

Molecular Pathways: Gene–Environment Interactions Regulating Dietary Fiber Induction of Proliferation and Apoptosis via Butyrate for Cancer Prevention

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Abstract

Gene–environment interactions are so numerous and biologically complicated that it can be challenging to understand their role in cancer. However, dietary fiber and colorectal cancer prevention may represent a tractable model system. Fiber is fermented by colonic bacteria into short-chain fatty acids such as butyrate. One molecular pathway that has emerged involves butyrate having differential effects depending on its concentration and the metabolic state of the cell. Low–moderate concentrations, which are present near the base of colonic crypts, are readily metabolized in the mitochondria to stimulate cell proliferation via energetics. Higher concentrations, which are present near the lumen, exceed the metabolic capacity of the colonocyte. Unmetabolized butyrate enters the nucleus and functions as a histone deacetylase (HDAC) inhibitor that epigenetically regulates gene expression to inhibit cell proliferation and induce apoptosis as the colonocytes exfoliate into the lumen. Butyrate may therefore play a role in normal homeostasis by promoting turnover of the colonic epithelium. Because cancerous colonocytes undergo the Warburg effect, their preferred energy source is glucose instead of butyrate. Consequently, even moderate concentrations of butyrate accumulate in cancerous colonocytes and function as HDAC inhibitors to inhibit cell proliferation and induce apoptosis. These findings implicate a bacterial metabolite with metaboloepigenetic properties in tumor suppression. *Clin Cancer Res*; 20(4); 799–803. ©2013 AACR.

Background

Considering that cancer susceptibility is determined by numerous gene–environment interactions, dietary factors are believed to alter the risk of cancer in general and colorectal cancer in particular. One of the most extensively studied dietary factors has been fiber, which is defined as the edible part of plants or their extracts, or analogous carbohydrates, that are resistant to digestion and absorption in the small intestine, but are used after partial or complete fermentation in the colon by resident microbiota (1). Fiber includes polysaccharides (e.g., resistant starch, cellulose, hemicellulose, pectins, and gums), oligosaccharides, and lignins. As human populations have shifted away from traditional, high-fiber diets toward processed foods containing refined sugars, colorectal cancer incidence has increased markedly. Colorectal cancer is now the third most diagnosed cancer in both men and women in the United

States, and it is also the third most deadly (2). This trend of increasing colorectal cancer incidence is particularly evident in China and developing countries that have rapidly adopted Western diets in recent years (3). The correlation between decreased fiber consumption and increased colorectal cancer incidence is also evident in developing countries because colonoscopies are being performed on a limited basis; in contrast, widespread screening and removal of precancerous adenomas in the United States has coincided with a recent plateau or slight decline in colorectal cancer incidence. More rigorous prospective cohort studies have also been performed and have yielded both positive and negative results (4–9). Nevertheless, the most recent expert report from the World Cancer Research Fund and the American Institute of Cancer Research has upgraded the evidence from probable to convincing that fiber has a protective effect.

It has not been established how dietary fiber might protect against colorectal cancer, but there are two general models that are not mutually exclusive (Fig. 1). First, insoluble fiber bulks luminal contents and speeds colonic transit to minimize the exposure of the colonic epithelium to ingested carcinogens such as nitrosoamines from charred meat. Second, the fact that bacteria in the lumen of the colon ferment soluble fiber into short-chain fatty acids (SCFA) is probably important. The most abundant SCFAs, such as acetate, propionate, and butyrate, are present in the

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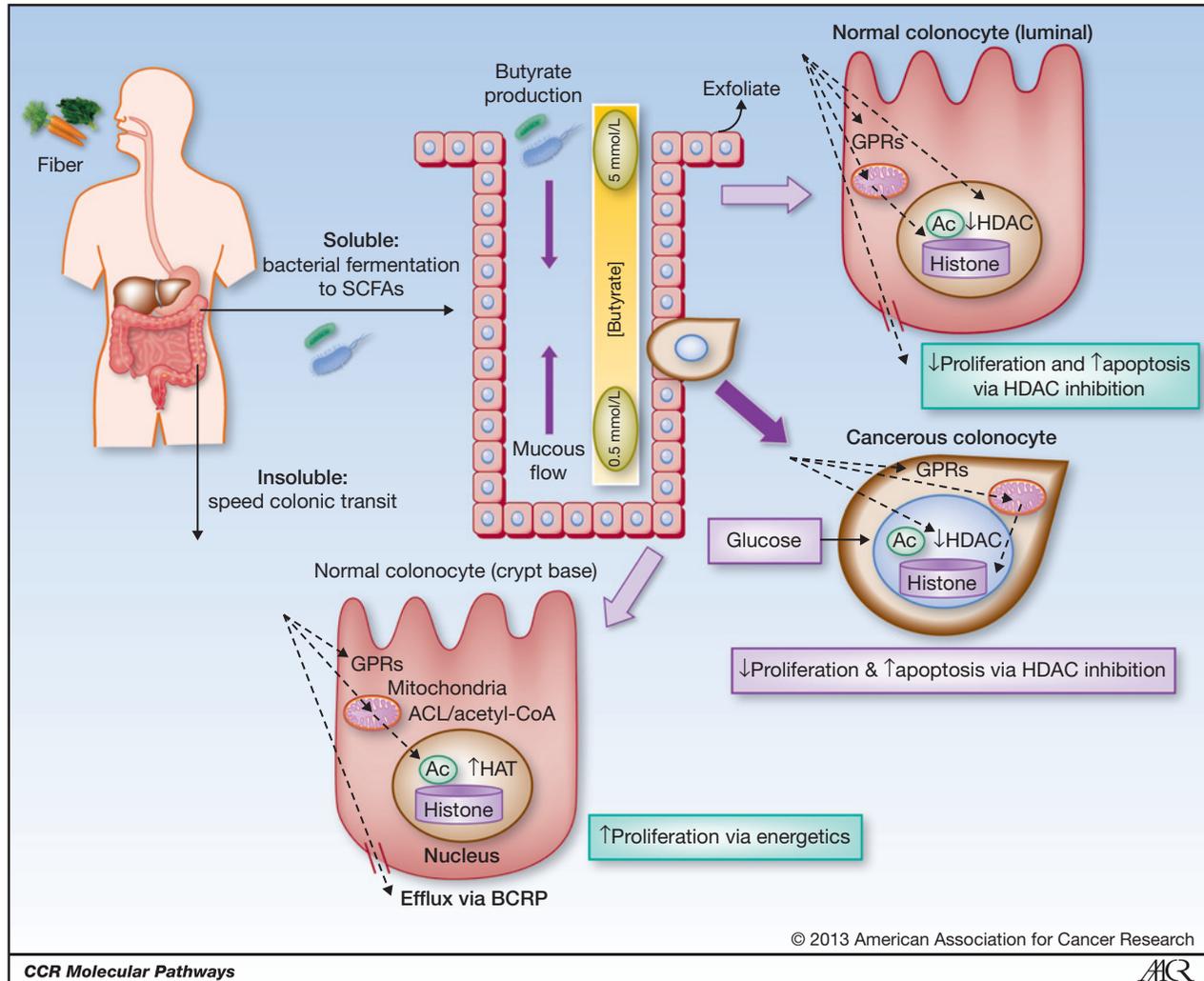


Figure 1. A model of how fiber might protect against colorectal cancer. Left, first, insoluble fiber bulks luminal contents and speeds colonic transit to diminish the exposure of colonocytes to ingested carcinogens. Second, soluble fiber is fermented by bacteria into SCFAs, including butyrate. Center, butyrate is believed to be present in a gradient with ≥ 5 mmol/L in the lumen and ≤ 0.5 mmol/L at the crypt base. This is due to bacterial density and fermentation being highest in the lumen, and the diffusion of butyrate into the crypt is impeded by the upward flow of mucous produced by goblet cells. Right, for normal colonocytes near the base of the crypt (bottom), butyrate is readily metabolized in the mitochondria (thick-dashed arrow). This supports cell proliferation via energetics but also increases histone acetylation via an ACL-acetyl-CoA-HAT mechanism. Intracellular butyrate levels are regulated via BCRP-mediated efflux, and butyrate can also function as an agonist of GPRs. For normal colonocytes near the lumen (top), the butyrate concentration exceeds the oxidative metabolic capacity of the cell. Therefore, higher levels of butyrate accumulate in the nucleus (thick dashed arrow) to increase histone acetylation via HDAC inhibition. This epigenetically regulates gene expression to inhibit cell proliferation and induce apoptosis as these cells exfoliate into the lumen. For cancerous colonocytes (middle), the ability of butyrate to accumulate as an HDAC inhibitor is particularly pronounced for two reasons. First, due to the Warburg effect, the preferred energy source is switched from butyrate to glucose. Second, butyrate is able to accumulate more efficiently because BCRP is often silenced so that there is less efflux.

lumen at very high (mmol/L) concentrations and serve as an energy source for many species of bacteria that inhabit the colon as well as the host (10). Butyrate is selectively taken up by the colonic epithelium (via MCT1, SMCT1, and other transporters) and provides colonocytes with approximately 70% of their energy (11) and is required for energy homeostasis (12, 13), whereas acetate and propionate are primarily transported to muscle and liver tissue, respectively (14). Butyrate is a particularly good candidate for colorectal cancer prevention not only because it is metabolized by colonocytes but also because it has more potent activity as a

tumor suppressor and a histone deacetylase (HDAC) inhibitor than the other SCFAs or any other known bacterial metabolites (15).

Tumor-suppressive effects of butyrate involve the Warburg effect and HDAC inhibition

Butyrate has been implicated in cancer prevention on the basis of >100 published studies demonstrating that it inhibits cell proliferation and/or stimulates apoptosis in a variety of tumor-derived cell lines (16–19). However, the bioavailability of butyrate is primarily restricted to the colon

because this is where it is produced and it is readily metabolized by colonocytes. Therefore, experiments with colorectal cancer cells lines are probably the only ones that are physiologically relevant. Because butyrate is a fatty acid and the preferred energy source of normal colonocytes, it undergoes β -oxidation in the mitochondria and relatively little accumulates in the nucleus (Fig. 1; refs. 12, 20). In contrast, glucose is the preferred energy source of cancerous colonocytes (Fig. 1), which increase their glucose uptake by >10-fold (via increased expression of glucose transporters [GLUTs]) and rely heavily on aerobic glycolysis with a concomitant decrease in oxidative metabolism within the mitochondria. This metabolic shift, referred to as the Warburg effect, occurs in a vast majority of cancers, including colorectal cancer, and is the basis of $2[^{18}\text{F}]\text{fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET)}$ used for tumor imaging in the clinic (21). *AKT*, phosphoinositide 3-kinase (*PI3K*), *KRAS*, and other mutations affect metabolism and contribute to the Warburg effect, which is selected for cancer cells. This is because the Warburg effect serves as a conduit for diverting carbons into biosynthetic pathways that are used by rapidly dividing cells to double their biomass (22, 23). As a consequence of the Warburg effect and metabolic reprogramming in cancer cells, butyrate is metabolized to a lesser extent and accumulates in the nucleus of cancerous colonocytes, in which it functions as an HDAC inhibitor to epigenetically regulate gene expression (Fig. 1; ref. 20).

HDAC inhibitors such as butyrate are global transcriptional regulators because they increase histone acetylation to alter the conformation/position of nucleosomes, which consist of 147-bp segments of DNA wrapped around histone octamers (two copies each of H2A, H2B, H3, and H4), and are the most fundamental unit of chromatin. Butyrate induces histone acetylation at the promoters of many genes. Some direct targets, such as the *p21* cyclin-dependent kinase inhibitor and the proapoptotic *BAX* and *FAS* genes, contribute to functional changes in cell proliferation and apoptosis (16–19). Butyrate can also promote cell differentiation by regulating genes such as the mucins. Thus, one may consider butyrate as a candidate "tumor-suppressor metabolite" analogous to the 2-hydroxyglutarate "oncometabolite," which is present at high levels only in *IDH* mutant gliomas and leukemias, in which it inhibits the TET and JmJc families of deoxygenases that demethylate DNA and histones to alter epigenomic profiles (24).

Butyrate also has potent anti-inflammatory effects

Butyrate was the first HDAC inhibitor to be identified (15), and this mechanism is also known to regulate the transcription of certain cytokine genes, including components of the $\text{IFN-}\gamma$, $\text{TNF-}\alpha$, and $\text{NF-}\kappa\beta$ signaling pathways, which are involved in both inflammation and cancer (16–18). These pathways are perturbed in intestinal epithelial cells and immune cells from individuals with Crohn disease and ulcerative colitis, and butyrate enemas strongly ameliorate colonic inflammation associated with these inflammatory bowel diseases (IBD) in

both rodent models and human patients (16). It is noteworthy that patients with colitis have a 10-fold increased risk of colorectal cancer (25). These observations are consistent with the well-accepted idea that inflammation exacerbates tumorigenesis, which is an emerging hallmark of cancer (26). This raises the possibility that butyrate might not only function autonomously in cancer cells but may also affect immune cells in the tumor microenvironment to influence colorectal cancer. Interestingly, activated lymphocytes undergo a metabolic shift similar or identical to the Warburg effect to support their rapid proliferation (27, 28), which can exceed that of a cancer cell and achieve doubling time of approximately 8 hours. Therefore, butyrate is not expected to be readily metabolized by activated T cells or B cells but should instead function as an HDAC inhibitor similar to cancer cells.

Butyrate increases histone acetylation by two distinct mechanisms

Recent work has shown that the role of butyrate in histone acetylation is more complicated than previously appreciated (20). When cells were exposed to a relatively low dose of butyrate (0.5 mmol/L), the intracellular concentrations of butyrate did not reach levels compatible with it acting as an HDAC inhibitor. The levels were only 0.04 to 0.40 relative to the IC_{50} , regardless of whether or not the cells were undergoing the Warburg effect; yet this 0.5 mmol/L dose stimulated histone acetylation and affected cell proliferation. At this dose, butyrate undergoes β -oxidation in the mitochondria, and this leads to acetyl-CoA condensing with oxaloacetate to form citrate in the first step of the tricarboxylic acid cycle. Citrate can exit the mitochondria, and the enzyme ATP citrate lyase (ACL) converts it to acetyl-CoA in the cytosol and nucleus (Fig. 1; ref. 29). Cytosolic acetyl-CoA is crucial for lipid biosynthesis, whereas nuclear acetyl-CoA is as an essential cofactor for histone acetyltransferases (by serving as the acetyl group donor; ref. 29). To demonstrate that butyrate can increase histone acetylation by this mechanism, flux experiments were performed in which ^{13}C -butyrate was added to cells, and ^{13}C -acetyl groups were detected on histones in an ACL-dependent manner (via depletion of ACL in RNA interference experiments) using liquid chromatography/tandem mass spectrometry (LC/MS-MS; ref. 20). Transcriptome-profiling experiments in siACL cells versus siMock cells identified genes that were regulated in an ACL-dependent manner and ACL-independent manner, respectively. Butyrate dosage influenced the utilization of each mechanism. At a 0.5 mmol/L dose, the ACL-acetyl-CoA-HAT mechanism (i.e., ACL dependent) was the prominent one, accounting for 75% of the genes that were upregulated compared with cells not treated with butyrate. However, the HDAC inhibition mechanism (i.e., ACL independent) became more prevalent at 2 mmol/L and was the prominent one at 5 mmol/L, accounting for 75% of the upregulated genes. This mechanistic shift can be explained by the observation that these cells reach their oxidative

metabolic capacity at 1 to 2 mmol/L doses of butyrate (30, 31). Therefore, when the dosage exceeds this range, butyrate molecules are not metabolized in the mitochondria and accumulate as HDAC inhibitors in the nucleus.

The transcriptome-profiling experiments also showed that the ACL-dependent genes at 0.5 mmol/L were enriched for cell proliferation functions, whereas the ACL-independent genes at 5 mmol/L were enriched for apoptosis (20). Because butyrate is believed to be present in a gradient with higher levels in the lumen and lower levels at the base of the crypt (due to the upward flow of mucous produced by goblet cells; Fig. 1), butyrate may promote homeostasis of the colonic epithelium, which proliferates rapidly but is continuously turned over (Fig. 1; ref. 20). Lower butyrate concentrations near the base of the crypts correlate with the position of proliferative cells, whereas higher butyrate concentrations in the lumen correlate with the position of cells that undergo apoptosis as they exfoliate into the lumen (Fig. 1).

Because colorectal cancer cells undergo the Warburg effect, even lower doses of butyrate will be metabolized to a lesser extent and will function as an HDAC inhibitor to promote apoptosis rather than proliferation, which is consistent with its tumor-suppressive effects. The differential effect of butyrate in normal versus cancerous colonocytes is made even more pronounced by differences in butyrate efflux. An ATP-binding cassette transporter in the multidrug resistance family, named breast cancer resistance protein 1 (BRCP), transports butyrate out of cells and modulates the ability of butyrate to affect cell proliferation (32). BRCP is expressed at high levels in the normal intestine but is downregulated in IBDs and in colorectal cancer cell lines compared with nontumoral intestinal epithelial cells (32, 33). This likely contributes to higher levels of butyrate in cancerous colonocytes and further increases HDAC inhibition.

Butyrate is also an agonist for G protein-coupled receptors

Butyrate functions in multiple cell compartments. In addition to being metabolized in the mitochondria (primarily for energetics but also for histone acetylation via the ACL-acetyl-CoA-HAT mechanism) and the nucleus (as an HDAC inhibitor), butyrate can also function at the cell surface as an agonist for certain G protein-coupled receptors (GPR), which function via the cAMP and phosphatidylinositol signaling pathways to have many effects. Butyrate binds to GPR109A, which is highly expressed on the apical membrane of colonic epithelial cells (34). GPR109A is silenced in human colorectal cancer, a mouse model of colorectal cancer, and colorectal cancer cell lines (34). GPR41 and GPR43 are bound by several SCFAs, including butyrate. GPR41 is intriguing because it mediates microbiota-induced effects on host metabolism including adiposity and the production of leptin and peptide YY that regulate feeding behavior (35). GPR43 is intriguing because it may be a tumor suppressor based on reduced or abolished expression in

colon adenomas and colorectal cancer cell lines (36). Consistent with butyrate having anti-inflammatory functions relevant to cancer prevention, butyrate and other SCFAs can signal through GPR43 on the surface of regulatory T cells to stimulate their expansion in response to fiber (37, 38).

Clinical-Translational Advances

Recent microbiome studies, which combine next-generation sequencing with computational analyses (39, 40), have revealed many differences in the relative abundance of commensal gut bacteria between individuals (41, 42). In some complex diseases, including cancer, significant differences between cases and controls have been reported. For example, at least two studies have reported that colorectal cancer cases have fewer butyrate-producing bacteria than controls (43, 44). This raises the possibility that some of the conflicting results from epidemiology studies that have investigated whether dietary fiber protects against colorectal cancer might be due to microbiome differences between participants. To reconcile these conflicting results, it would be useful to integrate epidemiology results with data from genome-wide association studies and microbiome studies because the efficacy of the fiber effect is probably influenced by both genetics and gut microbiota. This approach might make it possible to discriminate between those individuals who respond favorably to increased fiber consumption and those who do not.

A number of synthetic HDAC inhibitors are currently in phase III clinical trials or have already received approval by the U.S. Food and Drug Administration as chemotherapeutic agents, primarily for the treatment of hematopoietic malignancies, but are known to have adverse effects (45). A chemoprevention strategy involving diet (prebiotics fiber) and possibly dietary microbiota supplementation (probiotics butyrate-producing bacteria) is attractive because it would involve an endogenous HDAC inhibitor (butyrate) that does not have any known adverse effects (46). As opposed to synthetic HDAC inhibitors, which are delivered systemically, butyrate is produced only in the colon. This specificity precludes collateral damage from occurring in other tissues. Furthermore, unlike synthetic HDAC inhibitors, butyrate is a fatty acid readily metabolized by normal cells and is specifically targeted to cancer cells (in which it accumulates) because of the Warburg effect. Another translational possibility is to fortify foods with butyrate, but this would need to make use of a butyrate derivative such as tributyrin that it is not as readily absorbed in the upper gastrointestinal tract and yields high levels of butyrate in the colon (13, 46). However, as a practical consideration, it would be necessary to use nanoparticles or another approach to mask the unpleasant odor of butyrate derivatives.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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