

Biological functions of SLC5A8, a candidate tumour suppressor

V. Ganapathy¹, E. Gopal, S. Miyauchi and P.D. Prasad

Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912, U.S.A.

Abstract

SLC5A8 is a candidate tumour suppressor gene that is silenced in colon cancer, gastric cancer and possibly other cancers in humans. This gene codes for a transporter belonging to the Na⁺/glucose co-transporter gene family (*SLC5*). The cancer-associated silencing of the gene involves hypermethylation of CpG islands present in exon 1 of the gene. *SLC5A8* is expressed in colon, ileum, kidney and thyroid gland. The protein coded by the gene mediates the Na⁺-coupled and electrogenic transport of a variety of monocarboxylates, including short-chain fatty acids, lactate and nicotinate. It may also transport iodide. The normal physiological function of this transporter in the intestinal tract and kidney is likely to facilitate the active absorption of short-chain fatty acids, lactate and nicotinate. One of the short-chain fatty acids that serves as a substrate for *SLC5A8* is butyrate. This fatty acid is an inhibitor of histone deacetylases and is known to induce apoptosis in a variety of tumours including colonic tumour. Since butyrate is produced in the colonic lumen at high concentrations by bacterial fermentation of dietary fibre, we speculate that the ability of *SLC5A8* to mediate the entry of this short-chain fatty acid into colonic epithelial cells underlies the potential tumour suppressor function of this transporter.

Introduction

SLC5A8 was originally cloned by Rodriguez et al. [1] in an attempt to identify iodide transporters that might play a functional role in the physiology of thyroid gland. Iodide accumulation from the blood into thyroid follicular cells occurs via a Na⁺-coupled iodide transporter, known as Na/I symporter (NIS) that is expressed in the basolateral membrane of these cells [2]. Once inside the cells, iodide participates in the organification of thyroglobulin leading to the synthesis of protein-bound tetraiodothyronine (T₄) and triiodothyronine (T₃). A significant portion of iodide diffuses across the apical membrane of the follicular cells that lines the colloid lumen [3]. Pendrin, an anion exchanger, has been shown to be at least partly responsible for this diffusion process [4]. With the purpose of identifying new iodide transporters in thyroid gland, Rodriguez et al. [1] searched the EST database for mRNA sequences that are structurally similar to NIS. This search led to the identification of a gene that codes for a protein with 46% identity in amino acid sequence with NIS. This putative transporter represents the eighth member of the *SLC5* gene family and hence the gene is designated *SLC5A8*. The gene is located on human chromosome 12q13. The protein product of *SLC5A8* is expressed specifically in the apical membrane of thyroid follicular cells and apparently mediates passive transport of iodide [1]. Accordingly, the protein was named 'apical iodide transporter' (AIT) [1]. Even though the role of Na⁺ in the

transport process mediated by AIT was not investigated directly, the studies by Rodriguez et al. [1] implied that the transporter functions as a Na⁺-independent iodide transporter. This was surprising because all other known members of the *SLC5A8* gene family function in a Na⁺-dependent manner [5]. The expression of this transporter is significantly altered in a variety of benign and malignant human thyroid tissues [6]. *SLC5A8* is also expressed in kidney, though at much lower levels than in thyroid tissue, but the potential functional relevance of the protein in the kidney as a Na⁺-independent facilitative iodide transporter was not addressed [1].

More recently, the same gene was identified by Li et al. [7] as a putative tumour suppressor in human colon cancer. The expression of the gene is silenced in colon neoplasia by hypermethylation of CpG-rich islands in exon 1 of the gene. This region remains unmethylated in normal colon mucosa but gets methylated in 60% of primary colon cancers. Methylation of exon 1, which occurs as an early event detectable even in microscopic aberrant crypt foci in colonic mucosa, leads to silencing of the gene. Inactivation of the gene in colonic epithelial cells seems to confer a selective proliferative advantage, as evident from the suppression of colony-forming ability of colon cancer cell lines upon expression of the transporter. Silencing of *SLC5A8* may not be restricted to colon cancer. There is evidence that a similar aberrant methylation of *SLC5A8* leading to silencing of the gene occurs in gastric cancer [8] and in oligodendroglioma [9]. If *SLC5A8* is indeed a facilitative iodide transporter as suggested by Rodriguez et al. [1], the question arises as to the functional relevance of a passive iodide transporter to tumour suppression. Interestingly, studies by Li et al. [7] have led to

Key words: cancer, lactate reabsorption, niacin, short-chain fatty acids, *SLC5A8*, tumour suppressor.

Abbreviations used: AIT, apical iodide transporter; MCT, monocarboxylate transporter; NIS, Na/I symporter; SMCT, sodium-coupled monocarboxylate transporter.

¹To whom correspondence should be addressed (email vganapat@mail.mcg.edu).

a totally different conclusion with respect to the biological function of this protein. These investigators have shown that SLC5A8 is a sodium transporter. Based upon the fact that SLC5A8 is a member of the Na⁺/glucose co-transporter gene family, Li et al. [7] speculated that SLC5A8 is a Na⁺-coupled transporter for a hitherto unidentified organic or inorganic solute. But, the co-transported solute was not identified.

The abundant expression of SLC5A8 in human colon and kidney and its role as a tumour suppressor in a variety of cancers including colonic tumour raised doubts about the passive iodide transport as the primary biological function of this transporter. Therefore, we cloned this transporter from human intestine and mouse kidney and investigated its transport function. These studies have established that SLC5A8 is actually a Na⁺-coupled transporter for a variety of monocarboxylates, including short-chain fatty acids, lactate and nicotinate. Iodide may be a weak substrate for the transporter. In this short review, we summarize the functional aspects of this transporter and speculate on the potential link between its transport function and its ability to serve as a tumour suppressor.

Functional identity of SLC5A8 as a Na⁺-coupled transporter for short-chain fatty acids

The expression of SLC5A8 in *Xenopus laevis* oocytes led to robust induction of Na⁺-dependent uptake of a variety of short-chain fatty acids such as acetate, propionate and butyrate as well as lactate [10]. The transport process was electrogenic, as evident from substrate-induced inward currents under voltage-clamp conditions. The affinities of the transporter for its various substrates were in the following order: butyrate (81 ± 17 μM) > propionate (127 ± 14 μM) > L-lactate (235 ± 24 μM) > D-lactate (742 ± 330 μM) > acetate (2.46 ± 0.89 mM). Fatty acids with five to eight carbon atoms were also transported via SLC5A8 but less effectively compared to shorter fatty acids. The Na⁺-activation kinetics of the transport process revealed that multiple Na⁺ ions were involved in the transport mechanism. The charge-to-substrate transfer ratio for propionate was 3, suggesting that the transport process involves the co-transport of four Na⁺ and one propionate. Propionate exists mostly as an anion under the experimental conditions employed in the study. Therefore, a Na⁺/propionate stoichiometry of 4:1 provides the basis for the observed electrogenic nature of the transport process.

The substrate specificity of SLC5A8 is similar to that of the previously identified monocarboxylate transporters (MCTs) [11]. But, there is no sequence similarity between the members of the MCT family and SLC5A8. In spite of the similar substrate specificity, SLC5A8 and MCTs differ in their transport mechanisms. In contrast to SLC5A8, which functions as a Na⁺-coupled electrogenic transporter, MCTs function as H⁺-coupled electroneutral transporters. Based on these functional studies, we named SLC5A8

as SMCT (sodium-coupled monocarboxylate transporter). These results have been confirmed independently by Coady et al. [12]. The rodent SMCT, cloned from mouse kidney, also functions as a Na⁺-coupled transporter for short-chain fatty acids and lactate [13]. The transport function could be demonstrated not only in *X. laevis* oocyte expression system, but also in a mammalian cell expression system.

Interestingly, the charge-to-substrate ratio seems to differ depending on the substrate. Whereas this ratio is 3 for propionate, the value becomes 1 for lactate. Thus, the Na⁺/substrate stoichiometry seems to vary depending on the transported substrate (4:1 for propionate and 2:1 for lactate). Alternatively, the transport function may be associated with uncoupled Na⁺ movement, the magnitude of which differs for different substrates. α-Cyanohydroxycinnamic acid, an inhibitor of MCTs, does not interact with SMCT.

SMCT is also a Na⁺-coupled transporter for niacin, a water-soluble vitamin

MCTs are known to interact with nicotinic acid (niacin), a water-soluble B-complex vitamin [14]. Therefore, we examined whether nicotinate is a substrate for SMCT. These studies have shown that SMCT is indeed capable of mediating Na⁺-coupled transport of nicotinate (E. Gopal, J. Fei, S. Miyauchi, L. Zhuang, P. Prasad and V. Ganapathy, manuscript submitted for publication). The Michaelis-Menten constant for this monocarboxylate is ~250 μM. The transport process is electrogenic with a Na⁺/nicotinate stoichiometry of 2:1. The charge-to-substrate ratio is 1, a value that agrees with the stoichiometry data. In this respect, nicotinate is similar to lactate but differs from propionate. The transporter does not recognize nicotinamide as a substrate, indicating the essential nature of the free carboxylate group for interaction with the substrate-binding site. The transport of nicotinate via SMCT is competitively inhibited by lactate and short-chain fatty acids such as acetate, propionate and butyrate.

Physiological functions of SMCT

SMCT is expressed abundantly in the colon, ileum, kidney and thyroid gland. Based on the data that SMCT functions as a Na⁺-coupled transporter for short-chain fatty acids, lactate and nicotinate, we speculate that this transporter plays a role in the absorption of these monocarboxylates in the colon and kidney. Short-chain fatty acids such as acetate, propionate and butyrate are produced at high concentrations in colonic lumen [15,16]. These fatty acids serve as the preferred metabolic fuel for colonic epithelial cells. The transporter is expressed predominantly in the apical membrane of the colonic epithelium (P.M. Martin, E. Gopal and V. Ganapathy, unpublished work) and therefore it is likely that the transporter mediates Na⁺-coupled active absorption of these short-chain fatty acids into the cells. Luminal addition of short-chain fatty acids is known to stimulate Na⁺ absorption in the colon [15,16] and the Na⁺-coupled transport function of SMCT may be at least partly responsible for this phenomenon. Since SMCT is expressed not only in the colon

but also in the ileum, dietary nicotinate may be absorbed effectively by the Na^+ -coupled process mediated by this transporter.

The levels of short-chain fatty acids as well as nicotinate in circulation are very low and therefore active reabsorption of these monocarboxylates may not be the principal physiological function of this transporter in the kidney. Instead, we believe that lactate is the primary substrate for the transporter in the kidney. Lactate is present in blood in high concentrations (1–1.5 mM), but very little lactate is excreted in the urine. This indicates the presence of an efficient reabsorption mechanism for this monocarboxylate in the kidney. Na^+ -coupled lactate transport has been demonstrated in the apical membrane of kidney tubular cells [17–19]. We have recently shown that lactate transport in kidney apical membrane vesicles is Na^+ -dependent and is inhibitable by short-chain fatty acids [13], suggesting that SMCT is responsible for lactate transport in these membrane vesicles. Thus, SMCT represents the first mammalian transporter identified that has the ability to mediate Na^+ -coupled transport of lactate.

The physiological function of SMCT in thyroid gland remains unknown. It is very unlikely that SMCT is responsible for Na^+ -independent facilitative transfer of iodide across the apical membrane of thyroid follicular cells. Iodide may be a substrate for the transporter as evident from the studies by Rodriguez et al. [1], but the transport process is expected to be Na^+ coupled rather than facilitative. It is not immediately apparent what physiological function such an active transport system for iodide in the apical membrane of the follicular cells would serve that is relevant to the biology of the thyroid gland.

Relevance of the transport function of SMCT to its predicted tumour suppressive role

Down-regulation of the expression of SMCT in a variety of cancers including colon cancer suggests that this transporter may have a tumour suppressive role. Functional studies have established that SMCT is a Na^+ -coupled transporter for short-chain fatty acids, lactate and nicotinate. Is there a connection between the transport function of SMCT and its tumour suppressive role? Butyrate is a substrate for this transporter and this fatty acid is produced at high levels in the colonic lumen. Butyrate is an inhibitor of histone deacetylases [20]. We speculate that the ability of SMCT to mediate the entry of butyrate into colonic epithelial cells underlies the tumour suppressive role of this transporter. The acetylation status of histones is an important determinant of gene expression. Inhibitors of histone deacetylases have been shown to cause growth arrest and apoptosis in a variety of tumours and hence are being considered as potential therapeutic agents for the treatment of cancer [20]. The presence of high levels of butyrate in the colonic lumen under physiological conditions and active transport of butyrate into colonic epithelial cells via SMCT mean that these cells are

constantly subjected to inhibition of histone deacetylases. Numerous studies have associated butyrate levels in colon with decreased incidence of colon cancer [21,22]. Gut flora have often been suggested to play a role in the prevention of colon cancer. Fermentation of unabsorbed carbohydrates and dietary fibre by gut flora occurs predominately in the proximal colon resulting in higher concentrations of butyrate in the proximal colon than in the distal colon. Interestingly, most tumours occur in the distal colon where the concentrations of butyrate are relatively low. There is also epidemiological evidence indicating an inverse relationship between dietary intake of milk fermentation products and occurrence of colon cancer [23]. It is possible that butyrate-induced inhibition of histone deacetylases may alter gene expression in the colon in such a way that genesis and/or progression of tumour is suppressed. One possibility is that butyrate-induced changes in the expression of various enzymes and transporters may prevent or reduce the exposure of colonic epithelial cells to dietary carcinogens.

SMCT as a link between gut flora and intestinal and colonic function

The human microbiota is a complex ecosystem that plays a critical role in the health and disease of the host. The largest population of endogenous bacteria is present in the intestinal tract and most of it is found in the colon. The gut flora directs the assembly of the gut-associated lymphoid tissue, helps educate the immune system, affects the integrity of the intestinal mucosal barrier, modulates proliferation and differentiation of various epithelial lineages and regulates angiogenesis [24]. The presence or absence of gut flora has major implications for the development and function of the mucosal immune system. Gut-associated lymphoid tissue is rudimentary in germ-free animals. This is due to defective microbial education of immune responses early in development. Gut flora have significant effects on the expression of genes in the intestinal tract [25]. Recently, specific G-protein-coupled receptors have been identified for short-chain fatty acids in mammals and these receptors are expressed predominantly on the plasma membrane of immune cells [26–28]. The gut-associated immune cells are present in the lamina propria just under the epithelial cell layer. The effective concentration of short-chain fatty acids to activate their receptors is in the range of 0.01–1 mM. Because the luminal concentrations of short-chain fatty acids are quite high in the colon, these fatty acids are likely to reach high enough concentrations on the serosal side of the colonic epithelial cell layer, aided by their active absorption via SMCT. Therefore, the receptors on the gut-associated immune cells are expected to be activated under physiological conditions. Such an activation of the gut immune system by the metabolic products of gut flora has relevance to various physiological and pathological conditions related to intestinal inflammation and anti-tumour immunity. SMCT is expected to play a central role in this process as the transporter for these bacterial metabolic products in the colonic epithelium.

Conclusions

SMCT is a member of the Na⁺/glucose co-transporter family and functions as a Na⁺-coupled transporter for short-chain fatty acids, lactate and nicotinate. It is expressed abundantly in the colon, ileum, kidney and thyroid gland. The expression of this transporter is down-regulated in a variety of tumours, including colonic tumour. The ability of the transporter to mediate active absorption of butyrate, an inhibitor of histone deacetylases, may underlie its tumour suppressive role in the colon. Short-chain fatty acids also serve as ligands for specific G-protein-coupled receptors on gut-associated immune cells. Since the concentrations of these fatty acids in the colonic lumen are quite high, SMCT may facilitate transcellular transfer of these fatty acids from the lumen into lamina propria where the immune cells reside and thus provide a link between gut flora and gut immune system. In the kidney, the primary physiological function of SMCT is to mediate active reabsorption of lactate from the glomerular filtrate. Iodide may be a weak substrate for SMCT, but the precise role of the transporter in thyroid gland remains to be determined.

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