Journal of
Gastroenterology and Hepatology Research

Online Submissions: http: //www.ghrnet.org/index./joghr/
doi: 10.17554/j.issn.2224-3992.2017.06.738

Journal of GHR 2017 October 21; 6(5): 2419-2424
ISSN 2224-3992 (print) ISSN 2224-6509 (online)

EDITORIAL

Putative Anti-Cancer Action of Aloe vera Via Butyrate Fermentation

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Conflict-of-interest statement: The authors declare that there is no conflict of interest regarding the publication of this paper.

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Received: October 5, 2017
Revised: October 10, 2017
Accepted: October 13, 2017
Published online: October 21, 2017

ABSTRACT

The efficacy of *Aloe vera* has been known for various health and disease-related conditions. Butyrate, a well-known epigenetic histone deacetylase inhibitor, is causally implicated in tumorigenesis and tumor angiogenesis. Butyrate was shown to induce cell cycle arrest, differentiation, and apoptosis in a variety of cancer cells. Dietary factors, microbiota composition, and microbiota metabolism are intimately intertwined in a complex network, highlighting the importance of intestinal functions for health maintenance. In the present review, we discuss aloe vera fermentation as a tumor-suppressive process generating microbial-derived butyrate.

Key words: *Aloe vera* gel; Butyrate fermentation; HDAC inhibitor; Human cancer cells inhibitor

Yagi A, Yu BP. Putative Anti-Cancer Action of Aloe vera Via Butyrate Fermentation. *Journal of Gastroenterology and Hepatology Research* 2017; **6(5)**: 2419-2424 Available from: URL: http: //www.ghrnet.org/index.php/joghr/article/view/2155

INTRODUCTION

Many studies have documented the capability of epigenetic regulators from dietary sources to modulate gene expression in cancer cells through covalent modification of DNA as well as histone and non-histone proteins. Histone acetyltransferases and histone deacetylases (HDACs) are among the most studied targets for chromatin remodeling, control of gene expression, and anticancer therapy. Many HDAC inhibitors are known to induce, among others, tumor cells apoptosis, growth arrest, differentiation, inhibition of angiogenesis, and immunogenicity[1].

Our earlier reports[2] summarized that aloe vera fermentation with endophytic microbiota (*Firmicutes*; *Bacillus* and *Lactobacillus* and *Fungus*) produces butyric acid and the extract stimulates anti-inflammatory activity via the suppression of reactive oxygen species as well as both Cox 1 and 2 enzymes. We further discussed the possible role of butyrate as HDAC inhibitor and insulin sensitizer for age-related atrophic muscle[3]. Based on these data, it is likely that fermented butyric acid by aloe vera not only provides energy source for host tissues, but also exerts the anti-inflammatory and apoptotic effect that may elicit the prevention of colorectal cancer and colitis. In the present review, we explore possible prophylactic contributions of butyrate on HDAC inhibition, autophagy, calorie restriction, neurological disorders, and human cancer cells.

EPIGENETIC EFFECT OF BUTYRATE ON HDAC INHIBITION

Butyrate has been shown to exhibit the protective effect on inflammatory diseases such as ulcerative colitis (UC) and inflammation-mediated colorectal cancer. Zimmerman *et al*[4] showed

that colonic mucosa from patients with UC exhibit increased signal transducer and activator of transcription 1 (STAT1) activation, and this STAT1 hyper-activation is correlated with increased T cell infiltration. Butyrate effectively inhibited IFN-r-induced STAT1 activation, resulting in inhibition of iNOS upregulation in human colon epithelial and carcinoma cells in vitro. Their data suggest that butyrate delivers a double-hit: induction of T cell apoptosis to eliminate the source of inflammation and suppression of IFN-r-mediated inflammation in colonic epithelial cells, to suppress colonic inflammation.

Recently, Li *et al*[5] reported that a set of unique genes that are activated only after butyrate treatment, based on the analysis of differentially expressed genes in the bovine epithelial cells using RNA sequencing technology. A complementary bioinformatics analysis of the functional category, pathway, and integrated network, using Ingenuity Pathway Analysis, indicated that these genes activated by butyrate treatment are related to major cellular functions, including cell morphological changes, cell cycle arrest, and apoptosis. These results revealed molecular insight on the butyrate-induced transcriptomic changes, and will accelerate the discerning of the molecular fundamentals of epigenetic regulation.

ROLE OF BUTYRATE AS AN INDUCER OF AUTOPHAGY BIOGENESIS AND TUMOR CELL APOPTOSIS

Short chain fatty acids (SCFAs) are the major by-products of bacterial fermentation of undigested dietary fibers in the large intestine. SCFAs, mostly propionate and butyrate, inhibit proliferation and induce apoptosis in colon cancer cells, but clinical trials have produced mixed results regarding the anti-tumor activities of SCFAs. Tang *et al*[6] demonstrated that propionate and butyrate induced autophagy in human colon cancer cells to dampen apoptosis whereas inhibition of autophagy potentiated SCFA induced apoptosis. The observations indicate that propionate-triggered autophagy serves as an adaptive strategy for retarding mitochondria-mediated apoptotic cell death, whereas application of an autophagy inhibitor, chloroquine is expected to enhance the therapeutic efficiency of SCFAs in inducing apoptosis of colon tumor cells. As it turns out, reactive oxygen species (ROS) play an important role in the process of apoptosis in many cell types. Brodska and Holoubek[7] evaluated the role of ROS in DNA-damaging agents (actinomycin D or decitabine), which induced apoptosis of leukemia cell line CML-T1 and normal peripheral blood lymphocytes. The possibility of synergistic interaction between histone deacetylase inhibitors, butyrate and suberoylanilide hydroxamic acid (SAHA) is also reported. Brodska and Holoubek found that in cancer cell line, ROS production significantly contributed to apoptosis triggering, while in normal lymphocytes treated by cytostatic or cytotoxic drugs, necrosis as well as apoptosis occurred and large heterogeneity of ROS production was measured. Combined treatment with HDAC inhibitor did not potentiate actinomycin D action, whereas the combination of decitabine and SAHA brought synergistic ROS generation and apoptotic features in CML cell line. Zhang *et al*[8] investigated whether butyrate induced endoplasmic reticulum stress-mediated autophagy, and whether there was crosstalk between autophagy and the butyrate-induced apoptotic response in human colorectal cancer cells. These results suggested that butyrate-induced autophagy was mediated by endoplasmic reticulum stresses, and that preventing autophagy by blocking the endoplasmic reticulum stress response enhanced butyrate-induced apoptosis, thus providing novel insights into the anti-tumor mechanisms of butyric acid. Pant *et al*[9] showed that sodium butyrate induced autophagy via ROS in hepatoma cells. Butyrate (0-6 mM) incubation significantly increased intracellular ROS levels (45.25% compared to control), which in turn inhibited phosphorylation of akt and mTOR, leading to the upregulation of autophagy proteins, followed by the increased autophagosome formation (34.4% compared to control cells).The data showed that butyrate induced ROS, which in turn induced autophagy via inhibition of akt/mTOR pathway.

MODIFICATION OF GUT MICROBIOTA BY CALORIE RESTRICTION (CR)

Several recent studies reported modified gut microbiota by CR (Figure 1). The modifications were found in both microbial composition of human by Ruiz *et al*[10] and structural modification of experimental mice by Zhang *et al*[11], and it is viewed that these changes in microbial status by CR may well be one of the major contributing factors for its well-known resistive action against tumorigenesis promotion of health status and life span.

Regulation of histone modifications, such as histone acetylation and deacetylation, are well documented by CR by Li *et al*[12]. Evidences are produced by numerous studies performed on class III HDAC enzymes (Sir2 in yeast and its mammalian sirtuin orthologs) in multiple model organisms, wherein activation of this enzyme class has frequently been associated with CR and life span extension, suggesting that there may be a protective mechanism conferred by deacetylation during aging. In our earlier reports[13,14], we discussed that the healthy-promoting potential of a balanced gut microbiota status modulated by CR posits a possible close link between gut microbiota and healthy aging, and that butyrate effectively affects for insulin sensitivity, sirtuin activation through HDAC inhibitors on slowing aging process.

INHIBITORY ACTION OF BUTYRATE ON GLIOMA CELLS, AMYOTROPHIC LATERAL SCLEROSIS

The dietary natural source of butyrate through high fiber diet or butyrate produced by fermentation of non-digestive, such as acemannan in *Aloe vera* gel, is highly appealing approach to prevent a simple and selectively lower brain dysfunctions. Insulin receptor and its regulation by butyrate and other short chain fatty acids (SCFAs) were studied with a rat glioma cell line, C6, by Montiel

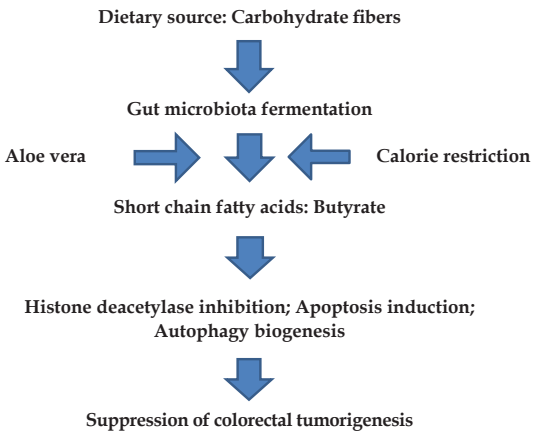

Figure 1 Anti-tumorigenesisi of Aloe vera via butyrate fermentation.

et al[15]. Intact C6 cells bind 125I-insulin in a rapid, reversible and specific manner. The results indicated that C6 cells bind insulin specifically, and that butyrate and other SCFAs down-regulate surface insulin-receptor number. Sawa *et al*[16] investigated the effects of HDAC inhibitors such as sodium butyrate (SB) and trichostatin A (TSA) on expression of vascular endothelial growth factor (VEGF) by human glioblastoma T98G, U251MG, and U87MG cells. The glioblastoma cells secreted three VEGF isomers, VEGF (189), (165), and (121), although the expression levels of VEGF differed between the cell types. Treatment with either 5mM SB or 100ng/ml TSA reduced VEGF secretion in conditioned media and reduced VEGF mRNA expression. Data suggest that HDAC inhibitor reduce VEGF secretion and modulate the expression of the other VEGF family members, and therefore it likely inhibits angiogenesis in glioblastoma tissues.

Malignant gliomas, the most common primary brain tumor, are known to invade the surrounding normal brain tissue. This often results in incomplete surgical resection, local recurrence, and poor responses to multimodal therapeutic interventions, such as radiotherapy and chemotherapy. Many glioma cells are known to be resistant to TNF-related apoptosis-inducing ligand (TRAIL), despite their expression of the TRAIL receptor. Therefore, a new therapeutic strategy should be developed to restore TRAIL-induced apoptotic potency to glioma cells. TRAIL is considered to be a promising candidate for anti-cancer therapy, since the cytotoxic activity of TRAIL is relatively selective for cancer cells compared to normal cells both *in vitro* and in vivo. Kim *et al*[17] found that in TRAIL-resistant glioma cells, co-treatment with nontoxic doses of butyrate and TRAIL resulted in a marked increase of TRAIL-induced apoptosis. This combined treatment was also cytotoxic to glioma cells overexpressing Bcl-2 or Bcl-xL (anti-apoptotic protein), but not to normal human astrocytes, thus offering an attractive strategy for safely treating resistant gliomas. Co-treatment with butyrate facilitated completion of proteolytic processing of procaspase-3 that was partially blocked by treatment with TRIAL alone. The authors found that treatment with butyrate significantly decreased the protein levels of survivin and X-linked inhibitor of apoptosis protein (XIAP), two major caspase inhibitors. Overexpression of survivin and XIAP attenuated butyrate-stimulated TRAIL-induced apoptosis, suggesting its involvement in conferring TRAIL resistance to glioma cells. Furthermore, the kinase activities of Cdc2 and Cdk2 (cyclin-dependent kinase is encoded by the Cdc2 gene) were significantly decreased following butyrate treatment, accompanying downregulation of cyclin A and cyclin B, as well as upregulation of p21. Cdc2-mediated downregulation of survivin and XIAP by butyrate may contribute to the recovery of TRAIL sensitivity in glioma cells.

Glioblastoma multiform (GBM) is the most common and aggressive type of malignant brain tumor. Patients afflicted with this disease unfortunately have a very poor prognosis, and fewer than 5% of patients survive for 5 years from the time of diagnosis. One such class of drugs that have generated great enthusiasm for the treatment of numerous malignancies, including GBM, is HDAC inhibitors. Preclinical data demonstrated the efficacy of various HDAC inhibitors as anticancer agents, with the greatest effects shown when HDAC inhibitors are used in combination with other therapies. As a result of encouraging preclinical data, numerous HDAC inhibitors are under investigation in clinical trials, either as monotherapies or in conjunction with other treatment such as chemotherapy, biologic therapy or radiation therapy. Shabason *et al*[18] presented a patient with GBM, and then discussed the pathogenesis, epidemiology and current treatment options of GBM. Finally, the authors examined the translation of preclinical studies that have demonstrated HDAC inhibitor; valproic acid, along with temozolomide and radiation therapy for the treatment of GBM.

To achieve an optimal therapeutic effect against glioblastoma, Guo *et al*[19] tested a strategy that combines baculovirus-mediated transfer of the p53 tumor suppressor gene with the use of SB. The authors observed a synergistic effect of the combination of the two treatments in provoking apoptosis in glioblastoma cells with mutant p53. In a mouse glioma xenograft model, the tumor inhibitory effect of baculovirus-expressed p53 was significantly enhanced by co-administration of SB. These findings suggest a new approach to treat glioblastoma using baculovirus-mediated gene transfer in combination with administration of HDAC inhibitor.

Nor *et al*[20] reported that the HDAC inhibitor SB markedly increases cell death and reduces colony formation in human medulloblastoma (MB) cell lines. SB increased the mRNA expression of Gria2, a neuronal differentiation maker, in D283 and DAOY cells and reduced the number of neuro-spheres in D283 cell cultures. SB reduced the viability of D283 cells when combined with etoposide. The data show that SB displays pronounced inhibitory effects on the survival of human MB cells and suggest that SB might potentiate the effects of etoposide. The study suggests that HDAC inhibition might promote the neuronal differentiation of MB cells and provides the evidence that an HDAC inhibitor might suppress the expansion or survival of MB cancer stem cells. Kim *et al*[21] found that SB or trichostatin A robustly protected against ischemia-induced loss of oligodendrocytes detected by confocal microscopy of myelin basic protein (MBP) immunostaining in the ipsilateral sub-ventricular zone, stratum, corpus callosum, and frontal cortex seven days post-permanent middle cerebral artery occlusion. Co-localization of 5-BrdU (+) and MBP (+) cells after SB treatment suggested the occurrence of oligodendrogenesis. SB also strongly upregulated vascular endothelial growth factor (VEGF), which plays a major role in neurogenesis, angiogenesis, and functional recovery after stroke. These SB-induced effects were markedly suppressed by blocking the TrkB signaling pathway with K252a. Permanent middle cerebral artery occlusion–induced activation of microglia and macrophages/monocytes-which has been linked to white matter injury-was robustly suppressed by SB in a K252a-sensitive manner. The results suggest that post-insult treatment with HDAC inhibitors is a rational strategy to mitigate white matter injury following ischemic stroke. Park and Sohrabji[22] reported that SB treatment reduced infarct volume and ameliorated sensory motor impairment in middle-aged female rats, when measured at 2 and 5 days post middle cerebral artery occlusion. At the early acute phase (2 days post stroke), SB treatment decreased brain lipid peroxides, and reduced serum levels of GFAP, a surrogate marker of blood-brain barrier (BBB) permeability. SB also reduced expression of inflammatory cytokine IL-1 in circulation and IL-18 in the ischemic hemisphere. These data provide the evidence that delayed (> 6 h) SB treatment post-stroke is neuroprotective in older female rats. Additionally, these data show that in addition to its well-known anti-inflammatory actions, SB may exert a biphasic effect after stroke, operating initially to reduce BBB permeability and oxidative stress in the brain, and later, elevating IGF-1 expression in peripheral tissues. The post-natal period is crucial for the development of gastrointestinal function. Cossais *et al*[23] characterized the postnatal evolution of enteric glial cells (EGC) phenotype in the colon of rat pups and studied the effect of SCFAs on their maturation. The authors showed an increased expression of the glial markers; glial fibrillary acid protein and calcium-binding protein S100β, during the

first postnatal week. As demonstrated by immunohistochemistry, a structured myenteric glial network was observed at 36 days in the rat colons. Butyrate inhibited EGC proliferation in vivo and *in vitro* but had no effect on glial marker expression. These results indicate that the EGC myenteric network continues to develop after birth, and luminal factors such as butyrate endogenously produced in the colon may affect this development. Stilling *et al*[24] provided a critical review of the literature on butyrate and its effects on multiple aspects of host physiology with a focus on brain function and behavior. The authors highlight the neuropharmacology of butyrate as follows: Butyrate is produced by specific gut bacteria, mainly in the colon, and is taken by the host. Butyrate affects multiple host physiological processes via specific transporters/receptors and as an HDAC inhibitor. Supra-physiological doses of butyrate exert potent neuro-pharmacological effects, facilitating synaptic tagging and capturing. Physiological levels of butyrate may influence the brain indirectly via regulating the immune system and vagus nerve activity. Microbiota–derived volatile butyrate may be involved in host behavior including social communication.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons. Zhang *et al*[25] used G93A transgenic mice as a model of human ALS. The mice were given 2% butyrate, a natural bacterial product, in the drinking water. In both ALS mice and intestinal epithelial cells cultured from humans, butyrate treatment was associated with decreased aggregation of the G93A superoxide dismutase 1 mutated protein. The findings highlight the complex role of the gut microbiome and intestinal epithelium in the progression of ALS and present butyrate as a potential therapeutic reagent for restoring ALS-related dysbiosis.

BUTYRATE INHIBITION OF HUMAN CANCER CELLS

Butyrate has been suggested to reduce colon cancer risks by suppressing the proliferation of tumor cells and to induce glutathione S-transferase (GSTs) in tumor cell lines, which may contribute to the detoxification of dietary carcinogens. Pool-Zobel *et al*[26] investigated the gene expression of drug metabolism genes in primary human colon tissue, premalignant LT97 adenoma and HT29 tumor cells cultured in an appropriate medium ± butyrate. The authors concluded that low GST expression levels were favorably altered by butyrate. An induction of the toxicological defense system possibly contributes to chemo-preventive properties of butyrate. Kim[27] investigated the role of reactive oxygen species (ROS) on human hepatocyte differentiation using SB-induced hepatocyte differentiation model. It was shown that intercellular ROS production was increased by SB. SB-induced urea production was significantly decreased with antioxidant treatment and SB-induced ornithine transcarbamylase and albumin transcription were also attenuated with antioxidant treatment. SB-induced increase in apoptosis was significantly inhibited by antioxidant treatment. ROS produced during the process of SB-induced human hepatocyte differentiation augments hepatocyte differentiation and apoptosis. Domokos *et al*[28] investigated the role of ROS induced by butyrate in tumor cells, comparing HT29R, an HT29-derived human colon cancer cell line refractory to butyrate-induced cell differentiation but highly sensitive to cell death, with the differentiation-positive HT29-12 and HT29-21 cell lines (exhibiting low sensitivity to butyrate-induced cell line death), with respect to levels of butyrate-induced ROS, and H_2O_2. A dose-dependent increase of ROS was induced in HT29R, but not in HT29-12 and HT29-21 cells, where, in contrast to HT29R, a dose-dependent increase of H_2O_2 release was induced by butyrate. The mode of butyrate-induced cell death in TH29R cells was of mixed type with necrosis predominating, which, however, switched to apoptosis as the major type of cell death in the presence of the drugs, resveratrol or cyclosporine A. The results suggest that ROS induced by butyrate in HT29R cells are products of cell death, while H_2O_2 induction in HT29-12 and HT29-21 cells is functionally related to cell differentiation. Dietary components influence the risk of human colon cancer including colon cancer through diverse mechanisms, which include the activation or inhibition of autophagy (type II programmed cell death (PCD). Tang *et al*[29] demonstrated that propionate and butyrate induce autophagy in human colon cancer cells to dampen apoptosis, whereas inhibition of autophagy potentiates SCFA-induced apoptosis. Propionate-induced autophagy originates from mitochondria defects associated with cellular ATP depletion and SOD generation, both of which contribute to AMPK activation and consequential mTOR inhibition. Remarkably, when autophagy is suppressed through either pharmacological or genetic approach, the colon cancer cells become sensitized toward propionate-induced apoptotic cell death (type I PCD). The authors reported that the novel role of SCFAs in orchestrating two type of programmed cell death. To define the potential mechanisms involved in butyrate-induced apoptosis, Fung *et al*[30] compared the global protein expression between HCT116 cells treated with butyrate and HCT116-BR cells, which are less sensitive to butyrate's apoptotic effects. The analysis has revealed that butyrate elicits an endoplasmic reticular stress response and that an adaptive cellular response to this stress may provide a mechanism for the development of resistance to butyrate-induced apoptosis in the HCT116-BR cells.

Oxidative stress plays an important role in the pathogenesis of inflammatory bowel disease, including Crohn's disease (CrD). High levels of reactive oxygen species (ROS) induce the activation of the redox-sensitive nuclear transcription factor kappa-β (NF-kβ), which in turn triggers the inflammatory mediators. Butyrate decreases pro-inflammatory cytokine expression by the lamina propria mononuclear cells in CrD patients via inhibition of NF-kβ activation. Russo *et al*[301] suggested that butyrate controls ROS mediated NF-kβ activation and thus mucosal inflammation in intestinal epithelial cells and in CrD colonic mucosa by triggering intracellular antioxidant defense systems.

Epidemiological studies suggest that colonic production of butyrate and estrogen may be involved in human susceptibility to colorectal cancer (CRC). Estrone (E1) can be produced by the aromatase pathway during the conversion of androstenedione (A) to E1. Rawluszko *et al*[32] studied the effect of butyrate on the CYP19A1 transcript and protein levels and on the conversion of A to E1 in HT29, DLD-1 and LoVo CRC cells. The authors demonstrated that although butyrate exhibited a protective role in CRC development, this compound may reduce aromatase activity and the production of E1 in colon cancer cells. Yamamura *et al*[33] investigated the effect of butyrate on the synthesis of anti-angiogenic and lymph-angiogenic factors in oral squamous cell carcinoma. Butyrate inhibits expression of lymph-angiogenic factors in HSC-3 cells. Therefore, it seems likely butyrate may have the potential as an anti-metastatic pro-drug for oral cancer. Wei *et al*[34] reported that high-fiber diet and butyrate significantly inhibited the growth lymphoma tumors. Butyrate induced apoptosis of lymphoma tumor cells and significantly up-regulated histone 3 acetylation level and target genes such as Fas, P21, P27.The results unraveled an instrumental role of fiber diet and their metabolites on lymphoma tumor and demonstrated an

intervention potential on the prevention and therapy of lymphoma. Xing *et al*[35] investigated the antioxidant effect of sodium butyrate on HepG2 cells under H_2O_2-induced oxidation stress. Butyrate (0.3 mM) attenuated cell death and accumulation of reactive oxygen species and improved multiple antioxidant parameters in H_2O_2-injured HepG2 cells. Butyrate inhibited glycogen synthase kinase-3β by increasing the p-GSK-3β (Ser9) level and promoted nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2), which increased the expression of downstream antioxidant enzymes. Butyrate protected HepG2 cells against oxidation stress by modulating Nrf2 pathway activity and mitochondrial function. Huang *et al*[36] investigated the effect of maternal dietary butyrate supplementation on energy metabolism and mitochondrial biogenesis in offspring skeletal muscle and the possible mediating mechanisms. Their results indicated that maternal butyrate supplementation during the gestation and lactation periods influenced energy metabolism and mitochondrial biogenesis through the G-protein-coupled receptors and PPAR-coactivator-1α pathway in offspring skeletal muscle at weaning. Butyrate role in nasopharyngeal carcinoma (NPC), an endemic malignant disease in South China and South Asia, was investigated by Huang *et al*[37]. The results revealed that enhanced store-operated Ca^{2+} entry and activated mitochondrial apoptosis axis may account for the mechanisms of cytotoxicity of butyrate in NPC cells, and that butyrate serves as a promising chemotherapeutic agent in NPC therapy. Recent emerging data on non-coding micro RNA (miRNA) for RNA silencing and gene expression modulation revealed their wide involvement in many cellular processes, like tumorigenesis and cell death. In this context, it is interesting to note that miR-22 in butyrate-mediated ROS release and induction of apoptosis was determined in hepatic cells by Pant *et al*[38]. Intracellular expression of miR-22 was increased when the Huh 7 cells were incubated with butyrate. Over-expression of miR-22 or addition of butyrate inhibited expression of anti-oxidative SIR-T deacetylase, thus enhanced the ROS production. Incubation of cells with anti-miR-22 confirmed the pro-oxidative nature of butyrate. Butyrate induced apoptosis via ROS production, cytochrome c release and activation of caspase-3, whereas addition of N-acetyl cysteine or anti-miR-22 reversed these butyrate-induced effects. Therefore, it is concluded that butyrate modulated both apoptosis and proliferation via miR-22 expression in hepatic cells.

SUMMARY

Butyrate has been shown to affect various cancer cells. Butyrate exerts its anti-cancerous effects by several mechanisms and has led to successful outcomes in phase I and II clinical trials. Accumulated data show that tumor targeting bacteria as food metabolites is a feasible strategy for tumor-selective therapy. Therefore, it is proposed that nonpathogenic anaerobic butyrate-producing bacteria with *Aloe vera* may be a versatile tool in tumor therapy as these bacteria can grow in anoxic and hypoxic regions of tumors and influence tumor cells by producing potent butyric acid. The probiotic/prebiotic strategies can modulate an endogenous HDAC inhibitor, butyrate, for anti-cancer chemoprevention without the adverse effects. Tumor targeting with nonpathogenic anaerobic bacteria with a higher capacity to produce butyric acid could be the direction of future research.

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