

Current topics

SLC5A8 (SMCT1)-mediated transport of butyrate forms the basis for the tumor suppressive function of the transporter

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Abstract

The identification of *SLC5A8* as a tumor suppressor gene in colorectal cancer marks, for the first time, the association of a plasma membrane transporter with tumor suppressive properties. The subsequent establishment of the functional identity of *SLC5A8* as a Na⁺-coupled transporter for short-chain monocarboxylates provides a mechanism for the tumor suppressive function of the transporter. Butyrate, a substrate for the transporter, is a histone deacetylase inhibitor and protective against colorectal cancer. This fatty acid is produced in the colonic lumen by bacterial fermentation of dietary fiber. *SLC5A8* mediates the concentrative entry of butyrate from the lumen into colonocytes. Consequently, the transport function of *SLC5A8* has the ability to influence the acetylation status of histones and hence gene expression in colonocytes. The ability of *SLC5A8* to deliver butyrate into colonic epithelial cells most likely underlies the tumor suppressive role of this transporter.

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Introduction

Cancer is the leading cause of death before the age of 65 years among men and women. Annual age-adjusted incidence rates for cancer have been stable since the mid-1990s, though death rates have shown a slight decrease. This has been attributed to improved screening and early detection of cancer, detection and removal of precancerous polyps, better therapy as well as the increased use of estrogen and progestin by women and the increased use of anti-inflammatory drugs by the general population (Rossouw et al., 2002; Thun et al., 1993; Weir et al., 2003). Despite these advances, the rapid aging and increasing size of the US population is expected to increase the cancer burden in this country; therefore, a better understanding of this disease is sorely needed.

Li et al. (2003) have recently identified *SLC5A8* as a candidate tumor suppressor gene that is silenced in approximately 60% of primary colorectal cancers. Silencing occurs primarily by intense CpG island methylation in exon 1 of

SLC5A8. When re-expressed in colon cancer cell lines in which it had been silenced, *SLC5A8* decreases colony formation by at least 75%. Silencing of *SLC5A8* occurs as an early and frequent event in the progression of colonic mucosa to neoplasia, detectable in over 50% of colonic aberrant crypt foci and adenomas. Subsequent studies in other laboratories have provided evidence for the silencing of *SLC5A8* in cancers in other tissues such as the stomach, thyroid gland, and brain (Dong et al., 2005; Hong et al., 2005; Porra et al., 2005; Ueno et al., 2004), suggesting that the postulated tumor suppressive role of *SLC5A8* may not be restricted to the colon. *SLC5A8* codes for a transporter belonging to the solute-linked carrier gene family *SLC5*. This gene family consists of several Na⁺-coupled co-transporters whose substrates include glucose, myo-inositol, iodide, choline, and B-complex vitamins (Wright and Turk, 2004). Even though the primary structure of *SLC5A8* indicated a transport function for the protein, the identity of the substrate that is transported by the protein was not known. Furthermore, how the transport function is linked to the tumor suppressive role of the protein also remained unclear. It is important to recognize that this is the first time a plasma membrane transporter has been suggested to function as a

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tumor suppressor. Therefore, a clear understanding of the relationship between the protein's transport function and tumor suppressive capability is of significant clinical and therapeutic importance. Since the initial discovery of this putative tumor suppressor, significant advances have been made in terms of understanding its transport function (Ganapathy et al., 2005). Here we review how the recently established identity of the transport function of SLC5A8 is related to the potential tumor suppressive role of the protein.

Identity of the transport function of SLC5A8

The original studies by Li et al. (2003), which described the identity of SLC5A8 as a potential tumor suppressor, investigated the transport function of the protein. These studies did show that the protein, when expressed heterologously in *Xenopus* oocytes, is capable of Na⁺ transport. However, since the protein belongs to a gene family which mostly consists of members which are Na⁺-dependent co-transporters, the ability of SLC5A8 to mediate Na⁺ uptake was not surprising. But, the question is: what is the identity of the substrate that is co-transported with Na⁺? Subsequently, studies from our laboratory showed that SLC5A8 is in fact a Na⁺-coupled co-transporter for a variety of short-chain fatty acids (Gopal et al., 2004; Miyauchi et al., 2004). The co-transported substrates include lactate, pyruvate, acetate, propionate, and butyrate. The substrate specificity of SLC5A8 is very similar to that of the previously known monocarboxylate transporters (MCTs) (Halestrap and Meredith, 2004), except that SLC5A8 is coupled to Na⁺ whereas MCTs are coupled to H⁺. Based on this transport function, we named SLC5A8 as Sodium-coupled MonoCarboxylate Transporter (SMCT). The functional identity of SLC5A8 has been confirmed independently by Coady et al. (2004). Recently, we have cloned a new transporter (SLC5A12) which is similar to SLC5A8 in terms of Na⁺-dependence and substrate specificity but differs in substrate affinity (Srinivas et al., in press). Accordingly, we refer to SLC5A8 as SMCT1 to distinguish it from SLC5A12 (SMCT2). SMCT1 is also capable of mediating the Na⁺-coupled transport of the B-complex vitamin nicotinate (Gopal et al., 2005a). All of the substrates of SMCT1 identified thus far are monovalent carboxylate anions. The transport process is electrogenic, associated with the transfer of net positive charge into the cells, indicating that more than one Na⁺ is involved in the transport process. Interestingly, the number of Na⁺ ions that are co-transported seems to vary depending on the co-transported anionic substrate. Since the transport process is electrogenic, the transport function is active, energized by a transmembrane electrochemical Na⁺ gradient. Compared to the recently identified SMCT2 (SLC5A12), SMCT1 is relatively a high-affinity transporter with affinities for the naturally occurring short-chain fatty acids, such as lactate, propionate, butyrate, and nicotinate, in the sub-millimolar range. Iodide, a monovalent inorganic anion, is also transported via SMCT1 (Rodriguez et al., 2002). But, recent studies from our laboratory have indicated that the transporter may function as an iodide channel, probably gated by short-chain fatty acids

(Gopal et al., in press). SMCT1 is expressed abundantly in the colon, kidney, and thyroid gland (Ganapathy et al., 2005). The principal physiologic function of the transporter may vary from tissue to tissue. In the colon, the transporter mediates the Na⁺-coupled entry of short-chain fatty acids from the lumen into colonocytes. In the kidney, the primary substrate for the transporter is lactate, and thus SMCT1 is responsible for the active reabsorption of this monocarboxylate from the glomerular filtrate. The handling of iodide may be the main function of the transporter in the thyroid gland. Iodide has to be transported across the apical membrane of the thyroid follicular cells into the colloidal lumen for subsequent iodination of tyrosine residues in thyroglobulin. SMCT1 is expressed in this membrane (Rodriguez et al., 2002); therefore, we believe that the iodide channel activity of the transporter is responsible for the release of iodide from the follicular cells into the colloidal lumen. Since pyruvate/lactate is produced as the byproducts of thyroid hormone synthesis within the thyroid follicular cells, the gating of the iodide channel by these monocarboxylates may have physiologic significance, providing a mechanism to link the synthesis of thyroid hormones to the release of iodide into the colloidal lumen.

Relevance of SMCT1 to colonic health

Short-chain fatty acids such as acetate, propionate, and butyrate are produced at high concentrations in the colonic lumen by bacterial fermentation of dietary fiber (Fig. 1, step I). The concentrations of these fatty acids in the lumen are in the range of 70–130 mM (Mortensen and Clausen, 1996). Dietary intake of fiber is known to be beneficial for colonic health. One of the mechanisms by which high fiber intake promotes colonic health is by providing the substrates for bacterial fermentation in the colonic lumen to generate short-chain fatty acids. These bacterial metabolites are believed to be the primary nutrients for colonocytes and promoters of cell differentiation (Fig. 1, step II). Of these short-chain fatty acids, butyrate is notable for its function as an inhibitor of histone deacetylases (Marks et al., 2001). Butyrate induces differentiation of colonocytes and promotes Na⁺, Cl⁻, and water absorption in the colon. At the same time, butyrate is also able to induce apoptosis in colonic tumor cells. The ability of butyrate to induce apoptosis in tumor cells is related to its ability to inhibit histone deacetylases and thereby influence gene expression. High dietary intake of fiber is known to be protective not only against colon cancer but also against inflammatory bowel disease. Recent studies have identified specific G-protein-coupled receptors (GPR41 and GPR43) on immune cells that interact with short-chain fatty acids as their physiologic ligands (Brown et al., 2005). Since the gastrointestinal system represents the largest immune system in the body, the production of high concentrations of short-chain fatty acids in the lumen may have relevance to the function of these gut-associated immune cells by providing endogenous agonists to GPR41 and GPR43 (Fig. 1, step III). Short-chain fatty acids have also been shown to induce leptin production and secretion from adipocytes through the G-protein-coupled receptor

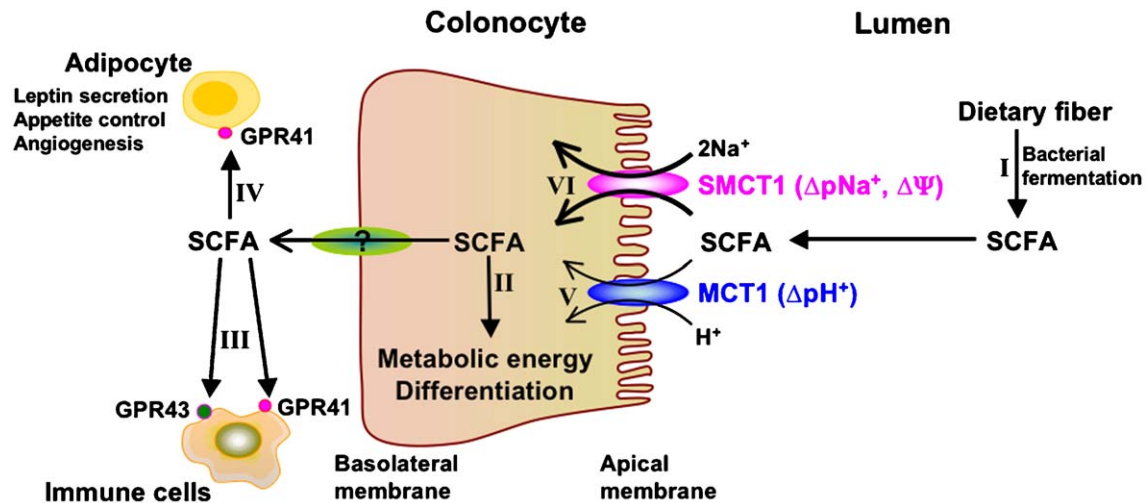


Fig. 1. Relevance of short-chain fatty acids (SCFA) to colonic health and the role of SMCT1 in this process. Step I, generation of SCFA from dietary fiber by bacterial fermentation; Step II, function of SCFA as the primary source of metabolic energy and as the promoters of cell differentiation; Step III, modulation of gut-associated immune function by SCFA by serving as the endogenous ligands (agonists) for GPR41 and GPR43; Step IV, stimulation of leptin secretion from adipocytes by SCFA and control of appetite, caloric intake, and angiogenesis; Step V, transport of SCFA via MCT1 by a H^+ -coupled, electroneutral process; Step VI, transport of SCFA via SMCT1 by a Na^+ -coupled, electrogenic process and consequent stimulation of Na^+ , Cl^- , and water absorption in the colon.

GPR41 (Xiong et al., 2004). Since leptin is an important hormone in the regulation of energy homeostasis (appetite control and energy expenditure) and angiogenesis, it is likely that bacteria-generated short-chain fatty acids in the colonic lumen influence caloric intake via the modulation of appetite control, and intestinal perfusion via modulation of blood vessel formation in the intestinal tract (Fig. 1, step IV). It is therefore clear that there must be mechanisms for these short-chain fatty acids to enter and traverse colonocytes to produce their biologic effects. MCTs have been once thought to be the principal transporters responsible for the entry of short-chain fatty acids into colonocytes (Cuff and Shirazi-Beechey, 2004). Specific isoforms of MCTs are expressed in the luminal membrane of colonocytes and these transporters recognize acetate, propionate, and butyrate as substrates. But, these transporters are not highly active because the transport function of MCTs is coupled to a transmembrane H^+ gradient rather than a transmembrane Na^+ gradient. Furthermore, the H^+ /substrate stoichiometry for MCTs is 1:1 and this makes the transport process electroneutral. This means that membrane potential has no relevance to the transport function of MCTs. Since the magnitude of the transmembrane H^+ gradient across the colonocyte apical membrane is negligible, there is very little driving force for the uphill entry of butyrate from the lumen into colonocytes via MCTs (Fig. 1, step V). Therefore, the discovery of SMCT1, which is also expressed at high levels in the luminal membrane of colonocytes, provides an important mechanism for the entry of short-chain fatty acids into colonocytes by an active process (Fig. 1, step VI). An energy-coupled entry mechanism via SMCT1, coupled to a transmembrane electrochemical Na^+ gradient, may also be a critical component of the transcellular transfer of these short-chain fatty acids from the lumen into the serosal compartment where they may function as ligands for G-protein-coupled receptors on immune cells and adipocytes to influence the biology of these cells. Colonic bacteria are known to influence

the function of gut-associated immune system, modulate intestinal mucosal barrier, regulate the proliferation and differentiation of various epithelial lineages in the gut, promote angiogenesis and blood perfusion in the gut, and reduce caloric intake. Short-chain fatty acids generated by the action of gut flora on dietary fiber seem to be the principal mediators of these functions which are essential to the maintenance of optimal colonic health. SMCT1, which mediates the entry of short-chain fatty acids from the lumen into colonocytes, serves as an obligatory link between the colonic bacteria and the host. The functional features of SMCT1 also provide the molecular basis for the stimulation of Na^+ , Cl^- , and water absorption in the colon by short-chain fatty acids. Since the absorption of short-chain fatty acids via SMCT1 is obligatorily coupled to co-transport of Na^+ , the presence of these bacterial metabolites in the colonic lumen would facilitate Na^+ absorption. This will enhance the absorption of Cl^- and water, driven by changes in the electrical and osmotic gradients, respectively, induced by the transport function of SMCT1. Moreover, it is also possible that SMCT1 may mediate the co-transport of water along with Na^+ and short-chain fatty acids as it has been demonstrated for the Na^+ /glucose co-transporter (Loo et al., 2002).

Tumor suppressive potential of SMCT1: the butyrate connection

SMCT1 is down-regulated in a variety of cancers including colorectal cancer, suggesting that this transporter is likely to be associated with a tumor suppressive function. How can SMCT1, a plasma membrane transporter, be associated with tumor suppression? This is the first time a plasma membrane transporter has been suggested to function as a tumor suppressor. The ability of SMCT1 to transport butyrate offers an important clue to the mechanism involved in the putative tumor suppressive function of this transporter. Butyrate is an inhibitor of histone deacetylases (Marks et al., 2001). The

acetylation status of histones in the chromatin is a key determinant of gene expression and histone deacetylase inhibitors have been shown to cause growth arrest and apoptosis in a variety of tumors (Marks et al., 2001). Therefore, histone deacetylase inhibitors have potential for use as therapeutic agents in the treatment of cancer. Since butyrate is produced at high concentrations in the colonic lumen by bacterial fermentation, this fatty acid represents an important endogenous inhibitor of histone deacetylases. SMCT1 as an energy-coupled active transporter for butyrate in the colon is critical for the function of this fatty acid as an inhibitor of histone deacetylases inside the colonocytes. There is ample evidence for an inverse relationship between the levels of butyrate in the colon and the incidence of colorectal cancer (Bingham et al., 2003). The bacterial fermentation of dietary fiber that produces butyrate occurs primarily in the proximal colon, an area that also has greater expression of SMCT1 than the remainder of the colon. Thus, colonocytes in the proximal colon are constantly subjected to butyrate-mediated inhibition of histone deacetylation to a much greater extent than those of the distal colon. Interestingly, colon cancer occurs more commonly in the distal colon. High fiber intake protects against colorectal cancer (Bingham et al., 2003). The beneficial effects of dietary fiber in terms of prevention of colorectal cancer are thought to be due, in large part, to its butyrate-forming capabilities (Manning and Gibson, 2004). Prebiotics (the undigestible food ingredients that constitute dietary fiber and have beneficial effects on the intestinal/colonic health in the host), probiotics (live bacteria taken either in the diet or as a dietary supplement, which improve microbial balance in the colon, promote fermentation of dietary fiber, and elicit beneficial effects in the host) as well as synbiotics (a combination of both) have anti-carcinogenic effects that are also related to the increased production of butyrate in the colon (Manning and Gibson, 2004). These findings suggest that butyrate-dependent alterations in gene expression in colonocytes via modulation of the acetylation status of histones may underlie the tumor suppressive function of this fatty acid. Butyrate may also influence the function of the gut-associated immune cells via its interaction with GPR41 and GPR43. We speculate that short-chain fatty acids, including butyrate, may be protective against inflammatory bowel disease because of their influence on immune function. Since chronic inflammation of the gut is linked to increased incidence of colorectal cancer, the modulation of the immune system by butyrate and other short-chain fatty acids may also contribute to the ability of these fatty acids to protect against colorectal cancer.

Even though butyrate has received the maximal attention among the short-chain fatty acids as the inhibitor of histone deacetylases, propionate also exhibits significant histone deacetylase inhibitor activity (Davie, 2003). In contrast, acetate has little or no ability to inhibit histone deacetylases. Because of the differences in the abilities of acetate, propionate, and butyrate to inhibit histone deacetylases, the relative amounts of these individual short-chain fatty acids in the colon may be important in terms of their collective role in the prevention of colorectal cancer. The fermentation products in the human

colon vary depending on the type and fermentability of dietary fiber and on the metabolic profile of bacterial flora in a given individual. Insoluble fibers (e.g., cellulose, lignin, psyllium) have low fermentability, but do have significant effects on the colonic function such as increasing the fecal mass and bulk and decreasing the transit time. In contrast, soluble fibers (e.g., pectin, guar gum, fructo-oligosaccharides, galacto-oligosaccharides) are highly fermentable and hence generate greater quantities of short-chain fatty acids in the colon than insoluble fibers. In addition, soluble fibers interfere with the digestion of starch in the small intestine and consequently increase the availability of starch for bacterial fermentation in the large intestine. Thus, soluble fibers may have advantages over insoluble fibers in terms of generation of short-chain fatty acids in the colon. Since the fermentation products vary depending on the bacterial genre and strain, the relative amounts of individual short-chain fatty acids also vary from person to person because of the differences in the composition of bacterial flora and in the chemical nature of dietary fiber. All these factors influence the role of dietary fiber and bacterial fermentation products in the prevention of colorectal cancer.

Relevance of histone acetylation status to malignancy

Cancer cells are characterized by uncontrolled growth, lack of terminal differentiation, and loss of cell cycle checks and balances. Abnormalities in gene expression and repression that occur in neoplasia are controlled, in part, by epigenetic changes such as post-translational modification of histones. Histones are highly basic proteins with positively charged, lysine-rich amino terminal tails that wrap around the anionic genomic DNA, packaging them into nucleosomes. The lysine residues in histones can be modified by methylation or acetylation. The pattern of these modifications controls the interaction of DNA with various regulatory proteins. Increasing evidence points to these epigenetic changes as a hallmark of cancer (Fraga et al., 2005).

Acetylation at the ϵ -amino group of lysine residues (i.e., change from $-\text{NH}_3^+$ to $-\text{NHCOCH}_3$) in the core histones H2A, H2B, H3, and H4 abolishes the cationic nature of these lysine residues and consequently decreases their affinity for binding to DNA. This loosens the interaction between the DNA and histones and hence increases the accessibility of DNA for transcription. The acetylation status of histones is determined by the relative activities of histone acetyl transferases (HATs) and histone deacetylases (HDACs) (Marks et al., 2001). Classical HDACs are divided into Classes I and II. Class I includes HDACs 1, 2, 3 and 8. These are expressed in most cell types and are related to the yeast transcriptional regulator RPD3. These are localized in the nucleus, and inhibited by depsipeptide and hydroxamates. Class II includes HDACs 4, 5, 6, 7, 9, and 10. These have a more restricted distribution, share similarity with the yeast deacetylase HDA1, and are inhibited only by hydroxamates. There is a third, non-classical group of HDACs which are homologous to yeast SIR2, NAD^+ -dependent, and insensitive to trichostatin. Aberrant hypoacetylation has been implicated in relation to abnormal gene

expression in numerous epithelial and hematological malignancies. This may occur due to mutations in the HATs or HDACs themselves or by aberrant recruitment of HDACs to the promoter regions of tumor suppressor genes, leading to their transcriptional repression. The acetylation status of histones can be enhanced by a diverse group of chemicals which function as histone deacetylase inhibitors (HDIs) (Marks et al., 2001). There are several known HDIs that can be divided into four groups (Drummond et al., 2005): short chain fatty acids (sodium butyrate, tributyrin, isobutyramide, AN-9, phenylbutyrate, valproic acid), hydroxamic acids (trichostatin, suberoylanilide hydroxamic acid [SAHA], pyroxamide, oxamflatin), cyclic tetrapeptides (trapoxin A, depsipeptide, apicidin), and benzamides (MS-275, CI-994) (Drummond et al., 2005).

Butyrate as an HDAC inhibitor and its relevance to tumor suppression

Butyrate is an endogenous inhibitor of HDACs and is generated at high concentrations in the colonic lumen by bacterial fermentation of dietary fiber. HDIs induce cell cycle arrest and prevent proliferation through p21 (an inhibitor of cyclin-dependent kinase)-dependent as well as p21-independent mechanisms. p21, which is induced by butyrate and other HDIs, inhibits CDK2 activity in G1/S-phase, reduces as well as dephosphorylates the retinoblastoma protein, suppresses c-myc expression and causes G1 cell cycle arrest (Kuefer et al., 2004). p21-independent pathways may involve activation of p19INK4d and p18INK4c, which are members of the INK4 family of CDK inhibitors or by stabilization of p27^{KIP1} protein in normal cells. HDIs may also delay the G2/M transition by triggering a p38 MAPK-dependant checkpoint. Tumor cell lines respond to high doses of HDIs by undergoing apoptosis rather than proliferative arrest. In these cell lines, p21 levels do not rise. The tumor cell lines lack an HDI-sensitive G2 phase cell cycle arrest checkpoint, resulting in the cells undergoing aberrant mitosis and subsequent apoptosis when treated with high doses of HDIs. This may be the mechanism by which normal cells are protected from apoptosis when exposed to HDIs (Burgess et al., 2001).

Butyrate-induced apoptosis in cancer cells was thought to be p53-independent (Hague et al., 1993). However, recent evidence suggests that butyrate causes apoptosis by acetylation of lysine residues on p53 itself as well as stabilization of p53 and upregulation of the p53 targets PIG3 and NOXA (Terui et al., 2003). Butyrate induces functional p53 expression, possibly through a p14(ARF)-dependent pathway, and suppresses the growth of p53-expressing cancer cells more efficiently than p53 null cells (Joseph et al., 2005). Butyrate, combined with wild-type p53 gene therapy, induces necrosis and complete tumor regression in nude mice xenografted with human cancer cell lines, an effect that is not seen with monotherapy (Takimoto et al., in press). Thus, butyrate may cause apoptosis through p53-dependent as well as p53-independent pathways.

Activation of the death receptor pathway components (TRAIL, DR5, Fas, and FasL) by HDIs in malignant cells may offer a p53-independent mechanism of apoptosis.

Butyrate induces tumor cell sensitization to Fas-mediated cytotoxicity (Bonnotte et al., 1998; Ogawa et al., 2004). Butyrate also increases susceptibility to the combination of TNF- α and IFN- γ (Chopin et al., 2004). Butyrate may upregulate Death Receptor 5 (DR5), specifically in malignant cells, sensitizing them to apoptosis by the new and promising chemotherapeutic agent, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), while not enhancing its effect on normal cells (Kim et al., 2004; Nakata et al., 2004). Another explanation for the selective apoptotic effect of HDIs on malignant cells and not on normal cells is that only the normal cells express DcR1 and DcR2, which are TRAIL receptors that do not have apoptotic effects when activated because they lack intra-cytoplasmic effector domains. DcR1 and DcR2 are decoy receptors for TRAIL and expression of these receptors in normal cells leads to the binding of TRAIL which in itself does not induce any intracellular signaling events but reduces the availability of the ligand for interaction with the death-inducing DR5.

Cox-2 over-expression plays an important role in the inflammation-carcinogenesis pathway of colorectal cancer. The expression of Cox-2 in stimulated colon cancer cells is suppressed by butyrate, as are several genes in the interferon- γ -mediated inflammatory pathway (Joseph et al., 2004; Tong et al., 2004). TGF- β suppresses proliferation in normal colonocytes; however, TGF- β resistance is acquired early by colorectal cancer (Fodde et al., 2001). HDIs restore TGF- β responsiveness in tumor cells by reversing the transcriptional repression of TGF- β receptor type I (Ammanamanchi and Brattain, 2004). In addition, butyrate decreases the metastatic and invasive potential of cancer cells by decreasing the activity of pro-metastatic matrix metalloproteinases (MMP-2) and other metastasis-associated genes as well as by inducing metastasis suppressors (Joseph et al., 2004; Kaneko et al., 2004; Zeng and Briske-Anderson, 2005). Butyrate can also cause apoptosis by inducing glutathione-S-transferases, triggering glutathione depletion and oxidative stress (Pei et al., 2004). This sensitizes chemo-resistant breast and perhaps thyroid cancer cells to doxorubicin cytotoxicity (Louis et al., 2005; Massart et al., 2005).

Butyrate may also offer protection against colon cancer by maintaining the expression of CEACAM1 in colonocytes. CEACAM1 is a tumor suppressor gene that is down-regulated in colon cancer as well as in breast and prostate cancer (Nittka et al., 2004; Shively, 2004). CEACAM1 is normally expressed on the apical membrane of the colonocytes and triggers anoikis when the cells lose contact with the basement membrane, thus preventing the loss of the monolayer. Genetic mutations,

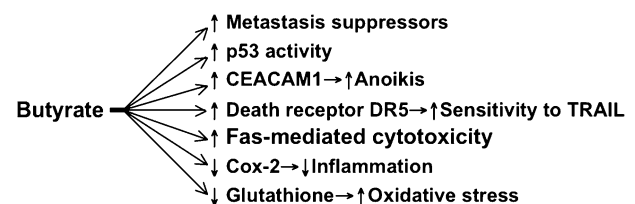


Fig. 2. Mechanisms by which butyrate functions as a tumor suppressor.

deletions, or promoter methylation do not seem to play any role in the cancer-associated silencing of CEACAM1. Instead, the silencing involves the transcription factor Sp2 in association with HDACs (Phan et al., 2004). Thus, butyrate-induced inhibition of HDACs may underlie the ability of normal colonocytes to express CEACAM1. Loss of CEACAM1 expression has been demonstrated in aberrant crypt foci and hyperplastic polyps, which are the earliest preneoplastic lesions in colorectal cancer (Nittka et al., 2004). These early lesions do not show loss or mutation of classical gatekeeper genes like the adenomatous polyposis coli (APC) gene, which is thought to play an initiating role in the progression from hyperplasia to neoplasia (Shively, 2004). However, these early lesions in colon cancer do demonstrate loss of SMCT1 (Li et al., 2003). Since butyrate-induced HDAC inhibition may be important for the normal expression of CEACAM1, the silencing of SMCT1 in early preneoplastic lesions may prevent the entry of butyrate from the lumen into colonocytes and thus lead to hyperactivity of HDACs and consequently to the silencing of CEACAM1. Loss of CEACAM1 in turn frees the cancer cell to proliferate in a multilayered, dysplastic manner without undergoing anoikis, thus instigating favorable conditions for mitogenesis and acquisition of mutations in other tumor suppressor genes such as *APC*. Thus, a wide variety of mechanisms operate in the tumor suppressive actions of butyrate, primarily through its ability to inhibit histone deacetylases (Fig. 2).

Conclusions

The identity of SMCT1 as a Na⁺-coupled transporter for butyrate offers a rational basis for the tumor suppressive role of this transporter. The abundant expression of the transporter in the luminal membrane of the colonocytes, the generation of butyrate in the lumen by the bacterial fermentation of dietary fiber, and the function of butyrate as an HDAC inhibitor may explain the beneficial role of normal gut flora and dietary fiber intake in the prevention of colorectal cancer and the tumor suppressive role of SMCT1. While the relevance of SMCT1 as a tumor suppressor in the colon is immediately apparent because of the presence of high concentrations of butyrate in the colonic lumen, the potential mechanisms by which this transporter may function as a tumor suppressor in other tissues such as the breast and prostate are not readily obvious. During lactation, breast epithelial cells synthesize large amounts of short-chain fatty acids, including butyrate. SMCT1 is expressed at high levels in mammary epithelial cells during mammary gland involution. The endogenous production of butyrate in lactating mammary gland may have relevance to apoptosis associated with mammary gland involution. Silencing of this transporter function may lead to the suppression of butyrate-induced apoptosis and enhance the risk of cancer. The normal concentrations of butyrate in circulation are very low. However, the expression of SMCT1 is silenced in cancer in other tissues such as the prostate in which there is no likelihood of butyrate-induced tumor suppression under normal physiologic conditions. It is therefore tempting to speculate that there may be other, hitherto unidentified, HDAC

inhibitors in the circulation that may function as substrates of SMCT1.

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