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Short-Chain Fatty Acid Transporters: Role in Colonic Homeostasis

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Abstract

Short-chain fatty acids (SCFA; acetate, propionate, and butyrate) are generated in colon by bacterial fermentation of dietary fiber. Though diffusion in protonated form is a significant route, carrier-mediated mechanisms constitute the major route for the entry of SCFA in their anionic form into colonic epithelium. Several transport systems operate in cellular uptake of SCFA. MCT1 (SLC16A1) and MCT4 (SLC16A3) are H⁺-coupled and mediate electroneutral transport of SCFA (H⁺: SCFA stoichiometry; 1:1). MCT1 is expressed both in the apical membrane and basolateral membrane of colonic epithelium whereas MCT4 specifically in the basolateral membrane. SMCT1 (SLC5A8) and SMCT2 (SLC5A12) are Na⁺-coupled; SMCT1-mediated transport is electrogenic (Na⁺: SCFA stoichiometry; 2:1) whereas SMCT2-mediated transport is electroneutral (Na⁺: SCFA stoichiometry; 1:1). SMCT1 and SMCT2 are expressed exclusively in the apical membrane. An anion-exchange mechanism also operates in the apical membrane in which SCFA entry in anionic form is coupled to bicarbonate efflux; the molecular identity of this exchanger however remains unknown. All these transporters are subject to regulation, notably by their substrates themselves; this process involves cell-surface receptors with SCFA as signaling molecules. There are significant alterations in the expression of these transporters in ulcerative colitis and colon cancer. The tumor-associated changes occur via transcriptional regulation by p53 and HIF1 α and by promoter methylation. As SCFA are obligatory for optimal colonic health, the transporters responsible for the entry and transcellular transfer of these bacterial products in colonic epithelium are critical determinants of colonic function under physiological conditions and in disease states.

Didactic Synopsis

Major teaching points

- Fermentation of dietary fiber by colonic bacteria yields short-chain fatty acids (SCFA)
- SCFA elicit beneficial effects on colon via extracellular and intracellular actions
- Extracellular actions of SCFA occur via receptors in apical membrane of colonic epithelium

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- Intracellular actions of SCFA in colonic epithelium involve inhibition of histone deacetylases (HDACs), generation of energy, and conversion into ketone bodies
- Entry of SCFA into colonic epithelium is mostly carrier-mediated
- MCT1 and MCT4 are H⁺-coupled transporters expressed in apical membrane (MCT1) and basolateral membrane (MCT1 and MCT4) of colonic epithelium, mediating SCFA influx from lumen and SCFA efflux into blood
- SMCT1 and SMCT2 are Na⁺-coupled transporters expressed solely in apical membrane of colonic epithelium, mediating SCFA influx from lumen
- SCFA transporters are defective in ulcerative colitis
- MCT1 and SMCT1 function as tumor suppressors in colon and are downregulated in colon cancer
- The tumor-suppressive function of MCT1 and SMCT1 might be related to dietary fiber content

Introduction

The bacteria that reside in normal colon are called commensals, with an overt connotation that only the bacteria benefit from their cohabitation with the host. It has become increasingly evident in recent years, however, that the cohabitation is actually beneficial to the bacteria and the host, highlighting the symbiotic nature of the relationship. The advantages to the bacteria are obvious; the host provides the bacteria a place to colonize and also all the nutrients for them to survive and proliferate. How does the host benefit from this relationship? Recent studies have provided unequivocal evidence for the obligatory role for colonic bacteria in maintenance of optimal colonic health. The intestinal tract represents the largest immune system in the body, necessitated by the presence of trillions of bacteria nearby in the intestinal and colonic lumen. However, as the bacteria in normal colon are beneficial to the host, the mucosal immune system actively learns to tolerate the bacteria without an overly antagonistic inflammatory response; interestingly, bacteria themselves play an essential role in educating the mucosal immune cells. The epithelial cells lining the intestinal tract (enterocytes, goblet cells, enteroendocrine cells, and Paneth cells in small intestine, and colonocytes, goblet cells, and enteroendocrine cells in colon) serve as a barrier between the bacteria and the host, and also act as an intermediary between the bacteria and the immune cells residing in the lamina propria. How do the bacteria communicate with host cells to elicit their biological effects? Chemical communication seems to be the major mode of interaction in this regard. Bacteria in the intestinal tract generate a broad range of metabolites that serve as messengers between the bacteria and the host. Bacteria generate these metabolites as intermediates in their constitutive metabolic pathways and also by chemical modification of specific dietary constituents by enzymatic reactions unrelated to their normal metabolism. Most of these metabolites, if not all, elicit beneficial effects on the host. Examples of such metabolites are short-chain fatty acids (SCFA) (acetate, propionate, and butyrate), lactate, tryptophan degradation products (indole-3-aldehyde, indole-3-acetic acid), and modified fatty acids (7, 8, 15, 34, 49, 108, 114, 116). Several molecular targets have been identified for these bacterial metabolites in colonic epithelium and mucosal

immune cells; this includes cell-surface G-protein-coupled receptors, intracellular receptors, and enzymes (7, 8, 15, 34, 49, 108, 114, 116).

Among the bacterial metabolites that elicit beneficial effects on the host, SCFA constitute a major component, both quantitatively and qualitatively (54,89,97,99,134). The molecular targets for SCFA in the host reside on the cell surface as well as inside the target cells. In colonic epithelium and mucosal immune cells, two cell-surface G-protein-coupled receptors, GPR109A and GPR43, are targets for SCFA with butyrate as the agonist for GPR109A and all three SCFA as agonists for GPR43 (7, 8, 15, 34, 49, 108, 114, 116). As these receptors are present in the lumen-facing apical membrane of colonic epithelium (20, 60, 106, 107, 120), luminal SCFA activate these receptors without the need to enter the cells. Activation of these receptors decreases cellular levels of cAMP and increases intracellular Ca^{2+} , which serve as second messengers to elicit biological effects (14, 69, 71, 78, 109, 115, 126, 133). In addition to these extra-cellular actions, SCFA also have intracellular actions, which obviously require their entry into colonic epithelial cells. Furthermore, SCFA have to traverse the epithelial cells to the serosal side to act on the immune cells present in the lamina propria. The entry from the lumen into cells as well as transcellular transit require transport mechanisms without which SCFA cannot produce their intracellular effects in colonic epithelial cells nor can they act on mucosal immune cells. As such, SCFA transporters expressed in colonic epithelium are major determinants of the beneficial effects of SCFA on the host. Defects in the expression and function of these transporters are closely associated with pathological conditions such as ulcerative colitis and colon cancer (33, 35, 125). In this article, we review what is known on the molecular identity, function, and regulation of SCFA transporters expressed in colonic epithelial cells.

Proton-Coupled Transporters for SCFA

Monocarboxylate transporter 1

Molecular identification and general functional features—The molecular identification of monocarboxylate transporter 1 (MCT1) has an interesting history. It was first cloned as a mutant protein from a specific clone of Chinese hamster ovary (CHO) cells with ability to transport mevalonate (3,5-dihydroxy-3-methylpentanoic acid), a precursor for endogenous synthesis of cholesterol (63). This clonal cell line varied from the parent CHO cell line in its ability to survive in the presence of low levels of mevalonate in culture medium when endogenous synthesis of mevalonate was inhibited with a pharmacological agent, indicating that the clonal cell was able to take up mevalonate. This was not the case with the parent cell line. The transporter responsible for mevalonate uptake in the clonal CHO cell line was then cloned and shown to exhibit mevalonate transport activity. Interestingly, the parent cell line that had no mevalonate transport activity also had mRNA and protein for the cloned transporter at levels comparable to those in the mutant cell line. Subsequently, it was found that the mutant cell line had a point mutation in the transporter resulting in the substitution of a single amino acid (phenylalanine at position 360 changed to cysteine) with a gain of function to transport mevalonate, a feature not shared by the wild-type transporter expressed in the parent cell line. The actual transport function of the wild-type transporter remained unknown. A couple of years later, the same investigative team that

cloned the mutant transporter identified the transport function of the wild-type transporter as a H^+ -coupled transporter for monocarboxylates such as lactate and pyruvate (36). Based on this transport function, the transporter was named monocarboxylate transporter 1 (MCT1). According to the Human Genome Organization nomenclature, MCT1 is referred to as SLC16A1 (53,82,92). The functional features of MCT1 include a H^+ :monocarboxylate stoichiometry of 1:1, rendering the transport process electroneutral, recognition of aliphatic short-chain (carbon chain length two to five) monocarboxylates as substrates, and stereoselectivity toward the isomers of lactate, preferring L-lactate over D-lactate (53, 82, 92). The Michaelis constant for L-lactate is in the low millimolar range (3–5 mmol/L). MCT1 is inhibited by α -cyano-4-hydroxycinnamate, bioactive flavonoids (e.g., quercetin and phloretin), and thiol-modifying agents (e.g., p-chloromercuribenzenesulfonate). There are, however, several newer inhibitors that are selective for MCT1 with inhibitor potency in nanomolar range (47,91,96,131); examples of such MCT1-specific inhibitors include AR-C155858 and AZD3965, both developed by AstraZeneca.

Structurally, human SLC16A1 consists of 500 amino acids with 12 putative transmembrane domains; both amino and carboxy termini are predicted to be on the cytoplasmic side of the plasma membrane. The human gene is located on chromosome 1 (1p13.2-p12) (37). The *MCT1* gene spans ~44 kb and consists of five exons, with only exons two to four coding for the protein (22, 110). The promoter region contains binding sites for several transcription factors, including nuclear factor for the κ light chain of immunoglobulin in B cells (NF- κ B), activator proteins 1 and 2 (AP1 and AP2), specificity protein 1 (Sp1), upstream stimulatory factor (USF), T-cell factor/lymphoid enhancer factor (TCF/LEF), and the oncogene c-MYC (22, 26, 51, 110).

MCT1 requires an ancillary protein for its trafficking to and localization in the plasma membrane; CD147, also known as basigin or EMMPRIN, a widely expressed glycoprotein, plays this role (66). In the plasma membrane, MCT1 and CD147 remain tightly associated. Interestingly, though the transport function of MCT1 is susceptible to inhibition by thiol-modifying agents, removal of all cysteine residues in MCT1 does not eliminate the susceptibility to inhibition by these agents, indicating that the inhibition is not due to direct modification of any of the thiol groups in MCT1 (132). The ancillary protein CD147 is responsible for this inhibition. This shows that CD147 not only functions as a chaperone but also is critical for the transport function of MCT1. In some cell types, MCT1 interacts with another protein, called embigin (132), which is closely related to basigin, both proteins forming a subgroup of the immunoglobulin superfamily. Though basigin or embigin could interact with MCT1 and function as a chaperone in *in vitro* ectopic expression studies, basigin is the endogenous chaperone for MCT1 in most cell types. The sites on MCT1 that interact with its chaperones basigin or embigin (75) or with the selective inhibitor AR-C155858 (88) have been determined; this was possible by homology modeling using the established structure of *Escherichia coli* glycerol-3-phosphate transporter (57). The transmembrane domain of basigin is in close proximity to the transmembrane domains 3 and 6 of MCT1.

MCT1 expression in the intestinal tract and its relevance to SCFA transport—

MCT1 has a wide tissue distribution pattern with mRNA and protein detected in almost all

human tissues (53,82). As lactate is a substrate for MCT1 and almost all tissues either generate or utilize this metabolite in biochemical pathways, it is not surprising that the transporter is expressed ubiquitously. Substrate selectivity of MCT1 includes several additional endogenous metabolites such as acetate, propionate, and pyruvate (53, 82). The primary ketone body β -hydroxybutyrate is also a substrate for MCT1; during starvation, the circulating levels of this metabolite increase markedly to millimolar concentrations from hepatic synthesis to serve as the metabolic fuel to neurons in place of glucose, and this metabolite crosses the blood-brain barrier via MCT1. The recognition of short-chain aliphatic monocarboxylates as substrates by MCT1 forms the basis of the connection of this transporter to the intestinal tract. SCFA generated by fermentation of dietary fiber by intestinal bacteria are short-chain monocarboxylates. In addition, dietary components such as yogurt and other milk fermentation products contain lactate. These bacterial metabolites and dietary constituents are absorbed into enterocytes in the small intestine and colonocytes in the colon very effectively, and some of them (e.g., acetate and propionate) appear in portal circulation in significant quantities (56, 97, 99). This means that the lumen-facing apical membrane and the serosa-facing basolateral membrane of these absorptive cells must have transport systems for these monocarboxylates for influx into cells across the former membrane and efflux from the cells into portal blood across the latter membrane. This phenomenon is particularly relevant to colon where SCFA are generated in large quantities. As such, numerous studies have examined the transport of SCFA, particularly butyrate, in apical membrane vesicles and basolateral membrane vesicles prepared from colonic epithelium (80, 98, 127, 129). In both membrane preparations, butyrate uptake was stimulated by an inwardly directed H^+ gradient and also by an outwardly directed HCO_3^- gradient, suggesting two potentially different mechanisms for butyrate uptake: $SCFA^-/H^+$ cotransport and $SCFA^-/HCO_3^-$ exchange. The cotransport process was not due to nonionic diffusion because the uptake was saturable and inhibitable by structurally similar SCFA. The exchange process was not due to any known anion exchanger because the uptake was not sensitive to anion-exchange inhibitors. Interestingly, though the presence of MCT1 protein was documented in the apical membrane and shown to participate in butyrate uptake by some investigators almost two decades ago (100, 101), the involvement of this transporter in SCFA uptake in colon did not receive much attention even after a decade later (8). It is now well accepted that MCT1 is solely responsible for $SCFA^-/H^+$ cotransport in the apical membrane (38, 100, 101). The transporter is expressed not only in the apical membrane but also in the basolateral membrane (2, 59, 120), suggesting a role in the efflux of SCFA from the cells to the serosal side. The electroneutral cotransport of $SCFA^-$ with H^+ via MCT1 could occur in either direction (influx or efflux) depending on the transmembrane concentration gradient for a given $SCFA^-$. As the concentration of SCFA is very high in the lumen, apical membrane MCT1 mediates the influx of SCFA into cells; those SCFA that are not degraded by cellular metabolic pathways would then leave the cells via MCT1 in the basolateral membrane to enter portal circulation. Regional distribution of MCT1 expression along the intestinal tract supports a role for the transporter in SCFA uptake; the expression is significantly higher in colon than in small intestine, matching the regional differences in SCFA production in the intestinal lumen (30, 38).

The localization of MCT1 in the apical versus basolateral membrane of colonic epithelial cells still remains a little bit controversial because of the evidence in published reports for localization selectively either in the apical membrane or in the basolateral membrane and, in some reports, localization in both membranes. The reasons for this discrepancy are not known. It is possible that there are species differences in the differential localization of MCT1 in the apical membrane versus the basolateral membrane in colonic epithelial cells. Given the fact that luminal components regulate the expression of the transporter in the apical membrane, experimental conditions such as fed state versus overnight starvation when the animals were used for the analysis could have also contributed to this controversy. The most plausible conclusion from the published reports, however, is that MCT1 is expressed in both apical and basolateral membranes, even though the relative abundance of the transporter protein might vary between the two membranes and the presence of the transporter in the apical membrane might depend on dietary conditions. Whichever is the location of MCT1 in the intestinal and colonic epithelial cells, the chaperone CD147 seems to follow MCT1 location. In differentiated Caco-2 cells, MCT1 is present in the apical membrane, and the interaction of MCT1 with its chaperone CD147 is necessary for the trafficking of MCT1 to this membrane (16).

Regulation of MCT1 expression in normal colon—As SCFA play a critical role on colonic health, the regulatory aspects of MCT1 in the intestinal tract have physiological and clinical significance. Decreased expression of the transporter would mean defective entry of SCFA into intestinal and colonic epithelial cells, thereby attenuating the beneficial effects of these bacterial metabolites that are mediated by their intracellular actions (e.g., histone deacetylase [HDAC] inhibition, decreased production of metabolic energy). The cell lines of intestinal origin, namely IEC6 from rat small intestine and Caco-2 from human colon, express MCT1; the participation of the transporter in H⁺-coupled uptake of butyrate and other SCFA has been documented in these cell lines (39, 52, 113). Regulation of MCT1 expression and function has been investigated primarily in Caco-2 cells. Activation of the atypical protein kinase C isoform PKC ζ with phorbol esters in this cell line upregulates MCT1 expression at the level of mRNA, protein, and transport function (103). The transcription factor AP2 mediates this effect. Somatostatin, a neuropeptide in the intestinal tract, also induces the expression of MCT1 as well as its ancillary protein CD147 via p38 MAP kinase signaling pathway (104). In contrast, the transcription factors USF1 and USF2 repress MCT1 expression without involving the Sp1 site in the MCT1 promoter (51). An important aspect of MCT1 regulation is the ability of butyrate to influence the transporter protein density in the plasma membrane (12, 13, 21, 137), suggesting that luminal butyrate is a key determinant of MCT1 function in colon. Detailed studies of this phenomenon have shown participation of two independent signaling pathways, one involving epigenetic modulation with butyrate-dependent inhibition of HDACs and the other involving the cell-surface receptor GPR109A for butyrate (12, 13). The GPR109A-mediated increase in MCT1 protein density in the plasma membrane is pertussis toxin-sensitive and is associated with decreased cellular levels of cAMP (12). The transcription factor NF- κ B also participates in butyrate-induced upregulation of MCT1, but this process is independent of protein kinase A and protein kinase C (13). The substrate-dependent regulation of MCT1 expression observed in Caco-2 cells might also occur *in vivo*. Oral feeding of dietary fiber (e.g., pectin, β -glucan)

increases the total cellular MCT1 protein levels and also the apical membrane expression and function of MCT1 in cecum and colon in animal models (64, 84). It is likely that these dietary manipulations increase the production of butyrate by bacteria in cecum and colon, which then induce the expression of MCT1. It is notable that the MCT1 promoter contains cis elements, which are expected to be responsive to Wnt signaling (110). As this pathway is important in the regulation of proliferation and differentiation of intestinal and colonic epithelial stem cells (24, 87), Wnt signaling might control the expression of MCT1 in the intestinal tract under normal physiological conditions.

Alterations in MCT1 expression in colon in disease states—SCFA protect against colonic inflammation and colon carcinogenesis at least partly by their actions inside the colonic epithelial cells. As carrier-mediated process is the primary pathway for the entry of these bacterial metabolites into colonic epithelium, the expression and function of SCFA transporters represent important risk modifiers for colitis and colon cancer. Based on the known beneficial effects of SCFA, one would expect that decreased expression of SCFA transporters would be associated with increased susceptibility and severity of colitis and colon cancer. As the Michaelis constant for MCT1-mediated butyrate uptake is in the 4 to 10 mmol/L range (21, 52, 104), it is likely that MCT1 is the principal contributor to butyrate uptake into colonic epithelial cells at butyrate concentrations normally found in the lumen (10–15 mmol/L). Therefore, changes in MCT1 expression in colon that occur in colitis and colon cancer might have clinical and therapeutic significance as such changes would indicate altered butyrate availability to colonic epithelial cells and provide clues as to mechanisms underlying the pathogenesis of the diseases.

In Caco-2 cells, treatment with the proinflammatory cytokine TNF- α decreases the expression of MCT1, suggesting that inflammation might be associated with suppression of MCT1 expression (130). In pigs as an animal model, high-protein diet leads to bacterial fermentation of proteins in the colon, resulting in increased production of TNF- α , and this condition is also associated with decreased expression of MCT1 (130). A similar phenomenon occurs in colitis in humans and in laboratory animals (123,124). In humans with colitis and in rats with dextran sulfate sodium-induced colitis, MCT1 expression is markedly decreased, accompanied with decreased uptake of butyrate (124). In the colon cancer cell line HT-29, treatment with proinflammatory cytokines IFN- γ and INF- α suppressed MCT1 expression and transport function (124). We examined the publicly available databases for changes in colonic expression of MCT1 in patients with ulcerative colitis (Table 1). Of the 12 different datasets examined, MCT1 mRNA abundance is decreased significantly in inflamed colon tissues compared to healthy or noninflamed colon tissues in 10 datasets. The magnitude of the observed changes in inflamed colon varies from dataset to dataset, ranging 17% to 95% of expression found in control tissue. These findings indicate that decreased availability of butyrate to colonic epithelial cells underlies colonic inflammation in ulcerative colitis. As butyrate is not only the primary metabolic fuel for colonic epithelial cells but also is an effective epigenetic modifier due to its ability to inhibit HDACs, which would have impact on a broad spectrum of biological processes via alterations in the transcription of multiple genes, any or all of these mechanisms might contribute to pathogenesis of ulcerative colitis. It has also to be emphasized that decreased

transport function of MCT1 results in decreased entry of butyrate and also other important biologically active monocarboxylates such as acetate, propionate, and lactate into colonic epithelial cells. Except for the role of these other bacterial/dietary substrates of MCT1 as a carbon source for energy production, little is known as to whether these monocarboxylates have additional biological effects in colon cells and whether their deficiency would impact on ulcerative colitis. For example, propionate is also an inhibitor of HDACs, almost as potent as butyrate (18,121), but this has not received much attention. Lactate and its metabolic product pyruvate are known to increase cellular levels of the multi-functional transcription factors HIF-1 α and HIF-2 α (81, 93), but its potential relevance to ulcerative colitis has not been investigated.

Colonic absorption of butyrate is coupled to electrolyte absorption even though the mechanisms underlying the association remain largely unknown at the molecular level (8). In this regard, it is important to note that exposure of Caco-2 cells to enteropathogenic *E. coli*, a food-borne human pathogen that causes diarrhea, decreases MCT1 protein density in the plasma membrane; nonpathogenic *E. coli* does not elicit this effect (11). Interestingly, the probiotic *Lactobacillus acidophilus* counteracts enteropathogenic *E. coli*; some unknown component in the culture medium secreted by *Lactobacillus*, which is heat-sensitive, mediates this effect (67). The decreased membrane localization and function of MCT1 in this model by a diarrheagenic *E. coli* strain would be expected to cause defective absorption of butyrate in colon, which would decrease electrolyte absorption thus contributing to diarrhea.

A close relationship between MCT1 and cancer is obvious and logical because of lactate as a substrate for MCT1 (53,76,92). Most cancer cells exhibit Warburg effect in which lactate is generated as the glycolytic end product even in the presence of sufficient oxygen. Unless this lactate is removed, cancer cells would be subject to detrimental effects of resultant intracellular acidification. As MCT1 is a lactate/H⁺ sym-porter, most cancer cells upregulate this transporter to eliminate lactic acid. Relevant to this functional role of MCT1 in cancer cells is the finding that the expression of this transporter is positively regulated by c-MYC (26). The relationship of MCT1 to colon cancer is not that simple as it is in cancers of noncolonic tissues. This transporter is responsible for the entry of SCFA into colonic epithelial cells from the lumen under physiological conditions. This is not the case in noncolonic tissues, which do not encounter butyrate or propionate at relevant concentrations. As butyrate and propionate are HDAC inhibitors, the function of MCT1 could be considered as tumor preventive in colon. Therefore, one would expect no benefit for colon cells to upregulate MCT1 during carcinogenesis. Instead, colonic epithelial cells could promote carcinogenesis by downregulating MCT1 expression as a means to avoid HDAC inhibition by preventing the entry of butyrate and propionate from the lumen.

Most studies have shown that MCT1 expression is indeed downregulated significantly in colon cancer. The tumor-associated decrease in MCT1 is demonstrable at mRNA and protein levels (68). We examined several publicly available datasets on gene expression in colon cancer and found the decrease in MCT1 expression in tumors to be significant in most cases (five of seven datasets); in none of these datasets is the expression of MCT1 increased (Table 2). This suggests that the availability of butyrate is decreased in colon cancer cells. In

normal colon epithelial cells, exposure to butyrate differentially affects the expression of various genes; interestingly, the changes in many of these genes in normal epithelium are opposite to that found in colon cancer tissue as would be expected if the downregulation of MCT1 in cancer cells leads to decreased entry of butyrate (23). It has to be noted, however, that some studies have reported significant upregulation of MCT1 expression in colon cancer (94, 95). Using publicly available datasets, we examined the relationship between the expression of levels of MCT1 and overall survival in patients with colon cancer (Fig. 1). We found a tendency, though statistically not significant, toward increased survival in patients with relatively higher expression of MCT1 when datasets for colon cancer and colorectal cancer were analyzed, and the survival was significantly better with higher expression levels of MCT1 when rectal cancer was analyzed separately. Thus it appears that the robust expression of MCT1 in the apical membrane of normal colonic epithelial cells serves an important role in preventing colon cancer and that colon cancer cells suppress the expression of this transporter to block the entry and the tumor-suppressive function of butyrate.

Consequences due to loss of function of MCT1 in humans and mice—Genetic mutations in MCT1 leading to loss of function have been identified in humans (83,128). Homozygosity for mutations causing inactivation of the transporter leads to defective utilization of ketone bodies in extrahepatic tissues and consequently results in profound ketoacidosis, aggravated during fasting or infections even in childhood (128). The affected children also have significant developmental delay. Lactate transport in erythrocytes is defective. Urinary excretion of ketone bodies is also elevated at least during the episodes of ketoacidosis. The patients might also experience intolerance to exercise. Intravenous administration of glucose alleviates these symptoms. Utilization of β -hydroxybutyrate, the predominant ketone body in circulation, occurs primarily in the brain, skeletal muscle, and cardiac muscle, and it is transported into cells and across the blood-brain barrier via MCT1. Therefore, the observed clinical consequences of loss-of-function mutations in MCT1 are logical. However, there is no information as to whether these patients exhibit any clinical or pathological phenotype in colon. Interestingly, mutations in MCT1 leading to increased expression of the transporter have also been reported (77, 90); these individuals have exercise-induced hyperinsulinism. The likely mechanism underlying this phenomenon is the stimulation of insulin secretion in response to increased MCT1-mediated entry and subsequent utilization of lactate for ATP production in pancreatic β cells, thereby increasing insulin secretion. Surprisingly, homozygous deletion of MCT1 in mice is embryonically lethal, associated with axon damage and neuronal loss in the brain (72, 73). Heterozygous deletion, on the other hand, results in resistance to development of obesity on a high-fat diet accompanied with increased insulin sensitivity and absence of hepatic steatosis. Even though the consequences and their severity vary between humans and mice with loss of function of MCT1, these findings highlight an important role for this transporter in energy homeostasis.

While the generation of SCFA is most pronounced in the large intestine in nonruminants, bacterial fermentation occurs mostly in the foregut in ruminants. This makes it relevant to understand the role of monocarboxylate transporters in SCFA absorption in the gut in ruminants. The total cellular levels of MCT1 protein in ruminants are in the following order: rumen > cecum > colon > small intestine (65). The expression of the protein, however,

seems to be restricted mostly to the basolateral membrane (3, 46), suggesting the participation of the transporter in the efflux of SCFA from the cells into the serosal side rather than in the influx from the lumen into cells.

Monocarboxylate transporter 4

Monocarboxylate transporter 4 (MCT4) is also a transporter capable of electroneutral H⁺/monocarboxylate symport; according to Human Genome Nomenclature, it is recognized as SLC16A3. The gene is located on human chromosome 17q25.3 and codes for a protein consisting of 465 amino acids and 12 transmembrane domains (70). The promoter contains binding sites for HIF-1 α . Compared to MCT1, MCT4 has much lower affinity for lactate and other monocarboxylates; the Michaelis constant for lactate transport is in the range of 25 to 30 mmol/L, almost 10 times higher than the corresponding value for MCT1 (53,82,92). Further, the affinity of MCT4 for pyruvate is much lower than for lactate, again in contrast with MCT1, which has relatively higher affinity for pyruvate than for lactate. In cancer cells, MCT4 is kinetically more suited to mediate lactate efflux than lactate influx. As such, the expression of MCT4 is upregulated in cancer and the transcription of MCT4 gene is enhanced by HIF-1 α (76, 92). Compared to MCT1, relatively less is known on the expression and function of MCT4 in the intestinal tract. There are, however, two reports that have documented the presence of MCT4 in the basolateral membrane of enterocytes in rat duodenum (61) and of colonocytes in human colon (38), indicating that MCT4 might be involved in the efflux of butyrate and other monocarboxylates from the cells into the serosal side. Portal blood does contain bacterially derived SCFA such as acetate and propionate; butyrate is present at relatively lower concentrations compared to the other two SCFA because of the preferential use of butyrate by colonic epithelial cells for metabolism and energy production. Uptake of butyrate via MCT4 has been demonstrated in the rat intestinal cell line IEC-18 (62), but it is not clear whether this cell line differentiates in culture to exhibit polarized apical and basolateral membranes; therefore, it is likely that the MCT4-mediated SCFA uptake monitored in this cell line represents transport function in the basolateral membrane rather than in the apical membrane.

Very little is known at present on the expression of MCT4 in the intestinal tract in disease states. Many of the publicly available databases also do not contain any information related to MCT4 expression in colonic diseases. Wherever available, analysis of the data shows that the expression of MCT4 is either not affected or slightly upregulated in colitis (Table 1) and colon cancer (Table 2).

Sodium-Coupled Transporters for SCFA

Sodium-coupled monocarboxylate transporter 1

Identification and function of SMCT1—Sodium-coupled monocarboxylate transporter 1 (SMCT1) belongs to the *SLC5* gene family and is identified as SLC5A8 (135). This transporter was originally discovered as a candidate tumor suppressor in colon (74). The gene is located on human chromosome 12q23.1, consists of 15 exons, and codes for a protein of 610 amino acids (74). Normal colon expresses the transporter but colon cancer tissues do not; the tumor-associated silencing of the gene occurs via DNA methylation,

primarily in exon 1. The promoter of SLC5A8 has been analyzed and the presence of binding sites for several transcription factors has been identified (136). Positive regulation of the gene expression has been documented with the transcription factors Sp1 and CCAAT/enhancer binding protein β (C/EBP β) (136). There also seems to be a role for C/EBP δ as evident from markedly reduced expression of the transporter in kidney in *c/ebp δ* -null mice (117). As most colon cancer cells silence the transporter gene by DNA methylation, treatment of these cells with inhibitors of DNA methylation induces the expression of the gene (74,119). Nontransformed colon epithelial cells constitutively express the transporter in contrast to colon cancer cells; ectopic expression of the transporter in colon cancer cells leads to cell death. These features seem to fulfill the requirements expected of a tumor suppressor. Nonetheless, the exact transport function of SLC5A8 remained unknown for some time even after the discovery of its potential tumor-suppressive function in colon (6,33,35,49). There was one initial study reporting that SLC5A8 is an iodide transporter expressed at high levels in the apical membrane of thyroid follicular cells, thereby naming the transporter as apical iodide transporter (AIT) (102). However, this finding has not yet been confirmed independently in other laboratories and also any potential connection of the iodide transport to the tumor-suppressive function is not readily obvious. Subsequently, SLC5A8 was identified as a Na⁺-coupled transporter for monocarboxylates, including the bacterially derived SCFA, endogenous metabolites such as lactate and pyruvate (17, 43, 86), the ketone body β -hydroxybutyrate (79), the B-complex vitamin niacin (42,44), the cyclic amino acid pyroglutamate (85), and even some of pharmacological and therapeutic agents that exist as anionic monocarboxylates at physiological pH (e.g., dichloroacetate, 3-bromopyruvate, and 5-aminosalicylate) (4, 5, 58, 122). The transport process is electrogenic with the influx of Na⁺ and monocarboxylate substrates causing depolarization, thus indicating cotransport of at least two Na⁺ with one monocarboxylate substrate.

Expression and regulation of SMCT1 in normal colon and in colon cancer—As a Na⁺-coupled active transporter for SCFA, some of which are inhibitors of HDACs, it made sense that the transporter functions as a tumor suppressor in colon. But this rationale requires the location of the transporter in the lumen-facing apical membrane of colonic epithelial cells under normal conditions. We showed that this was indeed the case. The transporter expression increases along the intestinal tract from duodenum to colon and the transporter protein is found exclusively in the apical membrane (20, 44) where it has access to luminal SCFA generated by bacterial fermentation. Non-transformed colonic epithelial cells express the transporter but almost all of the colon cancer cells examined show markedly decreased levels of the transporter; the decrease is due to DNA methylation (119). Ectopic overexpression of SLC5A8 in normal cells has no effect on cell viability in the presence or absence of butyrate, propionate or pyruvate, all of which are HDAC inhibitors, indicating that the ability of the transporter to mediate the influx of SCFA has no detrimental effect on normal colon epithelial cells (119). This is not the case with colon cancer cells. As the transporter is absent in these cells, exposure to low concentrations of butyrate, propionate or pyruvate does not affect their viability. In contrast, when SLC5A8 is overexpressed ectopically, the cells undergo apoptosis but only exposed to butyrate or propionate (119). This suggests that SLC5A8-mediated active uptake of SCFA into cancer cells underlies the cell death caused by SCFA. Interestingly, the original paper that described

the tumor-suppressive function of SLC5A8 also reported similar experiments; however, in that paper ectopic expression of the transporter was found to be sufficient to induce cell death in colon cancer cells in the absence of butyrate or propionate (74). This was puzzling because it was not readily apparent how the expression of the transporter protein could cause apoptosis unless coupled to the transporter-mediated uptake of an HDAC inhibitor such as butyrate. We then noticed that colon cancer cells are routinely cultured in the presence of pyruvate, an HDAC inhibitor. There was no effect of this metabolite on the viability of cancer cells because these cells did not express SLC5A8 to mediate concentrative uptake of pyruvate into cancer cells. But when the transporter was expressed ectopically, pyruvate present in the culture medium now entered the cells actively via SLC5A8 and induced cell death; this process involved HDAC inhibition. Analysis of publicly available databases shows downregulation of SLC5A8 in colon cancer in majority of cases (Table 2), which is expected from the tumor-suppressive role of SCFA and the potential role of SLC5A8 in mediating the cellular entry of these metabolites. But the transporter is also silenced in cancers of various noncolonic tissues (e.g., lung, prostate, pancreas, mammary gland, brain, stomach, and liver); the silencing is seen in cancer cell lines originating from these cancers and when the transporter is expressed ectopically, the cells undergo cell death (33). Obviously, the tumor-suppressive function of SLC5A8 in these tissues has nothing to do with SCFA; it is related to pyruvate as an inhibitor of HDACs. Pyruvate is present in circulation at concentrations sufficient to cause HDAC inhibition after actively transported into cancer cells of noncolonic origin via SLC5A8. The tumor-suppressive function of the transporter has been investigated in detail in breast cancer; here ectopic expression of SLC5A8 in breast cancer cells leads to cell death, but again only in the presence of monocarboxylates such as pyruvate that are an HDAC inhibitors (29). These findings highlight the tumor-suppressive activity of pyruvate and also explain why tumor cells possess robust activity to convert pyruvate into lactate, which is not an inhibitor of HDACs, and maintain low levels of pyruvate inside the cells (118). The effective conversion of pyruvate into lactate in tumor cells is a key component of Warburg effect; but this was always thought to be due to defective mitochondrial function resulting in ineffective oxidation of pyruvate, thus necessitating its conversion into lactate so as to maintain high level of glycolysis (35). The discovery that pyruvate is an inhibitor of HDACs but lactate is not adds a new dimension to the tumor-associated lactate production and represents a novel aspect of the Warburg effect.

In normal colon, bacteria-derived SCFA are the primary substrates for SLC5A8. This raised an interesting question as to whether colonic bacteria control the expression of this transporter. This indeed turned out to be the case (20). Normal mice express the transporter in ileum and colon, the expression restricted to the apical membrane. On the other hand, germ-free mice have markedly reduced expression of the transporter, both at mRNA and protein levels. Interestingly, when the colon of germ-free mice is colonized with bacteria, the expression returns to levels comparable to those found in conventional mice over period of about 4 weeks. These findings clearly point to colonic bacteria as a key determinant of SLC5A8 expression. The molecular mechanisms involved in this bacteria-dependent control remain however largely unexplored. *In vitro* studies with intestinal and colonic epithelial cell lines, certain probiotic strains positively regulate the expression of SLC5A8 and also

counteract the negative effects of proinflammatory cytokines or antibiotic treatment on the expression (10, 19). The strains that induce the expression include *Lactobacillus plantarum*, *Lactobacillus casei*, and *Lactobacillus GG*. Oral administration of these strains elicits a similar positive effect on Slc5a8 expression in ileum and colon *in vivo* in mice (10, 19). The ability of the probiotic to reverse the antibiotic-induced suppression of SLC5A8 expression is reproducible with tributyrin, a source of butyrate, suggesting that the bacterial fermentation product butyrate is at least partly responsible for the effects of the probiotic (19); beyond this, nothing much is known on this phenomenon at the molecular level. Nonetheless, the *in vitro* and *in vivo* data with the probiotics confirm the potential role of normal conventional bacteria as a regulator of SLC5A8 expression in colon. Notwithstanding the strong data in support of the expression of SLC5A8 mRNA in colon and its relationship to colon cancer, convincing demonstration of the transport function of this Na⁺-coupled transporter in normal human colon is lacking. Butyrate uptake in the human intestinal cell line Caco-2 or in human colonic apical membrane vesicles is predominantly Na⁺-independent with little evidence of Na⁺-coupled butyrate uptake activity that could be ascribed to SLC5A8 (52, 55). As such, the functional relevance of SLC5A8 to colonic absorption of SCFA across the apical membrane in humans still remains to be established.

Relevance of dietary fiber content to tumor-suppressive function of SLC5A8—

In vitro experiments with colon cancer cell lines have shown convincingly that SLC5A8 does function as a tumor suppressor. Expression profiles for SLC5A8 in colon cancer tissues from patients have also confirmed in a majority of cases the downregulation of the transporter in cancer (Table 2). However, we found no association between overall survival of colon cancer patients and the level of SLC5A8 expression in tumor tissues (Fig. 2). There was a weak correlation ($P = 0.047$) between the levels of SLC5A8 mRNA levels and overall survival when data for patients with colon cancer were analyzed separately. But, the trend correlating higher expression with lower overall survival goes against the tumor-suppressive function of the transporter. If the transporter functions as a tumor suppressor *in vivo*, higher level of expression in tumor tissues would be expected to be associated with a better overall survival and vice versa, but it is not the case. The same was true in experiments with *Slc5a8*-knockout mice (32). There was no difference between wild-type mice and the knockout mice in terms of disease progression and severity in experimental models of colitis and colon cancer. Again, if the transporter plays a protective role against colonic inflammation and carcinogenesis *in vivo*, deletion of the transporter would be expected to increase the progression and severity of colitis and colon cancer. Why then was not the case with *Slc5a8*-knockout mice? The answer to this puzzling question apparently lies in the relative contribution of SLC5A8 to the overall entry of SCFA from lumen into colonic epithelial cells under physiological conditions. SLC5A8 is a high-affinity transporter for SCFA; the Michaelis constant for SLC5A8-mediated transport of SCFA is in the low micromolar range (50–100 $\mu\text{mol/L}$), but the luminal concentrations of SCFA are at least three orders of magnitude higher. Under these conditions, SLC5A8 operates at a fully saturated level and the amount of SCFA entering the cells via this transporter might be much less relative to the entry mediated by the low-affinity SCFA transporter MCT1, which exhibits Michaelis constant for SCFA in the millimolar range. Therefore, it is plausible that under normal

in colon cancer, even though the molecular pathways contributing to this tumor-associated silencing of the gene remain unknown (48). Interestingly, it has been shown recently that the SCFA butyrate is a substrate for ABCG2-mediated efflux in intestinal cells (40, 41). Butyrate as an inhibitor of HDACs protects colon from carcinogenesis; as such, the decreased expression of ABCG2 in colon cancer might potentiate the tumor-suppressive function of this bacterial fermentation product.

Butyrate Transport via SCFA-/HCO₃⁻ Exchange: Indirect Role for MCTs?

With regard to electrolyte absorption in the intestinal tract, Na⁺ and Cl⁻ are absorbed and HCO₃⁻ is secreted in small intestine and colon; the two regions of the intestinal tract differ only in the handling of K⁺, which is absorbed in small intestine but secreted in colon. The secretion of HCO₃⁻ in colon is stimulated by SCFA (8,9). Studies with purified apical membrane vesicles from rat and human colon did provide evidence for an apparent SCFA⁻/HCO₃⁻ exchange; loading of the membrane vesicles with HCO₃⁻ stimulated the uptake of SCFA⁻ into vesicles (80,101,105). However, the exchange between extravesicular SCFA⁻ and intravesicular HCO₃⁻ was not sensitive to inhibitors of prototypical anion exchangers, potentially ruling out direct exchange between SCFA⁻ and HCO₃⁻ through a single transporter functioning as an anion exchanger. But, in the same studies, thiol-modifying agents inhibited the HCO₃⁻-dependent SCFA⁻ uptake and inwardly directed H⁺ gradient stimulated the uptake of SCFA⁻ into vesicles, both being the features of MCT1. The cooccurrence of Na⁺/H⁺ exchangers and Cl⁻/HCO₃⁻ exchangers in the same membrane along with MCT1 and the interrelationship among HCO₃⁻, H⁺, pH, and H₂CO₃ potentially enables functional coupling between the exchangers and MCT1. Furthermore, the same phenomenon of the stimulation of SCFA⁻ uptake by the presence of HCO₃⁻ on the trans side was also observed in colonic basolateral membrane vesicles (98,127). This membrane is not involved in HCO₃⁻ efflux *in vivo* but expresses MCT1 and MCT4. In addition, the SCFA⁻/HCO₃⁻ exchanger in colonic apical membrane postulated to mediate SCFA-stimulated HCO₃⁻ secretion has never been identified at molecular level. Taken collectively, it appears that the H⁺-coupled transporters for SCFA could have contributed indirectly for the SCFA⁻/HCO₃⁻ exchange observed in intact colon or in isolated membrane vesicles.

Conclusion

For a long time, the bacterially derived SCFA were believed to be absorbed in colon predominantly via nonionic diffusion, but now it is clear that the process is primarily carrier-mediated. Transporters for SCFA have been identified and are expressed both in the lumen-facing apical membrane and the serosa-facing basolateral membrane of colonic epithelial cells. SCFA, particularly butyrate, serve as the major metabolic fuel for colonic epithelium; therefore, the transporters responsible for the entry of SCFA are critical for colon health. Three transporters are important for the handling of SCFA by colon; MCT1 (SLC16A1) and SMCT1 (SLC5A8) in the apical membrane and, MCT1 and MCT4 (SLC16A3) in the basolateral membrane (Fig. 3). MCT1, a H⁺-coupled low-affinity transporter, is most likely the major contributor to SCFA entry into colonic epithelium under normal dietary conditions when luminal concentrations of SCFA are high whereas SMCT1, a Na⁺-coupled high-affinity transporter, plays a critical role in the process under conditions of low fiber content

in the diet with consequent decrease in the luminal concentrations of SCFA. Colonic bacteria and luminal butyrate promote the expression of MCT1 and SMCT1 in colon. SCFA protect colon against inflammation and carcinogenesis; hence, MCT1 and SMCT1 function as suppressors of colitis and colon cancer. Accordingly, the levels of these two transporters in colon are decreased significantly in disease states involving colitis and colon cancer. In conclusion, the SCFA transporters MCT1 and SMCT1 provide an important link between colonic bacteria and colon health and are obligatory for the beneficial effects of colonic bacteria and their fermentation products on colon. The ATP-dependent efflux transporter ABCG2, which also accepts butyrate as a substrate, is expressed in the apical membrane; it mediates the efflux of butyrate from the cells, but little is known on the relative contribution of this transporter to the overall handling of butyrate by the colonic epithelium.

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Figure 1.

Relationship between MCT1 (SLC16A1) mRNA expression levels in tumor tissues and overall survival of patients with cancer of colon and rectum. (A) Cancers of colon and rectum combined; (B) cancer of colon; and (C) cancer of rectum. OS, overall survival. In patients with rectal cancer, higher expression of SLC16A1 correlates significantly with improved overall survival. The trend, but not statistically significant, of positive correlation between SLC16A1 expression levels and overall survival is observed when data for patients with colon cancer and rectal cancer are combined. When data for patients with colon cancer are analyzed separately, there is no significant correlation between SLC16A1 expression levels and overall survival.

Figure 2.

Relationship between SMCT1 (SLC5A8) mRNA expression levels in tumor tissues and overall survival of patients with cancer of colon and rectum. (A) Cancers of colon and rectum combined; (B) cancer of colon; and (C) cancer of rectum. OS, overall survival. There is no statistically significant correlation between SLC5A8 mRNA levels in tumor tissues and overall survival of the patients. There is a weak correlation between the two parameters when data for colon cancer alone are analyzed, but this correlation seems to indicate that higher expression of SLC5A8 is associated with lower overall survival, a trend that goes against the tumor-suppressive function of SLC5A8.

Figure 3.

Polarized expression of short-chain fatty acid transporters in colonic epithelium. SCFA, short-chain fatty acids; MCT1, monocarboxylate transporter 1 (SLC16A1); MCT4, monocarboxylate transporter 4 (SLC16A3); SMCT1, sodium-coupled monocarboxylate transporter 1 (SLC5A8); ABCG2, ATP binding cassette transporter, gene family G, member 2. CD147 is the chaperone (ancillary protein) for MCT1 and MCT4.

Table 1

Expression of SCA Transmitters in Colitis (mRNA Levels)

Study	Samples	Transporter	FC	logFC, CI	P value
GSE1710	Ulcerative colitis: <i>n</i> = 10	SLC16A1	1.000	(-0.003, 0.003)	0.876
	Normal control: <i>n</i> = 11 *				
GSE3365	Ulcerative colitis: <i>n</i> = 26	SLC16A1	0.762	(-0.579, -0.205)	<0.001
	Normal control: <i>n</i> = 42 *	SLC5A12	1.730	(-0.011, 1.594)	0.053
GSE6731	Affected UC: <i>n</i> = 5	SLC16A1	0.258	(-2.843, -1.065)	0.003
	Normal sigmoid: <i>n</i> = 4 *	SLC5A12	1.217	(-0.562, 1.130)	0.694
	Affected UC: <i>n</i> = 5	SLC16A1	0.252	(-2.628, -1.354)	<0.001
	Unaffected UC: <i>n</i> = 4 *	SLC5A12	0.701	(-1.379, 0.353)	0.315
GSE9452	Unaffected UC: <i>n</i> = 4	SLC16A1	1.026	(-0.697, 0.771)	0.909
	Normal sigmoid: <i>n</i> = 4 *	SLC5A12	1.737	(0.344, 1.249)	0.012
	Inflamed UC: <i>n</i> = 8	SLC16A1	0.319	(-2.456, -0.837)	0.003
	Normal control: <i>n</i> = 5 *	SLC5A12	0.960	(-0.133, 0.015)	0.144
	Inflamed: <i>n</i> = 8	SLC5A8	1.069	(-0.081, 0.274)	0.259
	Noninflamed UC: <i>n</i> = 13 *	SLC16A1	0.394	(-2.363, -0.324)	0.050
GSE10616	Noninflamed UC: <i>n</i> = 13	SLC5A12	0.974	(-0.091, 0.015)	0.198
	Normal control: <i>n</i> = 5 *	SLC5A8	0.998	(-0.205, 0.199)	0.977
	Ulcerative colitis: <i>n</i> = 10	SLC16A1	0.811	(-1.698, 1.092)	0.652
	Healthy control: <i>n</i> = 11 *	SLC5A12	0.986	(-0.077, 0.035)	0.652
	UC inflamed sigmoid: <i>n</i> = 32	SLC5A8	1.071	(-0.170, 0.369)	0.652
	Normal uninflamed sigmoid: <i>n</i> = 24 *	SLC16A1	0.620	(-1.012, -0.367)	<0.001
GSE11223	UC inflamed sigmoid: <i>n</i> = 32	SLC16A3	1.017	(-0.429, 0.478)	0.912
	Normal uninflamed sigmoid: <i>n</i> = 24 *	SLC5A8	0.928	(-0.271, 0.054)	0.241
	UC inflamed sigmoid: <i>n</i> = 32	SLC16A1	0.980	(-0.070, 0.010)	0.164
	UC inflamed sigmoid: <i>n</i> = 32	SLC16A3	1.260	(0.222, 0.445)	<0.001
		SLC5A12	1.025	(0.007, 0.064)	0.029
		SLC16A1	0.952	(-0.115, -0.028)	0.003

Study	Samples	Transporter	FC	logFC, CI	P value
GSE13367	UC uninflamed sigmoid: $n = 25^*$	SLC16A3	1.239	(0.201, 0.417)	<0.001
		SLC5A12	1.009	(-0.016, 0.043)	0.362
	UC uninflamed sigmoid: $n = 25$	SLC16A1	1.029	(-0.002, 0.085)	0.144
	Normal uninflamed sigmoid: $n = 24^*$	SLC16A3	1.017	(-0.077, 0.127)	0.627
		SLC5A12	1.015	(-0.002, 0.046)	0.144
	UC inflamed: $n = 8$	SLC16A1	0.400	(-2.034, -0.611)	0.002
	Normal control: $n = 10^*$	SLC5A12	0.931	(-0.274, 0.069)	0.299
		SLC5A8	0.920	(-0.443, 0.204)	0.446
	UC inflamed: $n = 8$	SLC16A1	0.726	(-1.193, 0.269)	0.266
	UC noninflamed: $n = 9^*$	SLC5A12	0.925	(-0.255, 0.029)	0.224
		SLC5A8	0.907	(-0.422, 0.140)	0.303
	UC noninflamed: $n = 9$	SLC16A1	0.551	(-1.592, -0.130)	0.094
GSE14580	Normal control: $n = 10^*$	SLC5A12	1.007	(-0.179, 0.200)	0.910
		SLC5A8	1.015	(-0.309, 0.351)	0.910
	UC infliximab responder: $n = 8$	SLC16A1	0.173	(-3.531, -1.538)	<0.001
	Normal control: $n = 6^*$	SLC5A12	0.700	(-1.512, 0.481)	0.405
		SLC5A8	1.037	(-0.944, 1.049)	0.916
	UC infliximab responder: $n = 8$	SLC16A1	1.149	(-0.559, 0.96)	0.598
	UC nonresponder: $n = 16^*$	SLC5A12	0.819	(-1.001, 0.424)	0.558
		SLC5A8	1.293	(-0.314, 1.055)	0.558
	UC nonresponder: $n = 16$	SLC16A1	0.150	(-3.589, -1.881)	<0.001
	Normal control: $n = 6^*$	SLC5A12	0.855	(-1.078, 0.625)	0.592
		SLC5A8	0.802	(-1.049, 0.413)	0.510
	UC twin-pair: $n = 10$	SLC16A1	0.758	(-1.027, 0.226)	0.618
GSE22619	Healthy twin-pair: $n = 10^*$	SLC5A12	1.119	(-0.176, 0.501)	0.618
		SLC5A8	0.985	(-0.089, 0.045)	0.639
	Ulcerative colitis: $n = 15$	SLC16A1	0.408	(-2.255, -0.333)	0.043
	Healthy control: $n = 7^*$	SLC5A12	1.202	(-0.553, 1.084)	0.507
		SLC5A8	1.095	(-0.029, 0.290)	0.137
	UC with neoplasia: $n = 11$	SLC16A1	0.912	(-0.739, 0.474)	0.811

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Study	Samples	Transporter	FC	logFC, CI	P value
GSE47908	Normal control: $n = 5$ *	SLC16A3	1.001	(-0.385, 0.388)	0.993
		SLC5A12	0.814	(-0.456, -0.139)	0.006
		SLC5A8	0.677	(-0.980, -0.145)	0.029
	left-sided colitis: $n = 20$	SLC16A1	0.228	(-2.709, -1.552)	<0.001
		SLC5A12	0.968	(-0.102, 0.008)	0.121
	Normal control: $n = 15$ *	SLC5A8	0.958	(-0.173, 0.048)	0.259
		SLC16A1	0.261	(-2.508, -1.365)	<0.001
	Pan-colitis: $n = 19$	SLC5A12	1.040	(-0.016, 0.129)	0.165
		SLC5A8	0.956	(-0.203, 0.074)	0.352
	Normal control: $n = 15$ *				

* Reference group. UC, ulcerative colitis; FC, fold change; and logFC, CI, log of confidence interval for fold change.

Table 2**Expression of SCA Transports in Colon Cancer (mRNA Levels)**

Study	Samples	Transporter	FC	logFC, CI	P value
GSE9348	Early stage CRC: <i>n</i> = 70	SLC16A1	0.420	(-1.680, -0.823)	<0.001
	Control: <i>n</i> = 12 *	SLC5A12	0.750	(-1.002, 0.173)	0.164
		SLC5A8	0.752	(-0.957, 0.133)	0.164
GSE39582	Colorectal adenocarcinoma: <i>n</i> = 566	SLC16A1	0.440	(-1.536, -0.834)	<0.001
	Nontumoral colorectal mucosa: <i>n</i> = 19 *	SLC5A12	0.962	(-0.110, -0.004)	0.049
		SLC5A8	1.071	(-0.016, 0.215)	0.091
GSE33113	Stage II CRC: <i>n</i> = 90	SLC16A1	0.437	(-1.848, -0.538)	0.001
	Normal colon: <i>n</i> = 6 *	SLC5A12	0.451	(-2.223, -0.074)	0.036
	Colorectal cancer: <i>n</i> = 186	SLC16A1	0.967	(-0.28, 0.183)	0.680
GSE41258	Normal colon: <i>n</i> = 54 *	SLC5A12	1.264	(0.162, 0.513)	<0.001
	Primary tumor: <i>n</i> = 381	SLC16A1	0.661	(-0.835, -0.36)	<0.001
	Normal: <i>n</i> = 51 *	SLC16A3	1.049	(-0.178, 0.317)	0.581
TCGA-COADREAD		SLC5A12	0.335	(-2.049, -1.104)	<0.001
		SLC5A8	0.352	(-1.937, -1.076)	<0.001
		SLC16A1	0.600	(-1.007, -0.465)	<0.001
TCGA-COAD	Primary tumor: <i>n</i> = 286	SLC16A3	1.022	(-0.254, 0.318)	0.828
	Normal: <i>n</i> = 41 *	SLC5A12	0.336	(-2.096, -1.052)	<0.001
		SLC5A8	0.379	(-1.893, -0.904)	<0.001
TCGA-READ	Primary tumor: <i>n</i> = 94	SLC16A1	0.971	(-0.546, 0.46)	0.867
	Normal: <i>n</i> = 10 *	SLC16A3	1.208	(-0.218, 0.764)	0.342
		SLC5A12	0.328	(-2.711, -0.507)	0.008
		SLC5A8	0.263	(-2.81, -1.047)	<0.001

* Reference group, FC, fold change; logFC, CI, log of confidence interval for fold change.