Review

Effects of the intestinal microbial metabolite butyrate on the development of colorectal cancer

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Abstract

Colorectal cancer (CRC) is one of the major health threats in developed countries. Changes in dietary components, such as more protein and lipid intake, can increase the risk of CRC. Diet affects CRC in many ways. They regulate the composition and function of gut microbiota, which have an amazing metabolic capacity and can produce short chain fatty acids (SCFAs), such as propionate, acetate, and butyrate. Butyrate is a principal energy source for colonic epithelial cells and plays an important role in maintaining the stability of gut microbiota and the integrity of intestinal epithelium. However, there are few studies reviewing the anti-CRC potentials of butyrate. This review summarizes the recent research progresses in the effect of gut microbiota imbalance and the decrease in intestinal microbial metabolite butyrate caused by unbalanced diet on CRC development, and discusses the mechanisms of butyrate-induced anti-CRC activities, which may guide people to prevent CRC by improving diet structures.

Key words: Butyrate, Colorectal cancer, Gut microbiota, Diet.

Introduction

Colorectal cancer (CRC) is the second most common cancer in women and the third in men worldwide[1]. Epidemiological studies have shown that the occurrence and development of CRC is associated with an increase in meat diet and a reduction in dietary fiber, and that changes in food composition can affect CRC risk by affecting intestinal microbial metabolism[2, 3]. Studies in recent decades have proved that gut microbiota, which can be affected by diets, play an important role in the occurrence and development of CRC[4, 5]. After digestion and absorption, food with different structures left residue in the intestinal tract. As food flows through the colon, residues of dietary fiber can be fermented by gut microbiota to produce short chain fatty acids(SCFAs), such as propionate, acetate, and butyrate, which play plurifunctional roles on the gut microbiota and intestinal epithelial cells[6, 7]. Studies have shown that the primary energy source of the intestinal epithelia is butyrate, rather than glucose, which established the basis for the modern theory of mutualism between gut microbiota and intestinal epithelia[8]. Butyrate can inhibit the development of CRC and promote intestinal health through various mechanisms (**Figure 1**). This review summarizes the recent research progresses in the effect of gut microbiota imbalance and the decrease in the intestinal microbial metabolite butyrate caused by unbalanced diet on CRC development, and discusses the mechanisms of butyrate-induced anti-CRC

activity, such as suppression of neuropilin I expression, regulation of miRNA expression, promotion of endocan expression, hyper-activation of Wnt signaling pathway, and upregulation of P21waf1 and bax expression (**Figure 2**).
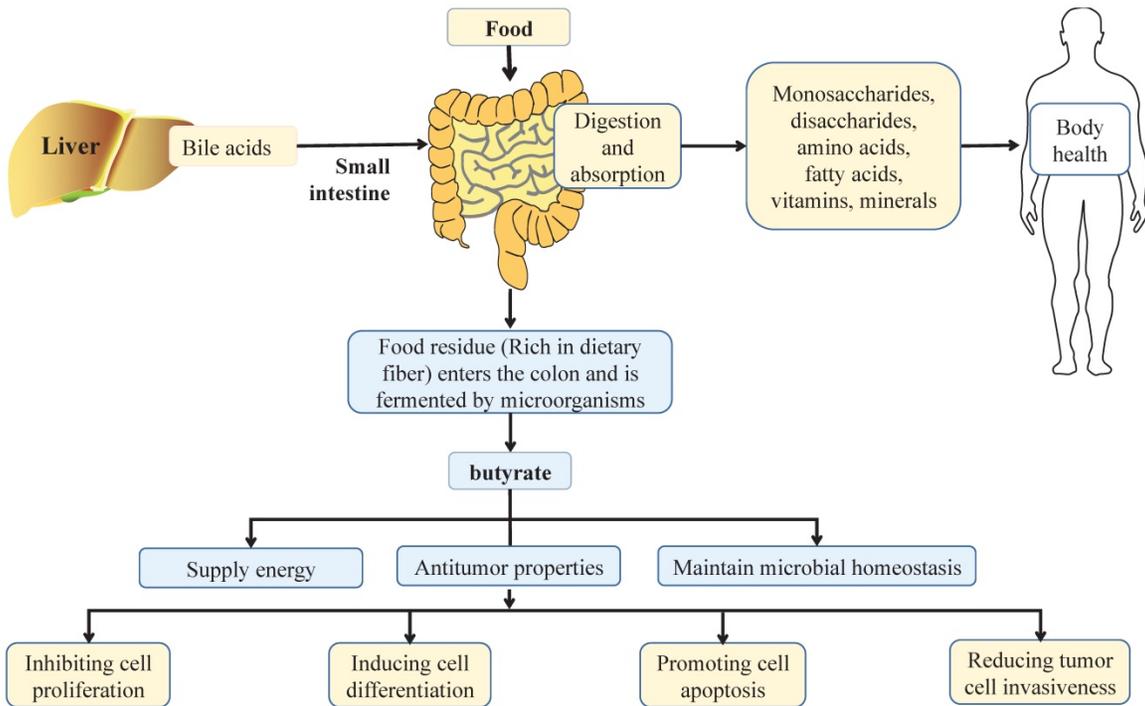

Figure 1. Effect of balanced diet on intestinal health. In a balanced diet, food is mainly digested by digestive enzymes and bile acids from liver in the small intestine, and more than 90% are absorbed in the small intestine. After absorption, nutrients are distributed throughout the body through the circulation of the blood, in which monosaccharides, disaccharides, amino acids, fatty acids, vitamins and minerals play important roles in maintaining the health of the body. When dietary residues flow through the colon, they are broken down by intestinal microbiota, which produce short chain fatty acids, such as butyrate. Butyrate is a principal energy source for the gut microbiota and intestinal epithelial cells, and plays an important role in maintaining the stability of gut microbiota and the integrity of intestinal epithelia. Notably, butyrate can also inhibit cell proliferation, induce cell differentiation, promote cell apoptosis, and reduce tumor cell invasiveness to exert its anti-tumor properties, thus playing an important role in the health of the colon.
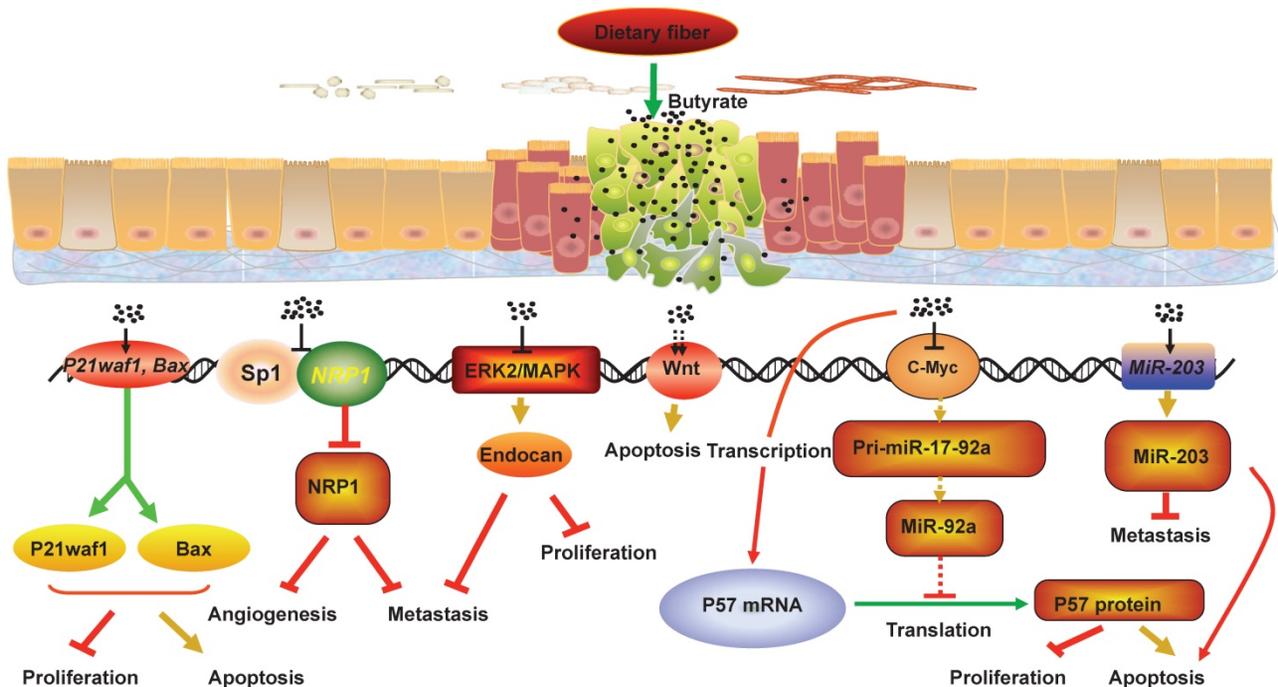

Figure 2. The mechanisms that butyrate suppresses CRC development. Fermentation of fiber in the lumen leads to production of short-chain fatty acids including butyrate. Butyrate can inhibit the development of CRC and promote intestinal health through various mechanisms. For example, butyrate can decrease the NRP-1 expression by inhibiting the transactivation of Sp1 to suppress the angiogenesis, metastasis and survival of CRC cells, and butyrate can promote the apoptosis of CRC cells by hyper-activating Wnt signaling pathway. In addition, butyrate can restrict cell proliferation, colony formation, cell invasion, and induce apoptosis in CRC cells through upregulating the expression of miR-203, P21waf1 and bax, and promoting endocan expression. Meanwhile, butyrate can increase p57 mRNA and protein levels by inhibiting c-Myc expression, which reduces miR-17-92a cluster transcription and the level of miR-92a. Collectively, the interactions between butyrate, NRP-1, Wnt, endocan, P21waf1 and bax, miR-203 and miR-92a mediate the anti-proliferation and pro-apoptosis effect of butyrate in CRC cells.

Effects of dietary factors on CRC

The geographical variation in CRC incidence is remarkable, as illustrated by the WHO's data that CRC incidence rate is relatively high in western developed countries while relatively low in developing countries, such as Asia and Africa. A previous study observed a significant change in the CRC incidence after Japanese migrate to Hawaii. Specifically, CRC incidence was low in Japan, and after these people migrated to Hawaii, their CRC incidence became as high as Hawaiian natives within one generation[9]. Further analysis showed that the CRC incidence varies from place to place, mainly due to different dietary habits. The high CRC incidence in western developed countries is induced by imbalanced diet, mainly demonstrated by a lack in fruits, vegetables, cereals, and other high fiber foods. It is well recognized that dietary fiber can reduce the CRC risk, whereas the meat diet can increase the CRC risk[10]. A study showed that up to 30 g/d dietary fiber intake could prevent CRC development[11]. Moreover, based on the characteristics of high fiber diet, gut microbiota is evolved into a model that is suitable for decomposing high fiber food residues. Fiber diet and intestinal microorganisms benefit mutually, and gradually become dependent on each other to form a stable equilibrium. These showed that dietary factors and the gut microbes play an important role in the CRC pathogenesis.

Effects of gut microbes on CRC

Gut harbors a large number of different microorganisms, such as bacteria, viruses, archaea, yeasts, and fungi. The gut microbiota has many important functions for human health[12, 13]. For example, gut microbiota can ferment dietary fiber to produce SCFAs, such as acetate, propionate, and butyrate[14]. Among the gut microbes, there are several types of anaerobic bacteria, such as *Faecalibacterium prausnitzii* and *Roseburia* species, which are the main butyrate-producing bacteria[15, 16]. In comparison with healthy controls, CRC patients have significant changes in composition of gut microbiota, such as the increase in *Fusobacteria*, *Porphyromonadaceae*, *Staphylo-coccaceae*, *Akkermansia spp.* and *Methano-bacteriales,* and the decrease in *Bifidobacterium*, *Lactobacillus*, *Ruminococcus*, *Faecalibacterium spp.*, *Roseburia*, and *T-reponema*[17-19]. In particular, butyrate-producing *Fusobacterium* was shown to inhibit CRC development[20, 21]. In addition, the microbial metabolites such as nitrogen components, were reported to be elevated, whereas others metabolites, such as butyrate, decreased in certain CRC patients[22]. Gut microbiota can also induce chronic inflammation, one of the major risk factors for CRC, by directly invading intestinal epithelial barrier or activating immune responses by binding to Toll-like receptors, which leads to the synthesis and secretion of cytokines. The abundance of gut microbes in the lumen is also closely related to the CRC occurrence. For example, the distribution of microorganisms in the distal colon is the densest in all parts of intestine, thus the probability of CRC occurrence in the colon is also the largest[23]. Food residue, intestinal microbiota, and intestinal epithelia, work together to maintain intestinal homeostasis. The structure of the gut microbiota is usually very stable, and the microbes in each part of the digestive tract from the mouth to the rectum are different[24]. The formation of this steady state is also determined by the long-term natural selection in human evolution. Plant food in the lower food chain is relatively readily available than meat food on the top of the food chain. Therefore, the long vegetarian diet of human determines the dependence of intestinal microbiota on fiber diet. When this long-term eating habits change, intestinal microbial disorder tends to occur. Butyrate, the metabolite produced by the fermentation of dietary fiber-rich food residues by the gut microbes, is an important mediator among dietary factors, intestinal microbiota and CRC. In comparison with healthy people, CRC patients have significant reduction in the number of bacteria that produce butyrate and other SCFAs[25]. Therefore, the change of intestinal microbiota caused by the diet change plays an important role in the occurrence and development of CRC.

Effects of the gut microbiota metabolites butyrate on CRC

In a balanced diet, gut microbes ferment fiber-rich food residue in colon and produce SCFA metabolites including butyrate. Butyrate can provide energy for intestinal epithelial cells[6, 26], and has anti-inflammatory and anti-tumor properties[27-29]. A recently proposed mechanism about the effect of butyrate on CRC progression is based on the down-regulation of butyrate transporter protein in CRC tissue, which will reduce the transport of butyrate in the intestine and reduce the metabolism of butyrate[30]. The atrophy of butyrate metabolism is associated with the pathogenesis of various colonic diseases[31]. It is well known that the pathogenesis of CRC is closely related to the dysfunction of intestinal epithelial barrier, which activates tumor-associated macrophages, leads to the production of inflammatory cytokines, and ultimately leads to tumor growth and progression[32]. Butyrate can promote the assembly of tight junction by activating the Amp-activated protein kinase (AMPK), and can induce the expression of MUC2 in human colon

cancer cell line LS174T cells, to maintain the function of intestinal epithelial barrier[33-35]. The movement of the gastrointestinal tract decreased and the subsequent intestinal transport slowed down in the long time, which also affected the incidence of CRC. Studies show that butyrate can interfere with the internal nervous system and regulate the excitability of neurons, causing the increase of colonic motility and the extracorporeal contraction reaction[36-38]. Therefore, butyrate is critical for intestinal microbial balance and colon health. In addition, a number of studies have shown that butyrate can inhibit the occurrence and development of CRC through various mechanisms (**Table 1**).

Table 1. Effects of butyrate on the development of colorectal cancer

Signaling pathway	Key molecules	Experimental systems	Main effects on colorectal cancer	References
NRP-1/VEGF	NRP-1	HCT116 HT29 Caco-2	Butyrate can decrease NRP-1 expression by inhibiting the transactivation of Sp1 to suppress the angiogenesis, metastasis and survival of CRC cells	[55, 56,57]
ERK2/MAPK	Endocan	RKO	Butyrate can inhibit CRC cell proliferation, clone formation and migration by promoting endocan expression through ERK2/MAPK signaling pathway	[61, 62]
Wnt	β-catenin	DLD-1 HCT116	Butyrate can promote the apoptosis of CRC cells by hyper-activating Wnt signaling pathway	[67-71]
p53	P21wafl bax	HT-29, SW48, SW1116	Butyrate can enhance the expression of P21waf1 and bax to inhibit the proliferation and induce apoptosis of CRC cells and block the cell cycle in G_1 phase	[72, 73]
microRNA	miR-203 miR-92a	SW837 HT-29 Caco-2 HCT116	Butyrate can up-regulate miR-203 expression and decrease miR-92a expression to restrict cell proliferation, colony formation, cell invasion, and induce cell apoptosis in CRC cells	[89, 91]

As a fatty acid, butyrate can be converted to acetyl-coA by beta-oxidation, and then completely oxidized through three carboxylic acid cycle. The metabolization of butyrate to acetyl-CoA is essential for stimulating the activity of histone acetyltransferase (HAT). Due to the Warburg effect, butyrate does not metabolize normally in cancer cells, but can be accumulated as a histone deacetylase inhibitor (HDACi) in the nucleus, thereby regulating the expression of downstream target genes. Histone deacetylase (HDAC) is very important for gene expression, and the expression of these enzymes in tumor cells increase and vary by tumor types [39]. For example, HDAC1 is highly expressed in prostate cancer, gastric cancer, lung cancer, esophageal cancer and breast cancer, and HDAC2 is highly expressed in

colorectal cancer, cervical cancer and gastric cancer. In addition, HDAC3 is highly expressed in breast cancer and colorectal cancer, while HDAC6 is highly expressed in nerve cell carcinoma[40, 41]. In most of tumors, acetylation will result in expression changes of some genes, which may be involved in signaling pathways such as ERK, Wnt, and further affect the protease system as well as the activities of some kinases (such as protein kinase C) [42]. On the structure, HDACi can induce cell death through different processes, depending on the target cells, including changes in gene expression, histone modifications, and epigenetic changes[43]. Inhibition of histone acetylation leads to chromatin lysis and reduction in concentration, making DNA more readily accessible. The decrease in the activity of histone deacetylase was associated with inhibition of tumor cell growth[44]. As an HDACi, butyrate has complicated roles in histone acetylation. Butyrate can influence the development of tumor by inducing cell cycle stagnation and apoptosis.

Butyrate reduces the expression of neuropilin by inhibiting the transactivation of Sp1(specificity protein 1) to suppress the angiogenesis, metastasis and survival of CRC

Tumor growth and metastasis are highly dependent on angiogenesis, which is the initial stage of new blood vessel formation[45]. Vascular endothelial growth factor (VEGF) is the core regulator of angiogenesis[46]. The expression of VEGF is up-regulated in many solid tumors, including primary and metastatic CRC[47]. Multiple studies have found that butyrate, as an HDACi, can down-regulate the expression of VEGF to inhibit the tumor angiogenesis[48, 49]. Neuropilin (NRP) is a receptor for VEGF, which is expressed in normal epithelial cells and is highly expressed in tumor cells[50, 51]. The binding of VEGF to Neuropilin-1 (NRP-1) promotes the migration and survival of CRC cells[52]. Clinical studies indicate that CRC with high expression of NRP-1 has a greater incidence of metastases, increased proliferation index and reduced numbers of apoptotic cancer cells than CRCs with low expression of NRP-1, suggesting that NRP-1 can protect CRC cells from apoptosis[53]. Silence of NRP-1 expression by siRNA can induce apoptosis of tumor cells, suggesting that the decrease in NRP-1 expression can be used to increase sensitivity of tumor cells to chemotherapy[54]. Previous studies revealed that NRP-1 is down-regulated by butyrate in CRC patients[55]. Meanwhile, *in vitro* experiment also showed that butyrate can down-regulated NRP-1 and VEGF at the mRNA and protein level in CRC cell lines through inhibiting Sp1 transactivation [56, 57]. HCT116,

HT29 and Caco-2 lines represent a range of CRC cell types. When different concentrations of butyrate were used to treat these three CRC cell lines, the expression of NRP-1 were suppressed, and the binding of VEGF to NRP-1 also decreased, thus inhibiting angiogenesis [49].

Butyrate inhibits CRC progression by up-regulating the endocan gene expression via ERK2/MAPK signaling pathway

Endocan is a sulfate proteoglycan and plays an important role in inflammation, angiogenesis, and metastasis of tumor cells[58-60]. The expression of Endocan in CRC tissues was lower than control tissues, and the Endocan expression was inversely correlated with the degree of malignancy[61]. A study showed that butyrate increased endocan expression at both transcription and translation level in the RKO colon cancer cells. Moreover, over-expression of endocan can significantly inhibit the proliferation, colony formation, and migration of RKO cells[62]. In addition, knocking-down of the endogenous endocan level reversed the inhibitory effect of butyrate on tumor proliferation and metastasis. This study also showed that butyrate increased the endocan expression through inhibiting ERK2/MAPK signaling pathway, thus suppressing the proliferation, migration, and colony formation of CRC cells[62].

Butyrate regulates Wnt activity levels and apoptosis in CRC cells

Wnt signaling pathway is deregulated in most cases of CRC[63]. The core protein in this pathway is β-catenin, which is bound by the adenomatous polyposis coli (APC) protein. When Wnt signaling is not activated, β-catenin is phosphorylated by glycogen synthase kinase 3β(GSK-3β) and targeted for degradation[64]. When Wnt signaling pathway is activated, the activity of GSK-3 kinase is suppressed, and β-catenin accumulates in the nucleus and binds to the Tcf/Lef (T-cell factor/lymphocyte enhancer factor) family to activate the transcription of target genes. However, in CRC, the mutation in the components of the pathway (e.g., β-catenin and / or APC) leads to constitutive transcriptional activation of the Wnt target gene, which ultimately leads to unlimited cell growth[65, 66].

Previous studies showed that butyrate induced higher Wnt activity and apoptosis in CRC cells, and Wnt levels were correlated with apoptosis levels. However, the underlying mechanism by which butyrate induced CRC cell apoptosis in hyper-activation of Wnt signaling pathway is not well understood[67-69]. The correlation between high Wnt activity and apoptosis induced by butyrate was also observed in DLD-1 and HCT116 cells with dominant negative (dn) Tcf4, in which the decrease of Wnt activity was associated with the decrease of apoptosis[70]. In addition, the CRC cells with higher Wnt activity had higher levels of apoptosis, while the samples with low or no Wnt activity had lower levels of apoptosis after the treatment of butyrate[70]. Meanwhile, another study confirms that butyrate can induce changes in expression of Wnt-signaling-specific genes in CRC cells[71]. Thus, the hyper-activated Wnt activity is causatively related to the induction of apoptosis in CRC cells treated with butyrate.

Butyrate enhances p21wafl and bax expression to inhibit proliferation and induce apoptosis of CRC cells

p53 is a tumor suppressor gene that has a high correlation with human tumor regression. *p53* mutation can be detected in more than 50% of human cancers. Some chemicals or food ingredients can induce the expression of *p53* target genes (such as *p21wafl*, *bax*) and potentially suppress tumor development. A study showed that butyrate induced *p21wafl* and *bax* expression both at mRNA and protein level in HT-29 cells[72]. In addition, butyrate with the concentration above 2.5 mmol/L can inhibit the growth of HT-29 cells. Furthermore, butyrate treatment increased the percentage of G1 phase cells. According to the results of Annexin-V/PI staining, the apoptosis rates of butyrate-treated group were significantly higher than those of the control group[72]. Studies using other CRC cell lines (SW48, SW1116, and SW837) also showed that butyrate could suppress cell proliferation and enhance cell apoptosis[73].

Butyrate upregulates miR-203 expression and downregulates miR-92a expression to restrict cell proliferation, colony formation, cell invasion, and induces cell apoptosis in CRC cells

The ability of butyrate to inhibit CRC cells is also associated with its regulation on microRNA (miRNA), such as the miR-106b family and the miR-17-92 family[74, 75]. miRNAs are small and non-coding RNAs that regulate cell cycle arrest, cell apoptosis, proliferation and differentiation[76, 77]. More than 1000 miRNAs have been identified, and many of them have hundreds of target genes[78]. Genetic or epigenetic modifications affect the expression of miRNAs in various human tumors[79]. Moreover, the disorder of miRNA expression is involved in the process of tumorigenesis through regulating the expression of proto-oncogenes and tumor suppressor genes[80]. In CRC cells, a variety of miRNAs have been

found to affect cell growth, migration and invasion[81-84]. MiR-203, as a tumor suppressor miRNA, has lower expression levels in CRC tissues than in non-tumor tissues[85]. Studies reveal that miR-203 could inhibit cell proliferation, invasion and metastasis, and promote cell apoptosis[86, 87]. A previous study of skin tumors found that butyrate was able to decrease the expression of miR-203 to inhibit tumor development[88]. Another study investigated the effect of butyrate on miR-203 expression in CRC cell lines, HT-29 and Caco-2. The results showed that butyrate could increase miR-203 to inhibit cell proliferation, clone formation, cell invasion, and induce apoptosis in CRC cells. A further study indicated that NEDD9, a target gene of miR-203, could partially reverse the effect of miR-203 on the colony formation and invasion of CRC cells[89].

In addition, another study showed that butyrate increased the expression of other miRNA families and clusters, including the oncogenic miR-17-92a cluster, also known as oncomiR-1 and C13orf25 in human sporadic colon cancer[90-92]. MiR-92a is overexpressed in CRC patients and associated with tumor metastasis and poor prognosis[93]. Further studies found that microbe-derived butyrate inhibits the oncogenic miR-92a by regulating c-Myc expression in CRC. Butyrate treatment on HCT116 and HT29 human CRC cells led to the significant decrease in miR-92a level. Other HDACi, such as hydroxamic acid (SAHA) and valproic acid, also have similar effects upon butyrate treatment. In addition, butyrate treatment also reduces the level of other members of the miR-17-92a family, including miR-17, miR-18a, miR-19a/b, and miR-20a. Exogenous supplement of miR-92a can reverse the expression of the tumor suppressor gene of p57, and growth inhibition and apoptosis induced by butyrate. Collectively, these findings reveal a new mechanism by which butyrate suppress CRC development through the interaction among butyrate, c-Myc, miR-92a, and p57, leading to the decrease in cell proliferation and increase in cell apoptosis[91].

Conclusions and future prospects

The geographical distribution of CRC is mainly due to the difference of dietary habits. Gut microbes rely on high fiber foods residues to maintain its normal structure and metabolism, wherein metabolite butyrate plays an important role in maintaining intestinal homeostasis and the health of intestinal epithelia. Butyrate is the basic energy source of the intestinal epithelium, and can inhibit intestinal inflammation. Above all, as an HDACi and a product of fiber fermentation, it can mediate the protective effect of dietary fiber against CRC by a variety of mechanisms. In recent years, studies on the interaction among dietary factors, gut microbes and metabolites in the development of CRC are very active. However, many unknown and the unanswered questions remain. Better understanding on the molecular mechanisms and signaling pathways induced by butyrate treatment will facilitate the development of new therapeutic means to cure CRC.

Acknowledgements

Abbreviations

CRC, colorectal cancer; SCFAs, short chain fatty acids; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; VEGF, vascular endothelial growth factor; NRP-1, neuropilin-1; Sp1, specificity protein 1; APC, adenomatous polyposis coli; GSK-3β, glycogen synthase kinase 3β; Tcf/Lef, T-cell factor/lymphocyte enhancer factor.

Competing Interests

The authors have declared that no competing interest exists.

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