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Review Article

The Role of Microbial Metabolites in Cancer and Inflammatory Diseases: Interaction with the Immune System

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Abstract
Microbiota is an aggregate of microorganisms that live in mammals including humans. These microorganisms, which include bacteria, viruses and fungi, reside in large numbers in the human intestine. Microbial metabolites resulting from microbiota play an important role in various types of cancer, including colorectal cancers, prostate, ovaries, and other types of cancers, and in various inflammatory diseases such as Inflammatory bowel disease (IBD), chronic kidney disease, cardiovascular diseases, etc. Types of microbial metabolites include reboxamycin, trimethylamine oxide (TMAO), lactacystine, and short-chain fatty acids which include butyrate, acetate, and propionate. All of these microbial metabolites play an important role in the physiological activity of the body and in inflammatory and cancerous diseases. Among various microbial metabolites, short-chain fatty acids have been studied and their role in immune cells, inflammatory diseases such as inflammatory bowel disease and cancers, is more pronounced than other microbial metabolites. In this regard, immune cells, especially those of acquired immunity, such as regulatory T-lymphocytes, play an important role in suppressing inflammation caused by inflammatory diseases and contribute to microbial metabolites in maintaining intestinal hemostasis. Microbial metabolites are effective elements in the development of gastrointestinal hemostasis and prevent unwanted inflammation after microbial infections, pathogens or any inflammatory disorders as far as possible. Microbial metabolites can help eliminate tumor cells by induction of apoptosis and specific mechanisms that will be discussed in this article. This review looks at the role of microbial metabolites in cancer and inflammatory diseases, especially IBD, and their association with the immune system.

Keywords: Microbiota, Microbial Metabolites, Cancer, Inflammatory Diseases, Immune System

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Introduction

Approximately 100 trillion microorganisms (including bacteria, viruses and fungi) reside in the intestines of a mature adult and form microbiota.[1] The microbiota is relatively stable throughout the intestine, but the absolute number of microorganisms varies significantly from the mouth to the rectum.[2] The microbiota of the intestine also varies from person to person. Intestinal microbiota in the early stages of life are acquired through the flora of skin commensal, vagina and the mother's face, and develop over the first two years of life. Microbiostatic evolution results from the interaction between host physiological processes and microorganisms that are obtained from the environment.[3-5] After the early stages of life, microbiota is a stabilizer and maintainer of its composition; however, this sustained composition is likely to change due to some fluctuations in adulthood in response to evolutionary and environmental events.[6] In late adulthood, the microbiota combination gradually changes, but it can still maintain its physiological functions.[7-10] Also, the acquisition of a stable and balanced microbiotype is essential for the development and maturity of a healthy immune system, which is indicated by immune deficiencies in bacteria-free animals under bacteria-free conditions.[3] The gastrointestinal colon is colonized with about 10^3 different microbial species, which mainly involve bacteria.[7] The colon contains about 10^{14} different bacteria (70% host microorganisms).[11] The intestinal microbiota plays an important role in regulating intestinal haemostasis. However, the microbiota components responsible for their effects have not yet been

identified. (i.e., which microorganisms are microbiota and which of the microbiota constituent microorganisms are responsible for the effect of microbiota are unknown).[12]

By definition, microbiota is a microbial community inhabiting a specific environment including bacteria, areca, viruses, and some single-cell cellular eukaryotes, but the whole genomic microbiota content is referred to as microbiome.[13]

Undigested food components that reach the colon are fermented by anaerobic bacteria to produce a wide range of microbial metabolites, reflecting the chemical diversity of the existing substrates and the significant biochemical capacity of microbiota. Non-digestible carbohydrates including plant cell wall polysaccharides, resinous starches and certain soluble oligosaccharides (e.g., fructo-oligosaccharides) are usually the primary substrates of microbial fermentation.[14] The metabolism of bacteria in the colon is not merely by fermentation, and it can also include aerobic respiration where nitrates, sulfates and various organic compounds act as electron receptors.[15] According to this, the role of microbial metabolites and their various types, including rebeccamycin, TMAO, lactacystin, short-chain fatty acids (SCFA), etc., will be discussed.

The most important types of short chain fatty acids (as a class of microbial metabolites) include butyrate, acetate and propionate.[16] Microbiotas provide nutritional functions as well as the proliferation and differentiation of intestinal epithelial cells through their metabolites, especially SCFAs. In the same vein, butyrate is known as an SCFA with stronger effects. Butyrate can alter the microstructure of the small and large intestines and may also increase the intestinal mucus maturation during development, or can restore the healing process after injury.[17]

Microbial metabolites also affect the immune system. The immune system in the intestine has evolved with microbiome (intestinal microbiota) to maintain intestinal health (Figure 1). Disrupting this hemostasis leads to inflammation and disease in the intestine. One of the roles of the immune system in maintaining intestinal health is the effect of regulatory T cells that inhibit inflammation of the intestine. The effect of microbial metabolites on immune system cells has been proven. For example, decreased SCFA level is associated with a decrease in the number of regulatory T cells and an increase in inflammation of the intestine.[18]

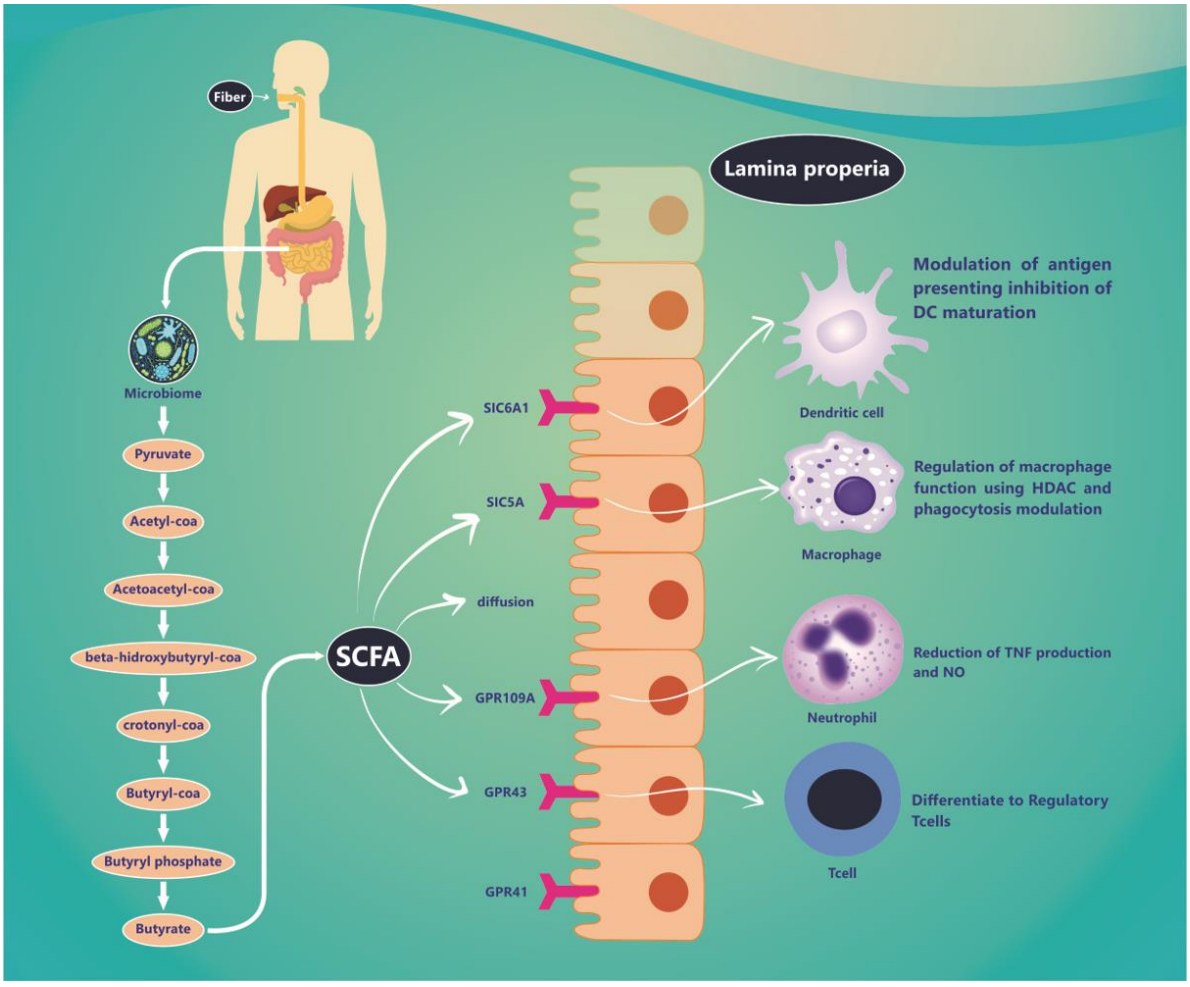

Figure 1. The Role of Intestinal Microbiota Metabolites in the Regulation of the Immune System. SCFA: Short-Chain Fatty Acids; SLC6A1: Solute carrier family 6 member 1; GPR109A: G protein-coupled receptor 109A.

The lumen of the intestine is in direct contact with a variety of foods, germs, and metabolites. The intestinal mucus is responsible for the selection of food and protection against toxins and germs. This mucus is believed to be responsible for a controlled physiological state of inflammation. This is a constant challenge for the intestine, especially the colon to control inflammation and prevent IBD.[19] Microbial metabolites also play an important role in various diseases, including gastrointestinal inflammatory diseases such as IBD and colon cancer.[11,20] Cancer is a group of diseases that is associated with uncontrolled growth and spread of abnormal cells. If not controlled, it leads to death.[21] Cancer is the leading cause of death in high-income countries and the second leading cause of death in countries with modest or low incomes. Of any four deaths in the United States, one is due to cancer.[22]

The role of microbial metabolites in various cancers, such as colon, lung, prostate, etc., has been proven in different studies.[15,23,24] Microbial metabolites have been used to isolate tumor cells in various cancers using cellular and microbial mechanisms discussed later in detail. Inflammation is the body's response to pathogens and various tissue damage. Inflammation results in the activation of innate and acquired immune responses and secretion of various cytokines such as TNF, interleukins, and the like. Inflammation affects various organs of the body and leads to various inflammatory diseases. Intestinal microbes live in their hosts through a two-way interaction, and if there is any change in their interaction, different inflammatory conditions will develop.[25]

IBD is a recurrent inflammatory disease, including Crohn's disease and ulcerative colitis. The incidence of this disease is unnoticeable. It affects many people in different countries. Different studies have shown that different microbial metabolites play a role in the pathogenesis of IBD, and it is suggested that some of them are diagnostic biomarkers for this disease.[26] There are many different microbial metabolites that affects the immune system and cause some inflammatory diseases. This study reviews the role of the interaction of some microbial metabolites with the immune system in some common cancers and inflammatory bowel disease.

Rebeccamycin

Rebeccamycin is a microbial metabolite isolated from the *Saccharothrix aerocolonigenes* culture medium, which was described in 1987 as a compound that induces prolonged survival of leukemic rats at a dose of 8 to 256 mg per 1 kg of weight.[27] Rebeccamycin causes a failure in the DNA of the bacterium. However, its lower solubility in the aquatic environment may cause problems for further investigations.[28] Its structure has been investigated using spectroscopic and x-ray crystallography methods. Rebeccamycin is structurally similar to many microbial metabolites, such as acetupyrsupurine, AT2433 A1, and B1 and K-252a, but unlike first-generation antibiotics, it can be produced in a large amount by fragmentation.[29,30] Rebeccamycin has several analogues. Over the past years, more than 150 different derivatives of rebeccamycin have been produced. Various activities have been described for rebeccamycin analogues. These analogues have strong anti-cancer activities. Some of these analogues are type 1 and 2 topoisomerase inhibitors or kinases based on their chemical structure. Rebeccamycin and its analogues can even bind to the DNA, even in the absence of topoisomerase. Rebeccamycin stabilizes interactions of topoisomerase-DNA, in which the DNA is cut but not mobilized.[29] In the following, we will deal with the role rebeccamycin plays in the treatment of cancers.

Since no study has yet been conducted on the role of rebeccamycin in inflammation, we will focus on the role of rebeccamycin in cancer. Different analogues of rebeccamycin inhibit cell proliferation and have multiple mechanisms affecting cancer cell lines, which inhibit the proliferation of cancer cells. Therefore, rebeccamycin analogues have an inhibitory effect on cancer.

The Role of Rebeccamycin in Cancer

Different analogues of rebeccamycin inhibit cell proliferation and have multiple mechanisms affecting cancer cell lines, inhibiting the proliferation of cancer cells. Therefore, rebeccamycin analogues have an inhibitory effect on cancer. One of the rebeccamycin analogues is NSC 655649, which is a synthetic antibiotic with cytotoxic activity and inhibits topoisomerase activity.[31,32] Unlike reboxamycin, NSC 655649 has no effect on type 1 topoisomerase, but effectively inhibits the activity of type 2 topoisomerase. These analogues have a strong anti-tumor activity in the culture medium against tumor cell lines. NSC 655649 also has anti-tumor activity against P388 cell lines, cellular sarcoma of retinal B16, lung cancer M109, human HCS-11 colon carcinoma and human lung cancer.[24] This analogue has been evaluated in different clinical trials. A Phase I study was performed on 45 patients with advanced solid malignancies who received a single dose of 20 mg/m^2 to 744 mg/m^2 of NSC 655649 (a Rebeccamycin analog with topoisomerase inhibitory properties) in 3 weeks. The antitumor activity of this analogue has been reported in 2 patients with ovarian cancer and a patient with soft tissue sarcoma. The high dose of this analog leads to suppression of the myeloid lineage.[33]

In another study, 31 patients with advanced solid malignancies received doses of 60 mg/m^2 to 188 mg/m^2 per day for 5 consecutive days. In this study, a half-life of 554 ± 154 hours was obtained for this analogue. The researchers also found that the analogue is metabolized in the liver. High concentrations of NSC 655649 were found in the biliary fluid of one of the patients. On the other hand, toxicity due to high doses of this analogue was reported to lead to neutropenia.[34] Another study evaluated the efficacy and

toxicity of NCS 655649, and a phase II clinical trial was conducted on people with advanced kidney cancer. The results showed that this analogue of rebeccamycin greatly improved the survival of patients, made them stable, and prevented the progression of the disease. Neutropenia and anemia have also been reported as toxic effects of this analogue.[35]

Also, another Phase II clinical trial on patients with recurrent small-cell lung cancer has been performed to evaluate the analgesic effect of rebeccamycin as an anticancer agent. Two patients who received the analogue of rebeccamycin had a partial response to treatment, while in 6 cases, the disease was stable without progression. The clinical improvement rate for the patients was 40% and the median survival rate was two months without progression. This study demonstrates the efficacy of analogue of rebeccamycin in recurrent cancer of small lung cells.[36]

Based on the above-mentioned results of various studies, it can be concluded that rebeccamycin has an anticancer effect not only in laboratory conditions, but also in patients with various cancers, if used properly. In the following, we introduce other microbial metabolites and their role in cancer.

TMAO (3-Methyl-N-Oxide)

TMAO is derived from an intermediate metabolite produced by Trimethylamines (TMAs).[37] This intermediate metabolite is oxygenated in the liver to yield the final form of TMAO, which has recently attracted scholarly attention.[38]

The metabolites produced from small and large nutrients by normal intestinal flora (microbiotas) have a very important impact on human health and disease. Eating high amounts of meat and low consumption of complex carbohydrates leads to an increased risk of type 2 diabetes and gastrointestinal cancers. The pathway in which high levels of meat consumption have adverse effects on human health is the Choline-Carnitine-TMAO pathway.[38]

Choline is an essential nutrient necessary for the production of all membranes, lipids and neurotransmitters, such as diathermic biosynthesis pathways.[39] The choline precursor is phosphatidylcholine, which is absorbed by the body by eating animal products, wheat and soy.[40] Choline is metabolized by Row's bacteria through the breakdown of the C-N bond, where *Formicocculi* and *Proteobacteria* are responsible for the activity that forms the TMA (trimethylamin) gas that can easily pass through the digestive wall.[41]

In the liver, the enzymes of the flavin mono-oxygenase family (FMO), mainly FMO1 and FMO3 isoforms, oxidize TMA to TMAO.[42] Also, TMA can be derived from carnitine, a compound that is mainly found in red meat. *Proteobacteria*, *Prevotellaceae* family and *Bacteroidetes*, along with phylum bacteria play a major role in converting carnitine to TMA.[43,44]

The level of TMAO in the blood of people has a direct relationship with food intake, and this relationship varies in different individuals due to the difference in their normal flora.[45-47] TMA also enters the human body through air pollution.[48] The importance of TMAO is due to its role as a diagnostic biomarker, and as an independent risk factor for conditions such as insulin resistance and gastrointestinal cancers.[38]

The Role of TMAO in Inflammatory Diseases

In one study on people with IBD, the plasma levels of TMAO in patients were evaluated in comparison with healthy individuals. According to the results of this study, the level of TMAO in IBD patients was reduced compared with the control group, and it was suggested that TMAO could be used as a diagnostic biomarker for IBD patients.[26]

Vascular endothelial dysfunction, a by-product of aging, is a major risk factor for cardiovascular disease among the elderly.[49,50] Although oxidative stress and inflammation are major contributors to endothelial dysfunction in aging, their role has not yet been adequately investigated.[51] Several pieces of evidence suggest that TMAO plays a role in the pathogenesis of cardiovascular diseases. A study was conducted on 2 groups of old (22-mo-old) and young (4-mo-old) rats, where one group was treated with DMB (3.3 dimethyl-1-butanol) as a TMAO inhibitor, and the other with no TMAO inhibitor. The results showed that compared to the young control group, the old control group has a high plasma level of TMAO, which decreases with DMB, while the control group increases the level of expression of inflammatory cytokines, and the expression of eNOS (endothelial nitric oxide synthase) is reduced in the aorta. According to the findings of this study, it is suggested that aging results in an increase in TMAO levels, which is accompanied with increased oxidative inflammation and stress, resulting in aging-associated endothelial dysfunction.[52]

TMAO has been shown to be associated with atherosclerosis and a risk of cardiovascular diseases.[53,54] One study examined the effect of TMAO on the function of endothelial cells and smooth muscle cells. TMAO leads to an increase in the expression of inflammatory genes. It also activates the NF-κB pathway and the MAP Kinase. Finally, it also induces endothelial activated leukocytes. NF-κB signaling is an indispensable factor for induction of inflammatory gene expression by TMAO.[55]

Inflammasome NLRP3 responds to internal and external risk signals in atherosclerosis.[56] In addition, the activation of thioredoxin-interactive protein (TXNIP) is a key event associated with inflammasome NLRP3 in conjunction with oxygen-reactive species.

It has been shown that the expression of inflammasome TXNIP-NLRP3 is stimulated by TMAO in human umbilical endothelial cells. TMAO significantly initiates oxidative

stress, activates the inflammatory TXNIP-NLRP3, releasing IL-18 and IL-1β inflammatory cytokines in a time-dependent and concentration-dependent process, while the production of eNOS and NO is inhibited. According to the findings of this study, TMAO induces endothelial inflammation and dysfunction through the activation of ROS-TXNI P-NLRP3, suggesting an inflammatory mechanism for a TMAO-associated increase in risk of atherosclerosis and cardiovascular diseases.[12]

According to the above-mentioned points, it can be concluded that TMAO is an effective factor in inflammation and plays a role in the various mechanisms mentioned above.

The Role of TMAO in Cancer

In a study on people with colorectal cancer, the serum TMAO level was studied. The results showed that TMAO has a high expression in colorectal cancer patients. The higher the TMAO in patients, the lower the survival rate. According to the findings of this study, TMAO is considered as a new independent diagnostic biomarker in people with colorectal cancer.[57] TMAO levels and their precursors are associated with the risk of colorectal cancer. Studies have shown that over 50 different genes exist between the frequency of TMAO and the risk of colorectal cancer.[58]

An increase in NOCs (N-Nitroso) plays an important role in insulin resistance and this increase in the level of these compounds depends on TMAO. TMA is a precursor to NOC, and specific NOC-induced mutations play an important role in colorectal cancer. The formation of NOC from TMAO requires nitrogenation and N2O3, and these call for a group of intestinal bacteria as a donor.[59,60] Another proposed mechanism for linking the risk of gastro-intestinal cancer with TMAO is that TMAO (as well as NOC), as a methyl group donor, causes epigenetic changes such as DNA methylation and causes malignant changes, and thus ultimately leads to cancer.[61] According to studies, TMAO which is recognized as a diagnostic biomarker in colorectal cancer plays an important role in colorectal and gastro-intestinal cancers, and the mechanism of its effect on carcinogenesis has been discovered to some extent.[62]

Lactacystin

Lactacystin is a metabolite synthesized by *Streptomyces* bacteria which modifies the amine end of the threonine residues in proteasome subunit X/MB1 (a very close homologue coded by the MHC family genes) and subsequently inhibits the protein processing and destruction associated with ubiquitin.[63] Lactacystin inhibits cell proliferation. This metabolite has been used extensively in studies on proteasome, a multi-functional complex with proteolytic properties that cause non-lysosomal intracellular destruction of the protein.[63] Lactacystin also binds to catepsin A, cathepsin B, and calpain 1.[23] This proteasome inhibitor has an inhibitory effect on cancer and has been used as an anticancer agent in cancer treatment researches.[64]

The Role of Lactacystin in Inflammatory Diseases

Most of the studies on lactacystin are related to its role in cancer.[65] However, in this section, we review the role of lactic acid inflammation. Although inhibition of the ubiquitin-protease system plays an important role in the pathogenesis of neurodegenerative diseases, studies have shown that protease inhibiting increases the expression of neuronal protective shock proteins. Gene expression evaluations in the primary neurons treated with lactacystin protease inhibitors show that several pathways are affected. Under the influence of proteasome, one of the factors that is affected by lactacystin is the increase in the expression of HSP-22 and HSP-70 heat shock proteins, which play a role in cell death due to protease inhibitory activity. Also, genes involved in cellular stress and inflammation are also overexpressed. According to the findings of this study, by applying its inhibitory effect on protease, lactacystin leads to an increase in the expression of inflammatory genes and has a promoting role in the inflammation.[66]

The Role of Lactacystin in Cancer

In a study on prostate cancer cell line LNCaP, it was reported that treating tumor cells with different concentrations of lactacystin leads to an increase in apoptosis.[23] Also, another study dealt with patients with lymphocytic leukemia and found that lactacystin induces apoptosis in cancer cells that cannot be induced by radiotherapy and chemotherapy. Even low concentrations of this protease inhibitor metabolism, lactacystin, leads to apoptosis in these cells. The inhibition of protease with lactacystin results in the inhibition of the transfer of the subunits of P50 and P65 from NF-κB, making these cells susceptible to apoptosis with TNF-α. According to the findings of this study, the use of lactacystin to inhibit protease is a new therapeutic finding in patients with acute lymphocytic leukemia.[67]

In another study on two gastric cancer cell lines, the relationship between NF-κB stability and lactacystin-associated cellular apoptosis was investigated. According to the findings of this study, Lactacystin has different inhibitory effects on gastric cancer cells the mechanism of which is induction of apoptosis by reducing the expression of NF-κB.[68]

Proteasome-ubiquitin dependent protein processing plays an important role in controlling apoptosis induced by radiation therapy in human lymphocytes. This control has been found to be altered in patients with chronic lymphocytic leukemia compared to healthy human lymphocytes exposed to apoptosis after radiation, but in some cases, no sensitivity to apoptosis has been reported. Lactacystin activates the apoptotic pathway in apoptosis-resistant, chronic, sensitive,

lymphocytic leukemia cells, with the same dose that has no effect on normal cells, so higher concentrations are required for this purpose. Therefore, the resistance of some chronic lymphocytic leukemia cells that begins with radiation does not have any relation to the increased sensitivity to lactacystin. The nuclear level of the NF-κB doping factor does not change after radiation or treatment with lactacystin, which indicates that other factors involved in controlling apoptotic death are altered during proteasomal inhibition. According to the findings of the study, the ubiquitin system plays an important role in controlling apoptotic death in lymphocytes among patients with chronic lymphocytic leukemia. The inhibition of the proteasome-ubiquitin dependent protein processing is a differential apoptotic stimulus among normal cells compared to chronic lymphocytic leukemia lymphocytes.[63]

Cisplatin is one of the most effective chemotherapy agents that is widely used in the treatment of firm tumors.[69] Cisplatin binds to the DNA and leads to induction of apoptosis. Some studies have shown that cisplatin induces endoplasmic stress and apoptotic signaling of the nucleus. Several physiological and pathological conditions result in endoplasmic endothelial stress, which in turn, results in the accumulation of folded or unfolded proteins in the lumen of the endoplasmic network. The accumulation of these proteins leads to the activation of the destructive mechanisms associated with ubiquitin-proteasome. In a study on HeLa cancer cell line, the effect of lactacystin as a protease inhibitor on cisplatin toxicity was evaluated. According to the findings of this study, the use of lactacystin results in an increase in the apoptosis associated with the cysplatin-induced endoplastic endothelial stress in HeLa cells.[64]

In the following, we will deal with the most important microbial metabolite that has received enormous scholarly attention in recent years and is of great importance in cancer and inflammatory diseases. In this regard, the role of this group of microbial metabolites will be discussed in a wider scope.

Short-Chain Fatty Acids

There are several microbes in the human digestive tract involving more than 10,000 different species.[70] These microbes have enzymes that can break the carbohydrates and turn them into useful metabolites that the host cells lack.[15] Non-digestible fibers such as proteins and peptides in the intestines can be metabolized by intestinal bacteria. The main product of these fermentation reactions are short chains of fatty acids that contain fatty acids with fewer than 6 carbon atoms and include formic acid (C1), acetic acid (C2), propionic acid (C3), butyric acid (C4), and valeric acid (C5). Short-chain fatty acids in the intestine are mainly C2, C3, and C4, which make up more than 95% of short-chain fatty acids (SCFAs). In general, C2 is formed from pyruvate with acetyl coenzyme A or the Wood-Ljungdahl pathway.[71] C3 consists mainly of succinate with succinate pathway or lactate with acrylate pathway. C4 is formed by acetyl coenzyme A and butyryl coenzyme A.[72]

The concentration of SCFAs in the colon and cecum is different. It is estimated that the total density is nearly from 70 to 140 mM in the proximal colon, and ranges from 20 to 70 mM in the distal colon. Short-chain fatty acids are used by colon cells after absorption or enter the bloodstream and other organs. In general, SCFAs enter the cells in different ways, namely through passive transmission, transmission with SMCT1/SLC5A8 and MCT1/S1C16A1 transporters, and via activating receptors with protein G. SLC5A8 which is a transducer coupled with Na^+ has high affinity to fatty acids, especially butyrate. It has a protective role against colitis and colon cancer in low-fiber diets. It also regulates the butyrate-dependent expression, IDO1 and ALDH1A2, in dendritic cells and also in completion of the regulatory T cells.[74] SLC16A1 is the transducer of SCFAs based on the H^+ gradient.[75] GPR41, GPR43, and GPR109a are receptors that can be activated by SCFAs. SCFA-GPR pathways play an important role in regulating immune responses.[76]

Short-Chain Fatty Acids in the Intestines and Circulation

SCFAs are the most abundant products derived from the fermentation process of non-digestible food fibers in the intestine by commensal bacteria.[72] In the intestine, SCFAs are mainly found in form of acetate, propionate, and butyrate, which make up more than 95% of the SCFA content.[78] Their concentration in the intestine is usually found by a ratio of 3: 1: 1.[79] Different types of SCFAs differ according to their location in the intestine, as will be described below.

Acetate and propionate are found in the small intestine, while butyrate is found mostly in colon and cecum.[80] Nearly from 400 to 800 milliliters of SCFAs are produced daily in a high-fiber diet, which is equivalent to fermentation of 10 g of dietary fiber. Various factors including nutrition, specific microbiota diversity, and specific amounts of commensal bacteria play an important role in the production of SCFAs.[81] After production, SCFAs can be absorbed into the epithelial cells of the colon in various ways, such as non-ionic release, carrier-mediated transport, and exchange with bicarbonate.[82] Additionally, SCFAs absorbed into the cecum, ascending colon, and transverse colon can enter the anterior mesenteric vein, while the absorbed SCFA flows into the descending clone and the sigmoid colon into the inferior mesenteric vein(s). Then they both flow into portal and hepatic veins.[83] Apart from this pathway, the SCFAs adsorbed into the rectum can enter the inferior vena cava through the pelvic network and play an important role in the circulation. After entering the circulation, SCFA metabolism affects the function of peripheral tissues, such as connective tissue, skeletal and hepatic muscles.[83]

Regulation of Immune Responses by Short-Chain Fatty Acids

It has recently been discovered that gut microbes play a role in regulating many body systems through their metabolites. Because short-chain fatty acids enter the bloodstream and other organs after production in the intestine, they can regulate the activity of various systems of the body such as the digestive system, the nervous system, and the endocrine system, and play a key role in regulating metabolic and immunological disorders. Short-chain fatty acids mainly do this by inhibiting histone deacetylases and activating receptors with G proteins, such as GPR41, GPR43, and GPR109a.[84] Short-chain fatty acids have been shown to affect the systemic autoimmune responses at different stages of inflammatory responses. They regulate the function of almost all types of immune cells and alter the expression of the gene, differentiation, chemotaxis, proliferation, and apoptosis. Through the inherent immune responses in mucosal areas, microbial products are identified by pattern recognition receptors, such as Toll-like receptors. Short-chain fatty acids affect the production of inflammatory cytokines by enhancing NF-κB through activating TLR in epithelial cells.[85] During inflammation, short-chain fatty acids stimulate neutrophil migration through the activation of GPR43[86] and modulate the production of reactive species and phagocytosis.[87] In addition, short-chain fatty acids inhibit production of inflammatory cytokines such as TNF-α in neutrophils.[88] They also regulate the function of dendritic cells that regulate immune responses not only through cytokine secretion, but also through their ability to interact with T lymphocytes. Butyrate and propionate inhibit the activation of bone marrow dendritic cells by inhibiting the expression induced by LPS of co-stimulatory CD40 molecules and secreting IL-6 and IL-12p40.[89] A recent study showed that dendritic cells exposed to butyrate facilitate the differentiation of naive T-cell into interferon-γ-secreting cells, which can be accomplished through immunosuppressive enzymes of indoleamine 2,3-dioxygenase and aldehyde-dioxygenase 1 A2 induced by butyrate.[73] Additionally, the dendritic mouse cells stimulated by propionate with reduced expressions of CD40, PD-L2, and CD86 have been shown to impair their ability to start effective Th2 function and lead to induction of Th2-dependent allergic airway inflammation.[90] Butyrate also modifies the function of digestive macrophages. Macrophage treatment with butyrate inhibits LPS-induced pre-inflammatory mediators, such as nitric oxide, IL-6, and IL-12, but has no effect on the production of TNF-α or MCP-1. These effects are independent of TLR signaling and GPR activation.[91] Short-chain fatty acids also play an important role in the regulation of acquired immune responses. Rats exposed to short-chain fatty acids have a higher regulatory T cells with Foxp3,[92] and these fatty acids lead to the polarization of naive T-cells to regulatory T-cells.[93] Butyrate has been shown to increase histone H3 acetylation and increase the differentiation towards regulatory T cells acting as an anti-inflammatory agent.[92] These findings suggest that short-chain fatty acids affect the histone acetylation of the transcription of genes that contribute to the differentiation of T cells.[18] A recent study reported that short-chain fatty acids led to an increase in IL-10 production in T lymphocytes, such as Th1, Th17, and regulatory T-cells.[94] It should also be taken into account that in this study, short-chain fatty acids facilitated the conversion of naive T-cells to Th1 or Th17 depending on the cytochrome environment. This function of short-chain fatty acids is primarily dependent on the histone deacetylase inhibitory activity.[94]

The Role of G Protein-Coupled Receptors (GPCRs) in the Regulation of Immune Responses by Short-Chain Fatty Acids

GPCRs, also known as free fatty acid receptors, include GPR41, GPR43, and GPR109. GPR41 and GPR43 are mainly activated by acetate, propionate, butyrate and other short chain fatty acids,[95] while GPR109 is mainly activated by butyrate and niacin.[97] Short-chain fatty acids mainly regulate immune responses by activating GPCRs, which are expressed in almost all immune cells such as epithelial cells, neutrophils and macrophages. Initial studies on GPCRs have shown that mice lacking GPCR show intense and incurable inflammation in dextran sulphate sodium (DSS)-induced colitis models, arthritis, and asthma.[98] With regard to these points, GPCRs play a crucial role in regulating immunity and inflammation.

The Role of Short-Chain Fatty Acids and GPCR in the Regulation of Intestinal Inflammatory Disease

Bacterial metabolites, such as short-chain fatty acids, are present in very high concentrations in the intestines. The intestine is the first place where short-chain fatty acids exert their own effects on the integrity of the intestinal epithelium or immune responses. Microbiota damage results in the reduction of short-chain fatty acids, which is associated with intestinal diseases such as IBD. According to one study, fecal microbiota in European children who are susceptible to IBD, was shown to contain less bacteria and lack effective bacteria in fiber digestion and SCFA production compared to African children.[99] In addition, Western nutrition has been reported to lead to microbial dysfunction, reduced SCFA, and high risk for colitis.[100] There is considerable evidence that SCFA concentrations are reduced in ulcerative colitis.[101] In addition, a recent study suggests that dysbiosis is associated with the reduction of butyrate-producing species, such as *Roseburia hominis* and *Eaecalibacterium prausnitizii*, in patients with ulcerative colitis.[102] GPCRs protect against inflammation of the intestine not only through the protection

of the intestinal epithelial barrier, but also through the regulation of immunity. Both GPR43 and GPR109a are important for regulating gut immunity. The GPR43 -/- and GPR109 -/- mice have been shown to suffer from DSS-induced colitis.[103] Several studies have found that SCFA can increase the production and inhibitory function of regulatory T-cells in the intestine in antibiotic-treated mice. GPR43 in colon T-cells is likely to induce the differentiation of the regulatory T-cell binding to SCFA. Rag-/-mice treated with propionate or SCFA have lower levels of colitis than those receiving water.[18] The butyrate bound to GPR109a inhibits dendritic cells and intestinal macrophages with the ability to induce the production of regulatory T-cells by increasing the production of IL-10.[103] Intestinal epithelial barrier plays an important role in preventing IBD inflammation. SCFAs affect the epithelial intestine by expressing high levels of GPR43 and act as regulators of physical barrier to and secretion of musine, antimicrobial peptides, chemokines, and cytokines. SCFAs may activate NALP6 through GPCR pathway, and also increase the secretion of mucus from intestinal goblet cells, an important barrier separating bacteria from epithelial cells.[104] Several studies have suggested that butyrate can lead to increased tight binding and epithelial permeability regulation.[106] Chronic hypoxia in mucous membranes of IBD patients leads to numerous changes in the intestinal environment. The stabilization of hypoxia-induced factors plays a protective role against inflammation and maintains gastrointestinal hemostasis.[107] A recent study has shown that SCFA is produced at a high level in oxygen deficit conditions, produces oxygen in the intestinal epithelial cells, maintains epithelial integrity, and protects the defensive barrier by stabilizing the HIF-induced factor.[108] On the other hand, administration of ethanol and 6-nitro benzenesulfonic acid in GPR43/GPR41/- mice decreased inflammation of the intestine, or inflammation with *Citrobacter rodentium* may decrease the secretion of pre-inflammatory cytokines and cytokines in the intestinal epithelial cells by increasing SCFA.[76] In general, SCFAs are involved in inflammatory and anti-inflammatory processes of IBD.

Chronic inflammatory diseases like IBD increase the risk of colorectal cancer, which is called colitis-associated cancer.[109] The intestinal microbiota is associated with inflammatory and tumorigenic processes. Therefore, their metabolites, such as SCFAs, may partially protect against intestinal cancer in a process dependent on GPCRs. In addition, GPR43 expression decreases in human intestinal cancer. Butyrate and propionate, based on the GPR43 pathway, induce apoptosis in cancer cells and prevent their proliferation.[110] GPR109a is a butyrate receptor in the intestine, expressed on the intestinal epithelial cells as well as immune cells. Lack of GPR109a increases the carcinogenesis induced by inflammation in the intestine.[103]

The beneficial effects of SCFA have been reported in patients with IBD. This was suggested long before the treatment of IBD patients with SCFAs or pre-biotic drugs that increase SCFA production.[111–113] SCFA treatment has been proven in patients with ulcerative colitis, which is also effective for patients with ameliorative colitis.[114] The combination of SCFA (sodium acetate, sodium propionate, and sodium butyrate) is used as a treatment with other therapies such as 5-aminosalicylic acid and corticosteroid therapy in the treatment of IBD and improves the efficacy and effectiveness of these treatments.[113] It can be concluded that SCFA affects intestinal immunity and IBD pathogenesis. However, due to limited symptoms or lack of patient consent, these treatments have not yet been established as a standard treatment.

Intestinal Carcinogenesis

SCFAs can directly exert their anticancer effects by HDAC inhibition and GPR activation. HDAC inhibition is associated with cell cycle stopping through SCFAs, leading to pre-apoptotic antidiabetic effects such as increased cellular differentiation in the intestinal cancer.[115] Human micronucleus analysis of human colon epithelial cells has shown that most of the responsive butyrate genes are used in the apoptotic, amplification and differentiation processes.[116] Butyrate, propionate and acetate promote apoptosis in several adenomatous and carcinoma cells by stimulating the expression of the regulatory genes of p53 and p21 cells, which decreases the expression of the anti-apoptotic protein Bcl-2, and increases the expression of the proapoptotic protein Bax.[117-119] Butyrate is the strongest inhibitor of cell proliferation and is consistently the strongest HDAC inhibitor, although acetate and propionate appear to boost the effects of butyrate in adenomatous cells.[121] Butyrate, propionate and acetate enhance cellular differentiation and inhibit cell migration in intestinal cancer cells in vitro by which they reduce invasion in carcinoma cells.[121-124] In addition, GPR43 and GPR109a both act as inhibitors of proliferation and enhancement of apoptosis in colon cancer cell lines independent of HDAC inhibition.[110,125] In addition, butyrate and other HDAC inhibitors inhibit cell growth in the intestinal cell lines by weakening the signaling pathway, Wnt, an active pathway in most colorectal carcinomas, and induce cell proliferation and tumorigenicity in these cancers.[126] Some conventional butyrate-resistant cancer cells exhibit resistance to apoptotic butyrate effects, which is related to the different degrees of weakening of Wnt.[127]

The clear contradiction in the effects of SCFAs in both providing energy for the growth and proliferation of normal cells and inhibiting the proliferation of cancer cells, is called the butyrate paradox.[128] This phenomenon is explained by Warburg effect where colon cells are dependent on glycolysis even in the presence of SCFAs for oxidation. Therefore, accumulation of SCFAs inhibiting the proliferation of

cancerous colonocytes is probably due to the effect on several target genes.[130] Another hypothesis suggests that the design and placement of epithelial cells inside crypts, protects stem cells against proliferation inhibition, while stem cells located in the deep areas of the crypt are provided with fewer SCFAs.[131] In a carcinogenic state where covering epithelial cells are impaired, SCFAs can reach the stem cells and inhibit cell proliferation.

The Role of Short-Chain Fatty Acids in Cancer

Applying SCFAs directly to the colon prevents the progression of colon cancer. Some studies have shown anti-carcinogenic butyrate effects in animals,[132] but other studies have shown no effect of butyrate on colon cancer.[134] In subsequent studies, very low concentrations of butyrate explain the effects observed, which probably indicates the need for high concentrations to be effective.

Because the direct use of SCFAs in colon is not for the prevention of physiologic colon cancer, some researchers have considered the use of pre-medications or food fibers with SCFAs to protect against intestinal cancer in animals. Pre-medications are inactive compounds that are metabolized to the active form of SCFAs. The advantage of using pre-medications is that unlike SCFAs that are rapidly metabolized by colonocytes, pre-medications provide high and stable concentrations after fast-absorption at the site of administration. An example of a premedication is tributyrin which is an ester compound of glycerol and butyrate. Metabolized by intracellular lipase, tributyrin releases the therapeutically effective butyrate directly into the cells over time.[136] Recent studies have shown that tributyrin protects carcinogen-treated rats against colon cancer.[137] In addition, a study on carcinogen-treated rats showed a 43% decrease in tumor in animals treated with tributyrin compared to 60% in controls.[138] Clarke et al also showed that carcinogen-treated rats fed with starch diets have more apoptosis in the distal colon compared with the control group.[139]

Inhibition of colon carcinogenesis by dietary fiber is attributed to intestinal butyrate-producing bacteria. In a study using a model of carcinogenesis in mice, lack of the SCFA receptor, GPR43 (Ffar2 -/-), was found to reduce animal survival. Dietary fiber can stop weight loss, diarrhea, and spread of intestinal polyps, but only in wild-type mice and not in Ffar2 -/- mice. These findings suggest that the GPR43 receptor is necessary to control the carcinogenesis of the colon associated with inflammation with dietary fiber.[140] Another study on mice shows an inhibitory role for the receptor butyrate GPR109a.[103] In rats exposed to a genetic challenge with azoxymethane, the removal of high-amylose corn starch resulted in high levels of SCFAs, high concentrations of acetate and butyrate in the colon, as well as high concentrations of SCFA, acetate and butyrate in the plasma of hepatic portal vein, in a dose-dependent manner. This intervention increases the thickness of the mucus, decreases single-stranded DNA fractures, and increases apoptosis rate in a dose-dependent manner in distal colon cells.[141] The effects of fiber depend on the source of fiber and the intestinal microbiota.[142] To investigate this, Donohoe et al., used cloned gnotobiotic mice with wild-type or mutant strains of a butyrate-producing bacteria to prove that food fiber has a strong tumor suppressor effect, but in a microbiostatic- and butyrate-dependent manner. As a result of what has been said thus far, since the use of SCFAs in human interventions is not possible, the use of preservatives or dietary fiber may provide SCFA effects to prevent colon cancer in humans. Also, there is still controversy over whether the food fiber plays a protective role in defense against colorectal cancer. However, the distinction between those who respond to the anticancer drug and those who did not is based on their precise microbial analysis.[142]

Mucosal Repair and Colon Protection

Van der Sluis[143] showed that mice lacking MUC-2 spontaneously develop colitis and colon cancer. It is suggested that the mucus layer plays an important role in colon homeostasis and protects against external damage. Administration of butyrate increases the expression of MUC2 gene after 7 days, but decreases the thickness of the mucus layer.[144] Ferreira et al.,[145] suggested that an improved intestinal barrier appears after administration of SCFA compound or butyrate alone in drinking water to colon mouse model.

In the surgical model of acute colon obstruction in rats where the pressure of explosion and intestinal wall was higher in the SCFA-treated group and the colon pressure was probably due to the inactive region of the intestine, it was shown that the activated region was stronger than the inactive region in the colon.[146] Another study on intestinal obstruction in rats suggests that interaoperative lavage with SCFA compound reduces necrotic ulcer size in suture and leads to rapid repair of mucous membrane cleavage.[147] Other animal studies have shown positive SCFA effects on mucosal repair and adhesion prevention.[148-150] Also, one review dealt with HT29 to assess the effects of SCFA on the growth of the human adenocarcinoma cell line. The results indicate that under culture conditions, propionate and butyrate inhibit HT29 cell lineage, while acetate does not have significant and meaningful effects. The antiproliferative effect of propionate or butyrate is accompanied with inhibition of FCS-induced activation of ornithine decarboxylase, a key enzyme of polyamine metabolism. Inhibition of growth induced by propionate or butyrate does not disappear with the addition of putrescine, which indicates that SCFAs do not exclusively act in the ornithine decarboxylase/polyamine. These findings suggest that propionate and butyrate, unlike acetate, lead to an increase in alkaline phosphatase activity, which represents a more

distinct phenotype than control group cells. Based on these findings, it is suggested that propionate and butyrate play an important role in the physiology of the colon and may have a protective effect against colon carcinogenesis.[151]

Butyrate

In the previous section, we discussed SCFAs as well as the role of propionate and butyrate as the main components of this family. However, due to its very important role in cancers as well as inflammatory diseases, butyrate will be discussed separately in terms of its characteristics.

The lower fecal butyrate level may be a biomarker for not only the risk of cancer, but also its progression and severity.[131] Clinical studies have shown that people with advanced colorectal cancer are more likely to be exposed to butyrate-producing bacteria and lower levels of butyrate compared to the control group.[153]

Several laboratory evidence has demonstrated the inhibitory effects of butyrate on tumorigenesis through numerous mechanisms.[154-156] The important effects of butyrate include anti-inflammatory and immune-regulating activities,[157] a reduction in the Wnt focal signaling pathway associated with colon carcinogenesis,[158] and inhibiting the proliferation and migration of neoplastic cells, limiting tumor angiogenesis, inducing apoptosis and enhanced differentiation of neoplastic colonocytes.[160] Butyrate plays an important role in strengthening the mucosal defense barrier by enhancing the expression of mucin-encoded genes and inducing trefoil factors, thermal shock proteins, antimicrobial peptides and transglutaminase activity.[132,161] Finally, there is evidence of the ability of butyrate to maintain the health of colon mucous membrane, which is mediated to some extent by activating the expression of FOXP3 and IL10 in regulatory cells. In studies on mice lacking specific pathogens, the gnotobiotic altered Schaedler-floracolonized mice and germ-free mice, SCFAs regulate the size and storage function of the colon's regulatory T-cells. This process leads to protection against colitis, possibly through interactions between GPR43 induction, direct activation of histone dastilase inhibition, and increased IL-10 gene expression.[18]

In another study, the 5-year history of the use of fiber and SCFA fecal concentrations in 344 advanced adenomatous patients was compared to that of 344 healthy age-matched subjects.[152] The consumption of fiber in patients with colorectal adenoma and SCFA levels significantly decreased. The microbiota combination and its amount were measured by PCR and pyrosequencing in 47 volunteers from each group, and the analysis of the essential components showed a distinct difference in the fecal microbiota communities of the two groups. *Clostridium*, *Roseburia*, and *Eubacterium* were significantly less prevalent in the group with advanced adenomatous disease than in the control group, while Enterococcus and Streptococcus spp were more common in the anomietic group. The rate of butyrate and bacteria producing high levels of butyrate was higher in the control group consuming high levels of fiber compared with those with colorectal adenomas that also consumed high levels of fiber, suggesting that lack of colonic butyrate is due to inadequate fiber consumption or a shortage of butyrate-producing bacteria promoting neoplasia.

The evaluative mechanisms suggest the association between the extent of fiber harvesting and neoplasia, and it has been shown that there is a pathway involving the biogenesis of oncogenic microRNAs through which butyrate can inhibit the proliferation of human cancer cell lines.[162] First, miR-92a levels have been proven to be seven times higher in human colorectal cancer tissue than in normal cells with cancerous tissue. Second, adding butyrate to a human cancer cell line has led to a decrease in miR-17-92a levels, a precursor and adult miR-92a. These findings, together with the inhibition of key MIC oncogenesis and increased expression of CDKN1C, lead to inhibition of proliferation and increased apoptosis of tumor cells. The same effects are achieved with the addition of histone dostazylase inhibitors, called suberoylanilide hydroxamic acid and valproic acid, indicating an epigenetic mechanism. A large number of human studies and trials suggest that the main mechanism for the ability of butyrate to inhibit proliferation and the risk of cancer is its ability to normalize oncogenic function of (miRNA) oncomirs.[163] For example, in a crossover controlled trail study, healthy volunteers took a large amount of red meat (300g per day) in their diet while the other group consumed a diet rich in red meat plus fiber for four weeks.[163] The consumption of high amounts of red meat resulted in increased expression of oncogenic miRNAs present in rectal mucosa and cell proliferation along with a reduction in the target of miR-17-92 gene at transcription levels.[163] All these effects increased with the elevation of butyrate synthesis due to fiber consumption. Laboratory evidence has been provided for the replacement of carcinogenesis that is based on enabling the SCFA produced by fiber fermentation to bind to the SCFA receptor, GPR43 in the colon.[165] In colon cancer-associated AMO-dextran sodium sulfate (DSS) colitis in a rat model, inclusion of resistant starch in diet reduced the tumor's multiplicity and the formation of adenocarcinoma.[165] Evidence has emerged of the mechanism involved in increasing the production of butyrate, which is dependent on increasing butyrate-producing bacteria and increasing the production of SCFA. Additionally, increasing the production of butyrate depends on increasing the expression of the SCFA receptor, GPR43 mRNA, which triggers reduction of inflammation by inhibiting the expression of cyclooxygenase 2, NF-κB, TNF-α, IL-1β, and cell proliferation.[165]

The Threshold of Butyrate Effect

It has long been discovered that butyrate can act contradictorily

under certain conditions by stimulating mucosal proliferation. This effect can be explained by various functions of butyrate on cell growth: as a major energy source, it can stimulate cell growth, while it can inhibit cell growth as an anti-neoplastic agent in a phenomenon called butyrate paradox. Evidence for this paradox includes observations of increased butyrate production, which stimulates epithelial growth through providing energy under famine or apoptotic conditions, while the production of butyrate under growth conditions is an inhibitor of proliferation and cancer risk.[166] Laboratory studies reveal paradoxical mechanisms and report that the production of butyrate is important and necessary. The low concentration of butyrate in intestinal crypts (0.5 mM) does not have an inhibitory effect on histone deacetylase, since the whole butyrate is used for cellular energy, while high levels of it (5 mM) can enter the nucleus and as histone deacetylase inhibitors, increase differentiation and apoptosis and inhibit proliferation of cancerous colonocytes.[129] In addition, in xenobiotic mouse models cloned with wild-type or mutated butyrate-producing bacterial strains, it has been shown that fiber plays a protective and inhibitory role in the tumor in a manner that is dependent on butyrate and microbiota.[142] However, it is not surprising to find studies that are not consistent with the hypothesis of carcinogenesis-inhibiting ability of butyrate. For example, a study has shown that a low-carbohydrate diet results in the reduction of butyrate-producing microorganisms and the production of butyrate in a mouse model of colon cancer. These findings are associated with decreased gut cancer development. According to cell culture studies, researchers suggest that intestinal microbiota stimulates colorectal cancer by providing butyrate, which results in β-actin excretion or proliferation and increased the risk of neoplastic deformation.[159]

There is good evidence that the high fermentation rate is as harmful as a low rate. In goats, consuming high amounts of cereals (65%) leads to epithelial surface layers, intracellular tight junction analysis, cell mitochondria damage and increased expression of IL-12 and IFN-γ mRNA. These effects are associated with increased microbial degradation, increased acidity and increased SCFA production.[167] These conditions have also been identified in the cow. During the feeding process, factors associated with the animal or the environment contribute to a fermentable carbohydrate stream from the small intestine to the colon that results in ejaculation, diarrhea and severe mucosal damage.[168] In addition, it is important to note that humans can also have similar conditions. The frequent loss of the small intestine, a condition called short-bowel syndrome, results in the delivery of a large amount of carbohydrates to the colon. In these conditions, colon microbiota performs an essential function for increasing fermentation with a re-use of up to 1,000 calories of carbohydrates per day.[169] However, the collapse of the microbiota compound may lead to an increase in the growth of D-lactate producing organisms and possibly D-lactic acidosis, and this form of lactate cannot be metabolized by humans.[159]

Conclusion

Microbial metabolites, produced by the metabolic processes of microbiota, have a protective role in various diseases, including inflammatory diseases and cancers. Any disorder in the microbial metabolites results in their inefficiency and various diseases. One of the factors that impairs the production of microbial metabolites is the consumption of foods with low-fiber and high carbohydrates and fat. One of the strategies that can be used to prevent diseases caused by the dysfunction of microbial metabolites is dietary considerations. A fiber-rich diet ensures sufficient production of microbial metabolites.

Among all the metabolites examined, the role of SCFAs in inflammatory and cancerous diseases is more prominent. Reduced levels of SCFAs are associated with an increase in the incidence of IBD and various cancers, including colon cancer. SCFAs, which contain a group of microbial metabolites, regulate the pattern of immune response to regulatory T-cells and prevent unwanted and over-immune inflammation by secreting anti-inflammatory cytokines, such as IL-10 and TGF-β. On the other hand, reducing the number of microbial metabolites leads to an increase in the severity of inflammatory IBD. The continuation of chronic inflammation in IBD leads to genomic instability, which is associated with increased epigenetic changes and leads to colon cancer. As a result, the presence of microbial metabolites can help to balance the immune response and maintain the hemostasis of the intestine and help prevent inflammatory bowel disease and cancers. The results of this overview reveal that microbial metabolites play the role of immune modulator and can be considered as immune-modulator agents. On the one hand, the regulation of cytokine T profiles suppresses any inflammation and prevents the spread of inflammatory diseases. On the other hand, by increasing apoptosis and inhibition of topoisomerase, cellular migration of cancer cells, histone deacetylase (HDAC), etc., the cancer cells are destroyed. With a small change in microbial metabolites as an essential and vital component of the body, the stage is set for the development of various inflammatory diseases and cancer. With regard to the therapeutic use of SCFAs for IBD and colon cancer, satisfactory results can be found, but there are other microbial metabolites, such as rebeccamycin that have anticancer effect in vitro but for therapeutics applications several clinical trials have been conducted. The same seems to be true with results in anticancer research. The microbial metabolites can also be used in vaccine design as adjuvant. However, further studies, especially in the molecular field,

are needed to provide more effective therapies for the treatment of cancer and inflammatory diseases. Table 1 represent some Microbial metabolite involved in cancer and inflammatory diseases.

Table 1. Microbial Metabolite Involved in Cancer and Inflammatory Diseases

	Inflammatory Disease	Cancer Type	Mechanism of Action
Rebeccamycin	Not reviewed	Hard tumor cell category, B16 reticulum cell sarcoma, M109 lung cancer, HCT116 human colon cancer and human lung cancer, ovarian cancer, soft tissue sarcoma, advanced kidney cancer, NSCLC	- Topoisomerase inhibitors 1 and 2 - Kinase inhibitors - Stabilizes the interaction of topoisomerase with DNA
TMAO	IBD, Cardiovascular Disease, Atherosclerosis	Colorectal, gastrointestinal tract	- Independent factor for conditions such as insulin resistance and gastrointestinal cancer - Known as a diagnostic biomarker in many cancers - Known as a factor in the methyl group, it causes epigenetic changes such as methylation, which leads to malignant changes and eventually cancer.
Lactacystin	Neurodegenerative disease	Prostate, lymphocytic leukemia, stomach, Hela cancer cells	- Inhibition of the ubiquitous-protease system - Increased expression of HSP-22 and HSP-70 thermal shock proteins - Increases the expression of inflammatory genes - Increased apoptosis by decreasing NF-KB expression
Short chain fatty acids (propionate, acetate, lactate)	IBB (Reduction of short-chain fatty acids and their receptors leads to IBD)	Colon cancer	- Effects on systemic autoimmune responses - Influence on production of inflammatory cytokines by increasing NF-κB by activating TLR in epithelial cells - Inhibition of the production of inflammatory cytokines such as TNF-α in neutrophils - Disrupts TH2 function by reducing CD40, PD-L2 and CD86 expression - Increase the number of trades - Increased IL-10 production in T lymphocytes such as Th1, Th17 and regulatory T cells - Playing a role as a therapeutic agent in patients with IBD - Has a protective role against intestinal cancer in a GPCR-dependent process - Induction of apoptosis in cancer cells by stimulating the expression of regulatory genes of p53 and p21 cell cycle and reducing the expression of Bcl-2 antiseptic protein and increasing the expression of Bax proappeptotic protein - Direct anti-cancer effects by inhibiting histone dysthylase (HDAC) and activating GPR - Increases cell differentiation and inhibits cell migration in intestinal cancer cells - Prevent the progression of colon cancer by taking SCFA directly into the colon

Authors' Contributions

All authors contributed to conception and design of the study, wrote the first draft of the manuscript, manuscript revision, and approved the submitted version.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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