**Introduction**

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer-related death. The pathogenesis of colorectal cancer involves a multi-step and multi-factorial process. Disruption of the gut microbiota has been associated with gastrointestinal diseases such as colorectal cancer. The genus *Bifidobacterium* is considered an important component of the commensal microbiota and plays important roles in several homeostatic functions: immune, neurohormonal, and metabolic. *Bifidobacterium animalis* subspp. *lactis* is a well-documented probiotic within the species *Bifidobacterium*. Mycosporin-like amino acids are low molecular weight amino acids demonstrated to exert prebiotic effects and to modulate host immunity by regulating the proliferation and differentiation of intestinal epithelial cells, macrophages and lymphocytes, as well as cytokine production. Their modulation of the metabolism of the immune system and transcription factors could exert a beneficial effect on colorectal cancer. *B. animalis* does not produce mycosporin-like amino acids. If one could create a *B. animalis*-producing mycosporin-like amino acids via genetic open reading frame engineering it should exert more potent immuno-stimulatory properties and, thereby, become a potent strain-specific microbial based therapy in colorectal cancer prevention.

**Keywords**

Mycosporin-like amino acids, *Bifidobacterium*, genetic engineering, colorectal cancer

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Mycosporin-like amino acids and gut microbiota

Mycosporin-like amino acids (MAAs) are low molecular weight (<400 Da) amino acids. MAAs act as absorbers of ultraviolet (UV) light and as photo protectants. MAAs also play a role in protecting against sunlight damage by acting as antioxidant molecules scavenging toxic oxygen radicals. MAAs are unique components of red seaweeds, and seaweed products are used as nutrional supplements in many foods. Bifidobacterium animalis subsp. lactis is a catalase-negative, rod-shaped bacterium which was first isolated in 1983. At the time of isolation, B. animalis subsp. lactis was considered as belonging to the species of Bifidobacterium bifidum.

Activity.

Several studies have shown that Bifidobacteria differ from other bacteria in their role in oligosaccharide metabolism and the capacity to perform fermentation. Bifidobacteria use the fructose-6-phosphate phosphoketolase pathway to ferment carbohydrates; through this pathway, indigestible fructans are converted into short-chain fatty acids (SCFAs), such as butyrate, propionate and acetate which have beneficial effect on intestinal immunity and metabolism. Bifidobacteria are the main sources of butyric acid production, and they are used as probiotic ingredients in many foods. Bifidobacterium animalis subsp. lactis is an anti-inflammatory agent that inhibits the adverse effects of ROS in epithelial cells. In in vivo experiments, the anti-inflammatory effects of MAAs were demonstrated. They can also reinforce intestinal barrier function.

Discussion

Bifidobacteria and colon cancer

Epithelial inflammation constitutes an important initiating factor in the development of colitis-associated CRC. Inflammation may arise after mucosal invasion by intestinal bacteria. Later, inflammation can induce persistent immune dysregulation and then neoplastic changes of the mucosa. Chung et al. demonstrated that Bacteroides fragilis triggers a pro-carcinogenic, multi-step inflammatory cascade that requires IL-17R and involves nuclear factor (NF)-κB signaling in colonic epithelial cells in the context of intestinal dysbiosis. When pathogenic bacteria invade the protective mucus layer of the colon, the equilibrium is disturbed and DNA damage begins with tumor formation along with chronic inflammation.

Abnormal patterns of DNA methylation in the intestinal tract can lead to the formation of aberrant crypt foci which are thought to later progress into adenoma and cancer and damage the intact barrier and intestinal epithelium. Aberrant DNA methylation and dysregulation of intestinal cell proliferation may precede the activation of oncogenesis, through ROS and p53, which are needed for neoplastic progression. DNA methylation is associated with CpG island ( CGI)-associated promoters in both intestinal epithelial stem cells and differentiated cells. Global hypomethylation leads to increased gene expression, heterozygosity and global loss of chromosomal stability. In addition, hypermethylation leads to inactivation of important tumor-suppressor genes.

These epigenetic changes play an important role in the formation of colorectal adenomas and carcinomas. Ghadimi et al. reported that Bifidobacterium restores epigenetically mediated changes in the human intestinal mucosal immune system via reducing histone acetylation and enhancing DNA hypermethylation. Disrupted methylation patterns can occur during inflammation in colonic disorders. They also showed that Bifidobacterium diminishes the expression of IL-17 and IL-23, which play an important role in inflammatory bowel disease. Schroeder et al. showed that Bifidobacterium strains promote mucus layer integrity and reverse abnormalities in the altered colonic microbiota. Colonic permeability is decreased, and the growth rate of the inner mucus layer increased in an intact colonic microbiota.

Bifidobacteria are the main source of butyrate production, and butyrate has potent anti-inflammatory and antitumor effects. A higher abundance of butyrate-producing bacteria was found in stools of native Africans with low CRC risk as compared to Afro-Americans with a higher risk. Clarke et al. reported that butyrate inhibits...
proliferation and induces differentiation and apoptosis of CRC cells. Increased levels of butyrate reduce the incidence of carcinogen-induced colon tumors. Free fatty acid receptor 2 (Ffar2) is a receptor for SCFAs (acetate, propionate and butyrate), and Sivaprakasam el al. showed that Ffar2 is downregulated in human colon cancers. They also reported that the administration of bifidobacterium alleviated intestinal inflammation and carcinogenesis in Ffar2−/−mice.51 Butyrate may play a role in mediating key processes in oncogenesis including genomic instability, inflammation and cell energy metabolism.

Krüppel-like factors (Klfs) are zinc-containing transcription factors that modulate proliferation, differentiation, growth and apoptosis. A total of 17 Klfs have been identified, and their biological structure and contribution to human diseases have been described by Bialkowski et al.52 Klf5 is highly expressed in crypt epithelial cells of the intestine and plays a critical role in regulating the proliferation of both normal intestinal epithelial cells and CRC cells.52 Klf4 is an inhibitor of cell growth and exerts contrasting effects on Klf5.53 Klf4 and Klf5 bind to similar DNA sequences. Klf5 inhibits the activating effect of Klf4 on the Klf4 promoter, and Klf4 abrogates the inhibitory effect of Klf5 on the same promoter.54 Engevik et al.55 reported that Bifidobacterium-associated mice have a 20-fold increase in the goblet cell differentiation marker Klf4 at the level of mRNA compared with germ-free controls. Bifidobacterium may play a role in mediating key processes in the modulation of Klf4 and Klf5 expression.

It seems that Bifidobacterium strains have protective and preventive effects on colonic microbiota composition and may have an impact on the epigenetic regulation of CRC.

**MAAs and colon cancer**

Harmful irradiation directly damages biomolecules, including lipids, proteins and DNA and induces oxidative stress through mutagenic free radicals. MAAs act as UV absorbers. In this way, MAAs play an additional role in the antioxidant system. In addition, MAAs modulate intestinal epithelial cell differentiation and cytokine production.26 NF-κB is aberrantly activated in tumor cells, contributing to their advantage in survival and proliferation. The modulation of NF-κB signaling in response to stress can also be a strategy for cytoprotection, as several survival pathways can be activated.32 It seems that modulation of NF-κB and tryptophan metabolism via MAAs has a beneficial effect on the immune system. Besides these properties, MAAs also inhibit thiobarbituric acid reactive oxygen species (TBAR),36 which are elevated in colon cancer.56

**Recommendation**

**Combination of Bifidobacteria and MAAs**

There are two biosynthetic pathways of MAAs. The first pathway57 is the shikimate pathway, also known as the synthesis pathway from aromatic amino acids. The second pathway is the pentose phosphate pathway.58 In both pathways, 4-deoxyxagalose is the common precursor. Transaldolase is an enzyme in the non-oxidative phase of the pentose phosphate pathway; Bifidobacteria strains contain transaldolase. Cyanobacteria are a phylum of bacteria that obtain their energy through photosynthesis and are the only photosynthetic prokaryotes able to produce oxygen. MAAs are an essential class of secondary metabolites of Cyanobacteria known for their protection against UV radiation and other stress factors.

A biosynthetic gene cluster for MAAs has been demonstrated in Cyanobacteria.59 Anabaena variabilis PCC 7937 (Cyanobacterium) is able to synthesize MAAs.59 A. variabilis PCC 7937 is not a component of commensal gut microbiota. It is a component of aquatic and terrestrial ecosystems. Genome studies identified a combination of genes,YP_324358 (predicted DHQ synthase) and YP_324357 (O-methyl transferase), which were present only in A. variabilis PCC 7937 and missing in other Cyanobacteria. Anabaena sp. PCC 7120 has been induced to produce MAAs using ORF after genomic transfer (YP_324358 and YP_324357 genes) from A. variabilis PCC 7937.60 It seems that Cyanobacterium is the source of MAAs, and we hypothesize that the genes of Cyanobacterium involved in MAAs biosynthesis could be transferred to the strain B. animalis subsp. lactis BB-12.61,62 Genetically modified Bifidobacteria can modulate the immune system to further reduce chronic inflammation and increase colonic mucosal stability. A greater degree of suppression of inflammation and increased mucosal stability might arrest colorectal tumorigenesis at different stages including tumor initiation, promotion, progression and metastasis.63 In addition, experimental data reveal the important role of NF-κB in colon tumor cells, as well as in the surrounding cancerous and reactive microenvironment.64,65 It can be predicted that this combination may be more effective in preventing CRC through the NF-κB pathway. In addition, elevated levels of TBARs are associated with colon cancer initiation and progression,58 and this combination can prevent cancer formation by lowering TBAR levels.

**Conclusion**

Significant progress has been made in recent years in recognizing the importance of the gut microbiota to CRC. Key findings include the discovery of oncogenetic mechanisms that link the gut microbiome to CRC, including reduced SCFA production, chronic inflammation, altered transcription factors and the immune response. Creating MAA-producing Bifidobacteria species via genetic engineering could result in a bacterium that is more potent in the prevention of CRC. MAAs produced via genetic engineering might be used not only as a probiotic but also as a pharmacological agent in CRC.
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Bozkurt et al.