, differentiation, and/or apoptosis in certain colon carcinoma cell lines, thus providing further evidence of the potential of gut microbial butyrate to prevent colon cancer. Another important mechanism by which butyrate interferes with...
colon carcinoma cells is the inhibition of HDAC, which leads to hyperacetylation of histone residues. The aberrant histone acetylation (Figure 3) leads to impaired transcription and silencing of genes involved in the control of cell cycle, differentiation, and apoptosis. As an HDAC inhibitor, the butyrate increases the expression of p21 (WAF1) by selectively regulating acetylation of the gene-associated histones and by inducing the cell cycle arrest at

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**Table 1** Probiotic metabolites and bioactive dietary compounds and their therapeutic and epigenetic effects in inhibiting colon cancer.

<table>
<thead>
<tr>
<th>Dietary phytometabolites (sources)</th>
<th>Epigenetic effect on carcinogenesis</th>
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<tbody>
<tr>
<td>Lycopene (tomatoes)</td>
<td>Demethylation of the GSTP1, RARβ2, and HIN-1 genes in breast cancer cells (MDA-MB-231 and MCF10A)</td>
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<tr>
<td>Probiotic metabolites (butyrate)</td>
<td>Control of epithelial cell proliferation and differentiation, production of essential nutrients and/or bioactive food components, prevention of overgrowth of pathogenic organisms, and stimulation of intestinal immunity. Inhibition of HDACs, histone hyperacetylation. Induction of differentiation and apoptosis in neoplastic cells. Transporter (Slc5a8)-dependent inhibition of HDACs. Reduced risk of development of colon cancer and dyslipidemia.</td>
</tr>
<tr>
<td>Curcumin (Curcuma longa)</td>
<td>Expression of c-jun, a proliferation-stimulating gene in immune cells; induction of apoptosis in immortalized NIH 3T3 and malignant cancer cell lines. Chemopreventive, antitumoral, radiosensibilizing, and chemosensibilizing activities against various types of aggressive and recurrent cancers.</td>
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<tr>
<td>Allyl mercaptan (Allium sativum)</td>
<td>Inhibition of DNMT.</td>
</tr>
<tr>
<td>Capsaicin (chili peppers)</td>
<td>Repression of transcriptional activity of β-catenin in human colorectal cancer cells; capsaicin content (based on the Scoville scale) mediated induction of significant growth arrest and apoptosis in human breast and leukemia cancer cell lines, with no significant effect on normal breast epithelial cells.</td>
</tr>
<tr>
<td>Caffeic acid phenethyl ester or artemisinin C (beehives)</td>
<td>Suppression of malignant peripheral nerve sheath tumor by blocking of the oncogenic PAK1 signaling pathways, e.g., in neurofibromatosis type 2 tumors by Bio 30, a water-miscible component rich in caffeic acid phenethyl ester.</td>
</tr>
<tr>
<td>Resveratrol (red grapes; Vitis vinifera)</td>
<td>Inhibition of DNMT; activation of SIRT1.</td>
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<tr>
<td>Phytoestrogens (e.g., genistein) (soy and other leguminous foods)</td>
<td>Inhibition of DNMT activity; inhibition of aromatase, protein tyrosine kinase, and S6 activities; inhibitory effects on angiogenesis; inhibition of DNA topoisomerase II; inhibition of HDAC, activation of HAT.</td>
</tr>
<tr>
<td>Apigenin (parsley; Petroselinum)</td>
<td>Inhibition of DNMT.</td>
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<tr>
<td>Ellagitannins and ellagic acids (tea, pomegranates, black raspberries, raspberries, strawberries, walnuts, and almonds)</td>
<td>Epigallocatechin-3-gallate (EGCG)-mediated antioxidant effects, e.g., delayed lipid peroxidation, depletion of endogenous lipid-soluble antioxidants. Free-radical-scavenging properties. Inhibition of DNMT and HAT.</td>
</tr>
<tr>
<td>Sulforaphane (cruciferous vegetables)</td>
<td>Activation of SIRT1, which is beneficial for regulation of cell metabolism, stress resistance, calorie restriction, oxidative stress, inflammation, cellular senescence, autophagy/apoptosis, autoimmunity, metabolism, adipogenesis, circadian rhythm, skeletal muscle function, mitochondrial biogenesis, and endothelial dysfunction.</td>
</tr>
<tr>
<td>Silymarin (Silybum marianum L.)</td>
<td>Inhibition of cell proliferation, which promotes cell-cycle arrest in human colon cancer cells.</td>
</tr>
<tr>
<td>Silybinin (Silybum marianum)</td>
<td>Activation of SIRT1.</td>
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**Abbreviations:** DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDACs, histone deacetylases; NF-κB, nuclear factor kappa beta; SIRT1, sirtuin 1.
the G1 stage. A novel contributory mechanism to the chemopreventive effect of butyrate is the downregulation of the key apoptotic and angiogenesis regulator neuropilin-1, which promotes tumor cell migration and survival in response to binding of vascular endothelial growth factor. The other mechanisms by which butyrate mediates apoptosis include upregulation of the proapoptotic protein BAK, caspase-3-mediated cleavage of poly(ADP-ribose) polymerase (PARP), and via mitochondrial pathways. A study by Thangaraju et al. suggests a novel mechanism of action of butyrate that involves GPR109A, a G-protein-coupled receptor for nicotinate that recognizes butyrate with low affinity. The receptor is expressed in the lumen-facing apical membrane of colonic and intestinal epithelial cells and recognizes butyrate as a ligand. Its expression is silenced in human colon cancer and murine models of CRC, in addition to some other colon cancer cell lines. The studies have shown that GPR109A acts as suppressor of colon tumors by mediating the tumor-suppressive effects of the bacterial SCFAs in the colon.

Hence, the biological and metabolic effects of butyrate are relevant to various physiological and pharmacological benefits. Although valuable information about the uptake and metabolic effects of butyrate is available, knowledge of the intracellular effects of butyrate is lacking, and this need to be addressed.

There is evidence that certain dietary ingredients may favorably enhance the biotransformation of ingested carcinogens, and the associated hypothesis is that the risk of developing CRC could be reduced if the prevalence of CRC is related to diet. Dietary fiber is another important factor that contributes to protection against CRC. Mechanisms include reduction in the transit time of the feces in the gut, which minimizes exposure of mucosal cells to carcinogenic biomolecules, along with absorption and utilization of bile salts, biogenic amines, and bacterial toxins. Findings on the chemopreventive role of dietary fiber and its gut metabolites, however, are still inconclusive.

**Figure 3** Probiotic-derived short-chain fatty acids act as histone deacetylase inhibitors and restore gene expression in tumor cells.

Abbreviations: Ac, acetylation of histone moieties; HAT, histone acetyltransferase; HDAC, histone deacetylase; SCFA, short-chain fatty acid; TF, transcription factor

PROBIOTIC METABOLITES AND EPIGENOMIC TARGETING

Fermented dairy foods containing lactic acid bacteria are the focus of attention as possible cancer-preventing dietary supplements. Manipulation of the gut ecosystem through probiotics and prebiotics to reduce the risk of colon cancer opens new areas of scientific investigation in gut disease. Epigenetic modification, which refers to the methylation of DNA through covalent addition of a methyl moiety to the nucleotide cytosine, has an important role in the regulation of gene expression. Virtually every step of carcinogenesis or tumorigenesis is dependent on epigenetic modifications in the cellular genome. In the mammalian genome, the majority (90–98%) of CpG sites are methylated, except for certain CpG-enriched areas (CpG islands) that are not methylated. A few genes, called imprinted genes, are regulated by
methylation of CpG islands in their promoter. These genes are precisely replicated but are reversed during inheritance. Hypomethylation of promoters is associated with increased efficiency of gene transcription.

Epigenetic modifications, including histone modification or acetylation as well as gene silencing mediated by noncoding RNAs, have also been noted. In human cancers, including CRC, the epigenetic events are of significant concern. Enhanced methylation of cytosine in CpG islands of tumor suppressor gene promoters impairs transcription and is used as a target to manipulate genes involved in the progression of carcinogenesis. Interestingly, the epigenetic alterations are reversible but have the potential to alter the transcriptome profile. Numerous bioactive dietary components, namely, curcumin (turmeric), genistein (soybean and other leguminous plants), polyphenols, and resveratrol (tea and fruits, etc.) (Table 1), can interact with various epigenetic targets. They alter the methylation and histone acetylation required for the activation or silencing of genes in the therapy or prevention of cancer. The probiotics and their metabolites can alter the population composition of gut bacterial species that can, in turn, alter the fermentation metabolites, particularly the SCFAs. Miscellaneous biological activities attributed to probiotics could be the result of epigenetic alterations that may explain the wide range of anticarcinogenic effects attributed to probiotics. New findings about the effect of probiotics on the production of SCFAs and about the epigenetic effects of SCFAs will add to current understanding of the associations between gut symbionts and the management of CRC through dietary interventions.

**IMMUNOMODULATORY AND ANTI-CARCINOCGENIC ATTRIBUTES OF PROBIOTICS**

Mounting evidence suggests that diet and the microbiota, independently or in conjunction with each other, can influence the risk of developing atopic diseases. Regulation of the host immune response is an important mechanism through which probiotics exert beneficial effects. The most widely studied and used probiotics with nutritional, therapeutic, and pro-health benefits are lactobacilli and bifidobacteria. The surge in allergic diseases in infants is attributed to a relative lack of microbial stimulation of their immune system, and probiotics might provide such stimulation. Cohort studies and clinical trials have shown that a combination of prenatal or early postnatal treatments could help in overcoming allergy symptoms such as atopic eczema in high-risk neonates and infants. A study assessing the effect of *Lactobacillus* GG administered at an early age in the treatment of food allergies and allergic inflammation showed that *Lactobacillus* GG was effective in the treatment of early atopic diseases in infants at high risk and that the gut microbiota might be a desirable source of probiotics or immunomodulators for the prevention of such diseases. However, subsequent studies using the same or different probiotics could not validate the above report, indicating there might be additional intrinsic differences between the probiotic strains employed and the populations examined.

Eukaryotic cells perceive and respond to prokaryotes, both commensals and pathogens, by various stimuli and signal cascades. Probiotics with immunomodulatory activities affect a variety of eukaryotic immunological and cellular functions by enhancing the epithelial barrier via interaction with Toll-like receptors, by augmenting the epithelial signal transduction pathways that subsequently lead to regulation of cytokine production to promote anti-inflammatory responses by stimulating the intestinal DCs, inhibiting the generation of interferon-γ-producing T cells, and generating higher levels of IL-10 by degrading antigens, enhancing mucosal barrier functions, promoting the onset of regulatory T cell activity associated with enhanced secretion of tumor growth factor β and IL-10 by peripheral blood mononuclear cells, by modulating immune responses differently, by improving the immunogenicity of vaccines, and by inducing production of cytokine tumor growth factor β, soluble CD14, and immunoglobulin A. Probiotics, viz., *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus rhamnosus*, each induced differential gene regulatory networks. Mucosal response to consumption of *L. acidophilus* was associated with 10 regulatory nodes that drive regulatory networks associated with interleukins, interferon, and insulin metabolism. Consumption of *L. casei* led to differential expression of genes that regulate cellular homeostasis and metabolism together with genes involved in mitotic growth and somatic cellular proliferation. The major gene transcriptional networks altered after consumption of *L. rhamnosus* included key transcription factors such as JUN, JAK2, STAT4, and IGF1, which affect cellular growth, proliferation, and development.

Proportions of SCFAs have been demonstrated to be reduced in IBD, possibly due to alterations in the composition of normal microbiota. Gut epithelial cells of gnotobiotic mice were found to have reduced expression of SCFA receptors, which returned to normal levels after the normal bacterial composition in these animals was restored. In addition to having immunoregulatory functions, the SCFAs also act as an energy source for mucosal cells. Moreover, HDAC inhibitors promote cell maturation and differentiation, epithelial barrier integrity through changes in gene expression, and expression of certain tight junction proteins like cingulin and occludins as well as cytoplasmic proteins such as ZO-1.
and ZO-2. Modulation of expression of specific tight junction proteins plays a role in cellular differentiation. The increased intake of fermentable dietary oligo- and polysaccharides or SCFAs seems to be clinically beneficial in the treatment of colitis. The effect is attributed to restoration of the normal gut microbiome, concurrent with an increase in the production of SCFAs, an increase in immune cell recruitment, and the development of healthy gut lymphoid tissue. Studies involving binding of SCFAs with G-protein-coupled receptor 43 (GPR43) show that SCFA-GPR43 interactions profoundly affect inflammatory responses and that stimulation of GPR43 by gut SCFAs is indispensable for the normal resolution of certain models of colitis, arthritis, and asthma. From the above studies, it could be inferred that restoration of the production of gut SCFAs by supplementation with pre- and probiotics could modulate the gut lumen milieu and perhaps play a role in preventing IBD.

**OPPORTUNITIES AND CHALLENGES**

The fields of nutrition, microbiology, and genomics are evolving tremendously and converging rapidly. Alterations in histone acetylation and methylation are common hallmarks of cancer. Aberrant epigenetic modifications may prime the mucosal cells for progression of cancer. However, epigenetic changes, unlike genetic aberrations in cancer, are reversible. Hence, the impact of food-derived ingredients and microbial metabolites on reversing epigenetic changes in mucosal cells is of great interest for preventing colon carcinogenesis. Identification of methylation markers specific for colon carcinogenesis would be highly useful for risk assessment, especially in individuals who are genetically susceptible to IBD or colon cancers. Probiotic metabolites and certain dietary phytometabolites (Table 1) have been shown to suppress carcinogenesis through various mechanisms. Since the information gathered to date seems incomplete, more studies are warranted to explore the novel mechanisms and strategies to prevent CRC and IBD.

The effectiveness of natural therapeutic and chemopreventive agents reflects the ability of such agents to counteract certain upstream signals, such as NF-kB, activator protein 1, tumor necrosis factor, β-catenin, etc. In addition, nonsteroidal and anti-inflammatory drugs with similar modes of actions, alone or in combination with probiotic supplementation, may prove to be safer than conventional chemotherapeutic treatments. It has become obvious that chemoprevention accompanied by probiotic therapy could be an inexpensive and acceptable approach in colon cancer. There is still, however, much to be learned about the epigenetic mechanisms that influence health and disease susceptibility, and how these mechanisms are affected by lifestyle and environmental factors. The studies described in this review indicate a beneficial impact of probiotics, probiotic metabolites, and certain dietary phytometabolites on colon carcinogenesis. The most pressing need is to establish whether these alterations confer health benefits to the host. Beyond this, an important challenge is to identify probiotic strain(s) that can safely elicit important immunological signals.

**CONCLUSION**

It is clear that gut microbial metabolites are of paramount importance for communicating with intestinal epithelial and immune cells. Insights into the cellular and molecular pathways that mediate initiation, progression, and dissemination of tumorigenesis have opened the door for the development of therapeutic approaches that are highly targeted and more efficient. Interest in the consumption of probiotics and bioactive dietary supplements has intensified in view of their safety and health benefits in inflammatory and immunological disorders. Evidence of the chemopreventive attributes of probiotics and their metabolites is based on the findings of studies on the activities of fecal enzymes and the detoxifications of certain mutagens or carcinogen-induced preneoplastic lesions and tumors. Advances in elucidating key epigenetic mechanisms and epigenomic alterations in cancer biology hold promise for developing novel approaches to prevent colon cancer. Furthermore, in light of the costs involved in healthcare, one of the most viable approaches is to increase the awareness and intake of probiotics and probiotic-fermented foods as cancer-preventive and therapeutic strategies. Clear-cut criteria for the design as well as the evaluation of the immunomodulatory and anticarcinogenic attributes of probiotic metabolites that can maintain healthy mucosal immune responses are needed. An open dialogue between basic and clinical scientists, regulatory authorities, food and nutrition industry, and consumers could bridge the gap between science and the marketing of probiotics with anticarcinogenic attributes.

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