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SLC transporters as a novel class of tumour suppressors: identity, function and molecular mechanisms

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Abstract

The role of plasma membrane transporters in cancer is receiving increasing attention in recent years. Several transporters for essential nutrients are up-regulated in cancer and serve as tumour promoters. Transporters could also function as tumour suppressors. To date, four transporters belonging to the *SLC* gene family have been identified as tumour suppressors. SLC5A8 is a Na^+-coupled transporter for monocarboxylates. Among its substrates are the bacterial fermentation products butyrate and propionate and the ubiquitous metabolite pyruvate. The tumour-suppressive function of this transporter relates to the ability of butyrate, propionate and pyruvate to inhibit histone deacetylases (HDAC). SLC5A8 functions as a tumour suppressor in most tissues studied thus far, and provides a molecular link to Warburg effect, a characteristic feature in most cancers. It also links colonic bacteria and dietary fibre to the host. SLC26A3 as a tumour suppressor is restricted to colon; it is a Cl^-/HCO_3^- exchanger, facilitating the efflux of HCO_3^-. The likely mechanism for the tumour-suppressive function of SLC26A3 is related to intracellular pH regulation. SLC39A1 is a Zn^{2+} transporter and its role in tumour suppression has been shown in prostate. Zn^{2+} is present at high concentrations in normal prostate where it elicits its tumour-suppressive function. SLC22A18 is possibly an organic cation transporter, but the identity of its physiological substrates is unknown. As such, there is no information on molecular pathways responsible for the tumour-suppressive function of this transporter. It is likely that additional SLC transporters will be discovered as tumour suppressors in the future.

Keywords

citrate metabolism; histone deacetylases (HDAC) inhibition; pH regulation; SLC transporters; tumour suppressors

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INTRODUCTION

A tumour suppressor is a protein that controls cell division and proliferation by keeping these processes in check. When its function is compromised either due to inactivating or gain-of-function mutations or suppression of expression, uncontrolled cell division and proliferation ensue. Often, in combination with other oncogenic events, loss of function of tumour suppressors results in cancer. The genes coding for such proteins are called tumour suppressor genes or anti-oncogenes. For a protein to be defined as a tumour suppressor, the protein has to have the ability to control cell proliferation, its normal function or expression has to be silenced during tumorigenesis and re-activation or re-expression of the protein in tumour cells or tumours has to block cell proliferation and tumour growth. A majority of the known tumour suppressors are involved in the regulation of gene expression or signal transduction. Loss of function of tumour suppressors can occur due to mutations in the coding region resulting in non-functional mutants, truncated proteins or proteins with new functions. Alternatively, the expression level of the normal protein might be decreased due to mutations in the gene promoter or silencing of the gene by epigenetic mechanisms (e.g. DNA methylation, histone methylation or histone acetylation).

Transporters are integral transmembrane proteins located in various cellular membranes including plasma membrane, lysosomal membrane, inner mitochondrial membrane, peroxysomal membrane and endoplasmic reticulum. These proteins mediate translocation of inorganic and organic solutes across these membranes and, depending on a given transporter, the process occurs with or without coupling to driving forces. For a long time, the only class of transporters that was receiving attention in relation to cancer was the drug efflux transporters (e.g. P-glycoprotein) present in the plasma membrane because of their role in the control of sensitivity of tumour cells to chemotherapeutic agents. Often times, cancer cells up-regulate one or more of these drug efflux transporters when chronically exposed to selective anticancer drugs, consequently inducing development of drug resistance. In recent years, considerable attention has turned to other plasma membrane transporters, with a selective focus on nutrient delivery to tumour cells (e.g. glucose and amino acids). The basis for this focus is logical because tumour cells have an increased demand for nutrients to support their rapid growth and they have to obtain these nutrients via plasma membrane transporters. Cancer biologists have recently become fascinated with Warburg hypothesis and ‘aerobic glycolysis’ as a unique metabolic pathway (i.e. metabolic conversion of glucose to pyruvate and then to lactate even in the presence of oxygen) that occurs in tumour cells [1–3], and as a consequence turned their attention to glucose transporters. This has resulted in the discovery that the facilitative glucose transporter SLC2A1 (GLUT1) is up-regulated in almost all cancers [4], which feeds glucose into aerobic glycolysis. Multiple signalling mechanisms including HIF-1α and the oncogenic protein c-MYC are responsible for this up-regulation. In some selective cancer types, the Na^+-coupled concentrative glucose transporters SGLT1 (SLC5A1) and SGLT2 (SLC5A2) are also up-regulated [5,6]. The increased uptake of glucose in tumour cells provides the basis for *in vivo* diagnosis of cancer in patients using positron emission tomography (PET) with appropriate ^{18}F-labelled non-metabolizable glucose analogues as the substrates of the glucose transporters (for example, 2-fluoro-2-deoxyglucose for GLUT1). Since these glucose transporters provide an essential

nutrient to tumour cells, they serve as tumour promoters and hence are potential targets for cancer therapy [4].

With a similar logic, amino acid transporters are now being noticed for their relevance to cancer [7,8]. Unlike the glucose transporters that provide glucose as the energy source and also as the carbon source for synthesis of other important biological molecules in tumour cells, amino acid transporters have an impact on a broader spectrum of biological processes. Amino acids are needed not only for the synthesis of proteins (all amino acids) but also for the synthesis of purines and pyrimidines (glutamine, aspartate, glycine and serine). Selective amino acids (leucine, glutamine, arginine, tryptophan) are also potent activators of mTORC1 signalling pathway [9]. Epigenetic modifications of DNA and histones are also dependent on some amino acids (serine and methionine). In addition, amino acids provide carbon source for the synthesis of other biological molecules necessary for cancer cell growth and survival. The transporters that have been shown thus far to be relevant to amino acid nutrition in cancer cells include SLC1A5 (also known as ASCT2, a Na^+-coupled electroneutral transporter for glutamine, alanine, serine and cysteine, which functions as an amino acid exchanger) [10,11], SLC7A5 (also known as LAT1, a Na^+-independent electroneutral transporter for a broad spectrum of neutral amino acids including most of the essential amino acids, which also functions as an amino acid exchanger) [12], SLC6A14 (also known as $ATB^{0,+}$, a Na^+- and Cl^--coupled electrogenic transporter for all amino acids except glutamate and aspartate, which functions almost as a uniporter mediating amino acid influx into cells) [13–15], SLC7A11 (also known as xCT, a Na^+-independent electroneutral cystine/glutamate antiporter) [16,17], SLC38A5 (also known as SNAT5 or SN2, a Na^+-coupled electroneutral transporter for glutamine, asparagine and histidine, which is coupled to the efflux of H^+) [18] and SLC38A2 (also known as SNAT2, a Na^+-coupled electrogenic transporter for glutamine and small amino acids such as alanine, glycine, serine and valine) [19]. As with glucose transporters, these amino acid transporters play an obligatory role in the growth of tumour cells by providing an important class of essential nutrients; accordingly these transporters are also tumour promoters and hence represent potential drug targets for cancer therapy [7].

One of the unique features of cancer cells is the excessive generation of lactic acid as a result of aerobic glycolysis; if not taken care of, this process will result in cellular acidification and consequently kill cancer cells. As expected, cancer cells have developed specific mechanisms to remove lactate and H^+ out of the cells in order to prevent cellular acidification. This process again involves plasma membrane transporters. The monocarboxylate transporters SLC16A1 (MCT1) and SLC16A3 (MCT4) are up-regulated in tumour cells [20]. Even though both these transporters are electroneutral H^+ /lactate co-transporters and in theory can work in either direction (i.e. entry of lactic acid into the cell or out of the cell), they function principally as efflux transporters for lactic acid in tumour cells because of the overproduction of lactic acid inside these cells; the concentration gradient for lactic acid across the plasma membrane dictates the direction of the function of these transporters in tumour cells. Another group of transporters that is critical for the maintenance of intracellular pH in tumour cells is the Na^+ /H^+ exchangers SLC9A1 (NHE1) and SLC9A3 (NHE3) [21–23]. Both mediate the efflux of H^+ out of the cells coupled to the influx of Na^+ in an electroneutral manner. These transporters are also up-regulated in cancer

as a means to prevent intracellular acidification. The amino acid transporter SLC38A5, whose function is also coupled to H^+ efflux [24,25], may also have a critical role in this process. Again, since all of these transporters play an essential role in the biology of cancer cells, their functions fuel tumour growth. As such, these transporters are also tumour promoters and are likely targets for the design of anticancer drugs.

The idea that plasma membrane transporters can also function as tumour suppressors has not gained traction in the field of cancer biology. In theory, any of the transporters whose function leads to intracellular acidification could function as a tumour suppressor; similarly, any of the transporters whose function is linked to increased DNA methylation or histone acetylation could also function as tumour suppressors. Interestingly, the Tumour Suppressor Gene Database, which lists 716 tumour suppressor genes, does include four genes coding for plasma membrane transporters in the list of tumour suppressor genes. To date, the database identifies 637 protein-coding genes and 79 non-coding genes as tumour suppressor genes; among the protein-coding genes lie the genes coding for the four transporters belonging to the *SLC* gene family (SLC5A8, SLC26A3, SLC39A1 and SLC22A18). The purpose of this review is to summarize the physiological functions of the protein products of these four genes and provide a logical basis for the tumour-suppressive functions of the four transporters.

SLC5A8

Discovery of SLC5A8 as a tumour suppressor in colon

SLC5A8 is a transporter belonging to the *SLC5* gene family that consists of several Na^+-coupled nutrient transporters; the members of this family include transporters for glucose and galactose, choline, mannitol, *myo*-inositol, biotin, pantothenate and iodine [26]. Li et al. [27] reported in 2003 that SLC5A8 is a putative tumour suppressor in colon; prior to this report, nothing was known on the function of this transporter or on its relevance to cancer. The findings of the study were that the transporter was expressed robustly in normal colon, the expression was silenced in colon cancer, the silencing was the result of DNA methylation at *SLC5A8* promoter both in primary tumours and in human colon cancer cell lines, inhibition of DNA methylation increased the expression of the transporter in colon cancer cells and re-expression of the transporter in colon cancer cells induced cell death. All these findings fit well with the definition of a tumour suppressor. Nonetheless, neither the exact function of the transporter nor how its transport function is associated with its tumour suppressive role was known. Li et al. [27] identified it as a sodium transporter simply based on the fact that it belonged to the *SLC5* gene family where the other known members were all Na^+-coupled transporters. The authors did demonstrate that expression of SLC5A8 in *Xenopus* oocytes increased intracellular concentrations of Na^+, thus establishing its function as a Na^+ transporter, but the identity of the substrate that was co-transported with Na^+ was not established. Immediately following this report, we and others have shown that SLC5A8 is in fact a Na^+-coupled transporter for short-chain fatty acids (SCFA) such as acetate, propionate, butyrate, pyruvate and lactate [28,29]. The transporter is expressed in colon selectively on the lumen-facing brush-border membrane of epithelial cells [30,31], indicating that its substrates under physiological conditions are acetate, propionate and

butyrate, which are generated in large quantities as a result of bacterial fermentation of dietary fibre (Figure 1A). The transport of these bacterial metabolites by SLC5A8 is energy-coupled and electrogenic with the involvement of transmembrane electrochemical Na^+ gradient. The transport process involves co-transport of the monocarboxylate substrate along with a minimum of 2 Na^+. How does this function relate to the tumour-suppressive role of SLC5A8? Butyrate and propionate, in addition to serving as the substrates for β-oxidation and citric acid cycle to generate metabolic energy, are also inhibitors of histone deacetylases (HDAC), particularly the isoforms HDAC1 and HDAC3 [32]. These endogenous HDAC inhibitors are present in normal colon lumen, and SLC5A8 mediates their concentrative entry into colonic epithelial cells with subsequent inhibition of HDACs. This provides a mechanism for the tumour-suppressive function of the transporter [33,34].

SLC5A8 as a link between colonic bacteria and the host

The human gut contains a large range of microorganisms, their number outnumbering human cells by 10 to 1. Though called ‘commensal bacteria’, the relationship between gut bacteria and the host is definitely not commensal but actually mutual. It is generally accepted that normal bacteria in colon protect against cancer, but little is known on the underlying molecular mechanisms except that the SCFA generated by fermentation of dietary fibre by these bacteria might be responsible for this phenomenon. This seemed logical because dietary fibre also is known to decrease the risk of colon cancer; if SCFA protect against colon carcinogenesis, it could be the common mediator linking the anticancer effects of dietary fibre and the normal colonic bacteria.

Relevance of HDAC inhibition to the tumour-suppressive function of SLC5A8

Two of the SCFA generated in the colonic lumen by bacterial fermentation of dietary fibre, namely butyrate and propionate, function as HDAC inhibitors. This provides some insight into the ability of these bacterial metabolites to reduce the risk of colon cancer. Cancer cells express higher levels of HDACs than normal cells [32], and inhibition of HDACs, particularly inhibition of class I HDACs (HDACs 1, 2, 3 and 8) is effective in cancer treatment [35]. Propionate and butyrate are inhibitors of HDACs 1 and 3 [32]; this provides a molecular basis for the anticancer effects of these bacterial metabolites. HDAC inhibitors induce cell cycle arrest and prevent proliferation in cancer cells through p21 (an inhibitor of cyclin-dependent kinase)-dependent as well as p21-independent pathways. p21-independent pathways might involve activation of the members of the INK4 (inhibitor of cyclin-dependent kinase 4) family of cyclin-dependent kinase inhibitors [36]. As p21 is a direct target for the tumour suppressor p53, the p21-dependent mechanism of tumour suppression by HDAC inhibitors is likely to involve p53. Butyrate does indeed induce p53 expression in cancer cells [37]; in addition, butyrate also activates p53 by increasing its acetylation status, resulting in increased expression of its target genes [38]. Acetylation/deacetylation represents an important mode of regulation of p53 activity, and HDAC1, a target for butyrate, is capable of de-acetylating p53 [39]. p53 being a pluripotent tumour suppressor, the involvement of this protein in the tumour-suppressive actions of butyrate makes sense. Butyrate and propionate are likely to activate p53 via their ability to inhibit HDAC1 and consequently increase the acetylation status of p53. As normal cells express low levels of HDAC1 and HDAC3, butyrate and propionate do not have any noticeable deleterious effects

on these cells [32,40]. It is important to note here that these SCFA actually elicit beneficial effects on normal colonic epithelial cells by facilitating their differentiation and also by providing an important source of metabolic energy [41,42]. Normal colon epithelial cell lines such as CCD841 constitutively express SLC5A8, and exposure of these cells to butyrate does not lead to apoptosis [40]. This is true even after additional expression of SLC5A8 by cDNA transfection. In contrast, colon cancer cell lines such as SW480 do not express the transporter; in the absence of this transporter, exposure of the cancer cell lines to butyrate does not induce apoptosis. However, when SLC5A8 is expressed by cDNA transfection, exposure of the cells to butyrate induces apoptosis [40]. The same phenomenon of the differential effects of SLC5A8/butyrate on normal cells compared with cancer cells is also evident with pyruvate, also a substrate for SLC5A8 and an inhibitor of HDAC1 and HDAC3 [32]. Activation of the death receptor pathway (TRAIL, DR5, Fas and FasL) by butyrate and propionate may represent a p53-independent mechanism for the tumour-suppressive functions of these bacterial metabolites. Butyrate enhances the sensitivity of tumour cells to Fas-mediated cytotoxicity [43–45] and also up-regulates the death receptor 5 (DR5) and TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) pathway [46,47]. Previously we reviewed the various molecular mechanisms by which butyrate is known to function as a tumour suppressor through its ability to inhibit HDACs [33]; these mechanisms include increases in p53 activity, metastasis suppressors, DR5/TRAIL-mediated cytotoxicity and oxidative stress.

The question that remained to be answered was how these metabolites get across the brush-border membrane from the lumen into colonic epithelial cells to have access to HDACs, their intracellular targets. The discovery of SLC5A8 as a transporter for these metabolites and the selective expression of the transporter on the lumen-facing brush-border membrane of colonic epithelial cells provide the answer to this question. The notion that SLC5A8 serves as an important biological link between colonic bacteria and the host in protection against colon cancer is further strengthened by the findings in mice that these bacteria play an active role in controlling the expression of the transporter in colon [48]. Although normal mouse colon expresses Slc5a8 robustly, the expression is markedly suppressed in germ-free mice; the expression reverts back to normal levels when germ-free mice are re-colonized with bacteria.

Relevance of the ubiquitous metabolite pyruvate to the tumour-suppressive function of SLC5A8

When Li et al. [27] discovered SLC5A8 as a putative tumour suppressor in colon, they were able to demonstrate that ectopic expression of the transporter in colon cancer cells induced cell death. This was before the discovery of the actual function of SLC5A8 as a Na^+-coupled transporter for the bacteria-derived HDAC inhibitors butyrate and propionate. Neither of these bacterial metabolites was included during the assay when the ability of SLC5A8 to induce cell death in cancer cells was demonstrated by these investigators. This indicated that the culture medium used in these studies must have contained some compound that was a substrate for SLC5A8 and also functioned just like butyrate and propionate inside the cells. Subsequent studies in our laboratory showed that pyruvate is the most likely compound with such characteristics [40,49]. Serum contains substantial amounts of pyruvate (~100 μM) and

this compound is added very often to cell culture media as a supplement. When SLC5A8 is expressed ectopically in colon cancer cells and cultured in the absence of pyruvate or butyrate, no cell death is observed [40]. When SLC5A8-expressing cancer cells are exposed to either of the compounds, cell death ensues, demonstrating that, similar to butyrate, pyruvate also induces cell death in cancer cells in an SLC5A8-dependent manner. Pyruvate is not only a high-affinity substrate for SLC5A8 just like butyrate but also an HDAC inhibitor just like butyrate [32]. Both in colon cancer cells and breast cancer cells, SLC5A8-mediated entry of pyruvate leads to inhibition of HDAC1 and HDAC3, resulting in apoptosis [32,49]. In both cases, normal cells express SLC5A8 that mediates the entry of pyruvate but do not undergo apoptosis in response to pyruvate. This tumour-cell selectivity of apoptosis induced by pyruvate-dependent inhibition of HDACs is similar to that of butyrate.

These findings relating pyruvate to the tumour-suppressive function of SLC5A8 explain why the transporter is silenced not only in colon cancer but also in cancers of all tissues that have been examined to date. This includes cancers of mammary gland, prostate, pancreas, stomach, thyroid, lung, head and neck and blood cells. Although butyrate is significant to colon because of its generation in the colonic lumen via bacterial fermentation of dietary fibre, it has little relevance to non-colonic tissues because butyrate is present in circulating blood only at concentrations less than 10 μM. In contrast, the blood levels of pyruvate are 10 times higher than that of butyrate; since the potencies of these two compounds to inhibit HDACs are comparable [32], pyruvate is more relevant than butyrate as an SLC5A8-dependent tumour suppressor in non-colonic tissues. In fact, pyruvate might also be relevant to colon during the initial stages of carcinogenesis when the epithelial cells become de-differentiated and lose their polarity. When this happens, SLC5A8 might actually come in contact with blood, thus getting access to pyruvate in the circulation (Figure 1B). Lactate, another substrate for SLC5A8 but not an HDAC inhibitor [32,49], is also present in blood at almost 10 times higher concentrations than pyruvate. SLC5A8 can mediate effective uptake of lactate into de-differentiated pre-malignant cells including the colonic cells. Prior to conversion into malignant cells, these cells have the ability to convert lactate into pyruvate via the isoform lactate dehydrogenase LDH-B. Thus, normal expression of SLC5A8 in cells during the pre-malignant stage might represent a tumour-suppressive mechanism via the transporter-mediated accumulation of the HDAC inhibitor pyruvate (Figure 1B). These cells also have the ability to oxidize pyruvate within the mitochondria.

Interestingly, the relationship between pyruvate and SLC5A8 also provides a link between the widespread silencing of the transporter in tumours and the Warburg effect. Tumour cells perform aerobic glycolysis, but do not metabolize pyruvate for energy production but rather convert this glycolytic end product into lactate (Figure 1C). Whereas normal and pre-malignant cells oxidize pyruvate involving pyruvate dehydrogenase and the citric acid cycle, malignant cells suppress mitochondrial oxidation of pyruvate through a decrease in pyruvate dehydrogenase activity and convert pyruvate into lactate through silencing of the lactate dehydrogenase isoform LDH-B and up-regulation of the isoform LDH-A. Thus, malignant cells effectively inactivate the tumour suppressor pyruvate by converting it into lactate. Therefore, it would be detrimental for these cells to continue to express SLC5A8; hence these cells silence *SLC5A8* via epigenetic mechanisms involving DNA methylation, thus preventing the entry of pyruvate into the cells. Since lactate is already generated at high

levels in malignant cells due to differential expression of the LDH isoforms, silencing of *SLC5A8* is also necessary to prevent the entry of lactate from the circulation. Since accumulation of lactic acid in malignant cells would result in acidification, these cells up-regulate the H^+-coupled monocarboxylate transporters MCT1 (SLC16A1) and MCT4 (SLC16A3) to remove lactic acid and avoid the potential detrimental effects of cellular acidification on cell survival and proliferation (Figure 1C). Recent studies have highlighted further the functional differences between pyruvate and lactate in relation to cancer. Whereas pyruvate is a tumour suppressor due to its ability to inhibit HDACs [32,49], lactate is actually a tumour promoter due to its ability to activate the G-protein-coupled receptor 81 (GPR81, lactate receptor) [50]. Accordingly, tumour cells maintain low levels of the tumour-suppressing metabolite pyruvate and high levels of the tumour-promoting metabolite lactate.

SLC5A8 methylation and the resultant silencing of the gene as early events in colon carcinogenesis

There is evidence that the silencing of *SLC5A8* might represent an important tumorigenic step in the colon neoplasia pathway. Li et al. [27] detected *SLC5A8* methylation in aberrant crypt foci, the earliest morphologically identifiable colon neoplasia. Similar findings have been reported by other investigators [51]. These reports suggest that the silencing of *SLC5A8* is an early event in colon carcinogenesis. The methylation of *SLC5A8* also correlates with tumour progression; the extent of methylation is greater in tumours with higher histological grades than in lower grade tumours [51].

In vivo evidence for the tumour-suppressive function of Slc5a8

It is very clear from several published reports that SLC5A8 does function as a tumour suppressor *in vitro*; the widespread silencing of the transporter by DNA methylation in almost all cancers examined thus far also indicates a similar possible tumour-suppressive role for the transporter *in vivo*. Despite this strong evidence, Frank et al. [52] have reported that deletion of *Slc5a8* in mice did not increase the incidence or progression of colon cancer in two different experimental model systems: inflammation-associated colon cancer (dextran sulfate sodium/azoxymethane model) and genetically driven colon cancer ($Apc^{Min/+}$ model). Quite puzzled by these rather unexpected findings, we wanted to see if this was also true in mammary gland, a tissue in which we have shown *in vitro* that SLC5A8 functions as a tumour suppressor [49]. If Slc5a8 has no relevance to breast cancer *in vivo* as was shown by Frank et al. [52] in the colon, it would confirm that the transporter does not function as a tumour suppressor *in vivo*; on the contrary, if Slc5a8 does function as a tumour suppressor in mammary gland *in vivo*, we will have to re-visit the transporter's function in the colon and seek answers as to why the studies by Frank et al. [52] failed to demonstrate the tumour-suppressive role of the transporter *in vivo* in this tissue. With this rationale, we obtained the $Slc5a8^{-/-}$ mouse line from Frank et al. [52], crossed it with MMTV-HRAS mouse, a model for spontaneous breast cancer, and evaluated the incidence, progression and lung metastasis of HRAS-driven breast cancer on $Slc5a8^{+/+}$ and $Slc5a8^{-/-}$ backgrounds. The results were surprising. Deletion of *Slc5a8* in MMTV-HRAS mouse promoted breast cancer. In every aspect of breast cancer progression that we monitored (tumour incidence, tumour growth and lung metastasis), the tumour-suppressive function of the transporter was clearly evident.

We recently published these interesting results as the first *in vivo* evidence for the tumour-suppressive function of Slc5a8 [53].

Why did then the studies by Frank et al. [52] fail to show evidence of Slc5a8 as a tumour suppressor in colon *in vivo*? We believe that the high concentrations of butyrate in the colonic lumen (~15 mM) and the kinetic features of SLC5A8 with regard to its interaction with butyrate offer a logical solution to this puzzle. SLC5A8 is a high-affinity transporter for butyrate with a K_m in the micromolar range [28,29] and butyrate, when present at high concentrations, diffuses or is taken up into colon cells by the low-affinity H^+-coupled monocarboxylate transporters. As such, SLC5A8-mediated butyrate entry into these cells represents only a small fraction of the total entry process, rendering the transporter dispensable for butyrate to inhibit HDACs when dietary fibre intake is high (Figure 2). SLC5A8 is obligatory for HDAC inhibition in colon cells only when the luminal butyrate levels are low (e.g. low-fibre diet). This is not the case in mammary gland where pyruvate, rather than butyrate, is the physiological substrate for the transporter and the tumour-suppressive metabolite, and the circulating levels of pyruvate (~100 μM) are close to the K_m for SLC5A8. With this logic, we hypothesized that SLC5A8 is not obligatory for the beneficial effects of butyrate in colon to protect against colon cancer when the dietary fibre intake is optimal; but the transporter becomes essential when the levels of butyrate in the colonic lumen are low as would be the case with a low-fibre diet. Frank et al. [52] conducted their experiments with mice fed a normal diet with optimal fibre content, which might explain why their studies did not show any difference between wild-type mice and *Slc5a8*$^{-/-}$ mice in colon carcinogenesis; under these dietary conditions, Slc5a8 is dispensable for the protective effects of butyrate.

We hypothesized that the outcome of the experiments conducted by Frank et al. [52] would have been markedly different if the mice were fed a low-fibre diet. With this logic, we repeated the same experiments that Frank et al. [52] did, but with two different dietary conditions: high-fibre content (HF-Diet) and low-fibre content (LF-Diet). We examined the progression of dextran sulfate sodium (DSS)-induced colitis and DSS/AOM (azoxymethane)-induced colon cancer in wild-type mice and *Slc5a8*$^{-/-}$ mice using these two different dietary conditions [54]. First, we used 2 % DSS to investigate the influence of Slc5a8 on colonic inflammation. The mice tolerated DSS for 6 days with no mortality irrespective of the genotype (WT and *Slc5a8*$^{-/-}$) when fed the HF-Diet; but there was a marked difference between the two genotypes when the mice were fed a LF-Diet. Wild-type mice survived the treatment with no mortality whereas all mice with *Slc5a8*$^{-/-}$ genetic background died within the 6-day treatment period. At the end of the 5th day, we examined the colon in surviving mice for gross appearance and histology. There was profound bleeding in the colon of *Slc5a8*$^{-/-}$ mice but not in wild-type mice when fed the LF-Diet; in contrast, there was no difference between the two genotypes when fed the HF-Diet. There was also massive inflammation and infiltration of immune cells in the colon of *Slc5a8*$^{-/-}$ mice but not in wild-type mice when fed the LF-Diet; again there was no difference between the two genotypes when fed the HF-Diet. These studies show for the first time the conditional nature of Slc5a8 as a blocker of colonic inflammation *in vivo*, a function that depends on the dietary fibre content. We then examined the incidence of colonic polyps using the DSS/AOM model of colitis-induced colon cancer. There was no difference

between wild-type and *Slc5a8*$^{-/-}$ mice in the number of colonic polyps when fed the HF-diet; in contrast, the number of polyps doubled in *Slc5a8*$^{-/-}$ mice compared with wild-type mice when fed the LF-diet. These studies show for the first time the conditional nature of Slc5a8 as a blocker of colon carcinogenesis *in vivo*, a function that depends on the dietary fibre content.

Role of SLC5A8 in mucosal immune function

The gut-associated immune system performs a paradoxical role in being tolerant towards the commensal bacteria. Butyrate is receiving increasing attention as the mediator of this tolerogenic phenotype. Dendritic cells (DCs) are the primary antigen-presenting cells in the intestinal tract, enabling the immune system to recognize the luminal bacteria as 'foreign'. Therefore, it is possible that the bacterial fermentation products suppress the development of DCs to impart a tolerogenic phenotype to the mucosal immune system. This indeed seems to be the case [54,55]. Butyrate shows a marked negative effect on DC development from bone marrow progenitor cells. These progenitor cells develop into granulocytes (Gr-1^{+} /CD11c^{-}) and DCs in the presence of GM-CSF (granulocyte/macrophage-colony stimulating factor); the DC development is drastically suppressed by butyrate. Butyrate transport via SLC5A8 with subsequent inhibition of HDACs is responsible for the suppression of DC development. Therefore, SLC5A8/butyrate-induced HDAC inhibition is a key event in the suppression of DC development, thus contributing to the tolerogenic phenotype of the mucosal immune system.

The bacteria-derived butyrate and propionate as endogenous HDAC inhibitors also impact on the function of DCs in colon. Among the immune cells associated with colonic mucosa, DCs are unique in the sense that they pass their dendritic structures between colonic epithelial cells, forming a tight junction and also gaining access to luminal contents. This is essential for the physiological functions of DCs as the sentinel to sample the luminal contents for foreign antigens and mount an appropriate immune response. DCs express IDO1, a tryptophan-catabolizing enzyme. Depletion of tryptophan and also generation of tryptophan metabolites via DC-associated IDO1 in the microenvironment induce arrest of proliferation of effector T cells, forcing them to anergy and apoptosis [56]. IDO1^{+} DCs also promote the conversion of naïve T cells into tolerogenic FoxP3^{+} Tregs [57]. Colonic epithelial cells as well as DCs in the lamina propria express high levels of IDO1, contributing to the tolerogenic phenotype of the mucosal immune system. Recent studies have shown that Slc5a8 and the bacterial-derived HDAC inhibitors butyrate and propionate are critical for these phenomena in the mucosal immune system [54]. The bacterial metabolites induce IDO1 in wild-type DCs but fail to do so in *Slc5a8*$^{-/-}$ DCs. This is also true *in vivo*; IDO1 expression is markedly lower in the colon of *Slc5a8*$^{-/-}$ mice compared with wild-type mice. In addition, the Slc5a8/butyrate-mediated HDAC inhibition in DCs also promotes the conversion of naïve T cells into immunosuppressive Tregs and blunts the generation of pro-inflammatory IFN-γ^{+} CD4^{+} T cells. The same studies have also shown that Slc5a8 and the bacterial-derived HDAC inhibitors are also important determinants of retinoic acid signalling, both in colonic epithelial cells and in colon-associated DCs. Recent studies have demonstrated a similar phenomenon with propionate, also a HDAC inhibitor and a substrate for SLC5A8; oral administration of propionate in mice promoted the

conversion of naïve $CD4^+$ T cells into immunosuppressive Tregs (interleukin 10-positive) and suppressed the conversion of naïve $CD4^+$ T cells into pro-inflammatory Th1 (interferon γ-positive) and Th17 (interleukin 17A-positive) cells, which ameliorated the progression of autoimmunity in the brain in an experimental model of multiple sclerosis in mice [58,59]. These studies suggest that the immune cells are trained in the gut in the presence of the bacterial-derived SCFA to fight autoimmunity elsewhere in the body. Even though the role of Slc5a8 in the observed actions of these SCFA has not been investigated in this particular study [58], the transporter is likely to play a role in the entry of these bacterial metabolites into immune cells to elicit their actions.

These findings show that SLC5A8 plays a critical role in the establishment of a tolerogenic phenotype in the mucosal immune system, thus maintaining a relatively immunosuppressive state in the colon in the presence of luminal bacteria. The tolerogenic phenotype of the mucosal immune system against commensal bacteria, though quite perplexing, is essential to maintain the symbiotic relationship between the colonic bacteria and the host. These findings are directly relevant to colon cancer. Carcinogenesis in colon in a significant proportion of patients is driven by inflammation; therefore, suppression of inflammation reduces the risk of colon cancer. The modulation of the mucosal immune system by SLC5A8 thus represents another arm in the tumour-suppressive function of the transporter.

Clinical and therapeutic significance of SLC5A8 as a tumour suppressor

The clinical relevance of the conditional nature of SLC5A8 to function as a tumour suppressor and inflammation blocker in colon in connection with the dietary fibre content is readily apparent. It emphasizes the importance of optimal dietary fibre intake for the prevention of colitis and colon cancer. Dietary modifications leading to increased butyrate production in colon could represent an effective means to prevent inflammation and carcinogenesis in colon. This strategy may include the use of selective dietary fibres (prebiotics) and butyrate-producing strains of bacteria (probiotics). In fact, based on the silencing of *Slc5a8* in germ-free mouse colon, it is likely that the SCFA in the colonic lumen resulting from bacterial fermentation of dietary fibre control the expression of the transporter; a low-fibre diet suppressing the expression and a high-fibre diet promoting the expression. As such, decreased fibre content in diet might be detrimental in two ways: decreased production of the protective metabolites such as butyrate and propionate and also decreased expression of the tumour suppressor SLC5A8. There are also polymorphisms in SLC5A8; the dbSNP site at NCBI lists 13 polymorphisms with amino acid changes in the protein-coding region and the frequency of the alleles vary significantly depending on the race and ethnicity [60]. We have reported that at least some of these polymorphisms have functional consequences [40]. Therefore, specific polymorphisms that compromise the transport function could synergize with a low-fibre diet as a significant modifier of the incidence and severity of colitis and colon cancer, thus explaining at molecular level the racial/ethnic disparity in colon cancer morbidity and mortality that is known to exist in the general population [61–63].

We analysed The Cancer Genome Atlas (TCGA) database to determine if there is a significant correlation between survival probability in cancer patients and the level of

SLC5A8 expression in their tumours. We could not find any statistically significant correlation between the two parameters in patients with breast cancer or colon cancer. Based on the strong evidence for the tumour-suppressive function of the transporter, one would expect that the level of expression of SLC5A8 in tumours would correlate directly with survival probability; higher expression levels for the transporter in tumours meaning increased survival and the vice versa. But this was not the case. This however does not necessarily undermine the tumour-suppressive function of the transporter. There are many critical variables in human studies, including the polymorphisms in the transporter that impact on the transporter activity and also the variations in dietary composition among the study subjects that is expected to have a huge impact on the expression levels of the transporter. As such, without accounting for these important variables, it is difficult to interpret in a meaningful manner the lack of correlation between the expression levels of SLC5A8 and the overall survival of patients with colon cancer or breast cancer.

It is logical to predict the use of SLC5A8 in cancer prevention; the higher the expression and function of the transporter, the greater is the protection against carcinogenesis. But, as the expression of the transporter is silenced in cancer, how can the transporter be exploited for cancer treatment? One way is to reactivate the expression of the transporter in cancer cells so that the endogenous HDAC inhibitor pyruvate can actively get into these cells and suppress their growth and proliferation. This approach is feasible because the mechanism of tumour-associated silencing of *SLC5A8* in all cancers involves hypermethylation of its promoter. Therefore, inhibitors of DNA methylation would re-activate the transporter expression. *In vitro* studies have unequivocally shown that treatment of cancer cells of various types with the DNA methylation inhibitors 5′-azacytidine, 5′-azadeoxycytidine or procainamide induces the expression of *SLC5A8* [33,34]. Some of these compounds are already in clinical use for treatment of certain specific types of cancers (5′-azacytidine, also known as Vidaza) or in advanced stages of clinical trials (decitabine). Of course, these inhibitors are expected to induce (and also suppress) the expression of a wide range of genes; therefore, it might be difficult to precisely quantify the contribution of the re-activated SLC5A8 to the therapeutic efficacy of such inhibitors.

There is some evidence that the tumour-suppressive function of SLC5A8 does not necessarily depend solely on the ability of the transporter to mediate the cellular entry of HDAC inhibitors [64]. Ectopic expression of the transporter in cancer cells leads to altered localization of the anti-apoptotic protein survivin involving protein–protein interaction. In cancer cells that have no expression of SLC5A8, survivin is localized primarily in the nucleus where it functions to elicit its anti-apoptotic effects. But, when SLC5A8 is expressed ectopically in these cells, survivin appears in the plasma membrane, nuclear survivin gets depleted and total cellular levels of survivin are decreased. All these effects are observed even in the absence of any of the SLC5A8 substrates that function as HDAC inhibitors (pyruvate, propionate or butyrate). In the presence of such compounds however, the SLC5A8-mediated effects on survivin levels and cellular location are amplified. The tumour-suppressive ability of SLC5A8 independent of its transport function is an important and interesting angle with regard to the biological connection between the transporter and cancer, but more research is needed to understand this phenomenon in detail.

Recent studies have indicated that re-activation of SLC5A8 in tumour cells using DNA methylation inhibitors could be exploited for cancer therapy by improving the clinical efficacy of other anticancer drugs that use the transporter to get into tumour cells. Dichloroacetate (DCA) is an example. DCA, a small molecule inhibitor of pyruvate dehydrogenase kinase 1 (PDK1), has been in use for treatment of congenital lactic acidosis and metabolic disorders [65]. DCA-induced PDK1 inhibition activates the pyruvate dehydrogenase complex in the mitochondrial matrix thereby promoting mitochondrial oxidation of pyruvate through the citric acid cycle. It has been well established that cancer is a metabolic disease with increased glycolysis and suppressed mitochondrial function and glucose oxidation [1–3]. Thus, an ideal strategy in cancer therapy should incorporate the ability to reverse the metabolic remodelling and increase mitochondrial oxidation in cancer cells. The ability of DCA to activate mitochondrial function and promote the oxidation of pyruvate is the basis for the recent scientific and public interest in DCA as a potential therapeutic drug for cancer treatment [66]. A recent clinical trial in glioblastoma patients has shown that DCA is an effective anticancer drug [67]. However, high concentrations of the drug need to be used to attain therapeutic efficacy, which results in peripheral neuropathy [68]. Further, when DCA is used at high concentrations for long periods of time, it actually induces cancer in some tissues [69]. As a result, the current perception is that the utility of DCA as an anticancer drug is limited in humans. Paradoxically, DCA inhibits PDK1 at micromolar concentrations (50–100 μM) in cell-free systems; therefore, it was not clear why significantly higher doses are needed to inhibit this enzyme in cancer cells and in cancer patients. This discrepancy between the cell-free system and the intact cell system remained an enigma until recently when it was shown that SLC5A8 is a high-affinity transporter for DCA ($K_t \sim 36$ μM), energized by a transmembrane electrochemical Na^+ gradient [70]. Since the transporter is silenced in tumour cells, DCA does not get into these cells effectively and consequently high concentrations of DCA are needed to elicit its therapeutic effect. Such high doses of the drug are accompanied invariably by detrimental side effects. Our previous studies have shown that this might indeed be the case [70]. DCA induces tumour-cell selective apoptosis at very low concentrations when SLC5A8 is functionally expressed in breast, colon and prostate cancer cells. These findings suggest that, when used in combination with DNA methylation inhibitors such as Vidaza, DCA would have its anticancer effect at relatively lower concentrations without unwanted side effects that are associated with higher concentrations of DCA.

SLC26A3

Discovery of SLC26A3 as a tumour suppressor in colon and identification of its transport function

SLC26A3 was discovered more than two decades ago as a putative tumour suppressor in colon by a technique known as subtractive hybridization using cDNA libraries from matched normal colon and colon carcinoma [71]. The gene is expressed principally in colon, and its expression is markedly decreased in colon cancer and colon cancer cell lines. The protein product was named DRA (down-regulated in adenoma). Down-regulation of *DRA* correlates with colon cancer progression [72]. Based on the presence of potential nuclear targeting motifs, the protein was originally predicted to function as a transcription factor [71], but

subsequent studies showed that *DRA* encodes a membrane protein that specifically localizes to the lumen-facing brush border membrane of differentiated colonic epithelial cells [73]. The exact function of the protein in normal colon remained unknown until it was found that mutations in DRA caused congenital chloride diarrhoea, identifying DRA as a transporter with a role in colonic absorption of the anion chloride [74]. It belongs to the *SLC26* gene family that consists of multifunctional anion exchangers [75]. Mechanistic studies have revealed that DRA (SLC26A3) functions as an electroneutral Cl^-/HCO_3^- exchanger with a stoichiometry of 1:1 [74,76,77]. This provides the molecular basis for the loss-of-function mutations in this transporter as the cause of chloride diarrhoea [78]. *In vivo*, SLC26A3 is functionally coupled to SLC9A3 (NHE3) in the brush border membrane of colonic epithelial cells, thus mediating electroneutral absorption of sodium chloride in colon (Figure 3A). The co-operation between the two transporters is made possible through formation of a transport metabolon with the NHE3 kinase A regulatory protein (E3KARP) serving as the linker between the two transporters [79]. The supply of HCO_3^- to SLC26A3 and H^+ to SLC9A3 comes from the action of carbonic anhydrase II (CAII) on H_2CO_3 [80]; thus, CAII forms a component of the transport metabolon. When the transport functions of SLC26A3 and SLC9A3 are coupled, there is no change in intracellular pH or in transmembrane potential difference. The basolateral membrane of colonic epithelial cells, which faces the blood, expresses another isoform of Na^+/H^+ exchanger (NHE1 or SLC9A1), which plays a role in maintenance of intracellular pH.

Molecular basis of the tumour-suppressive function of SLC26A3

The relationship between the down-regulation of *SLC26A3* and tumour progression in colon has not been investigated in depth. Chapman et al. [81] reported several years ago that ectopic expression of the transporter in colon cancer cell lines resulted in growth suppression. Even though the transporter is expressed almost exclusively in colon, the phenomenon of growth suppression was not selective to colon cancer cells; similar results were seen in breast, kidney and cervical cancer cell lines. The conclusion from the study was that SLC26A3 does play an active role in colon in suppressing tumour progression. Surprisingly however, the ability of the transporter to decrease proliferation in cancer cells had nothing to do with its transport function. Ectopic expression of a mutant form of SLC26A3 that had no transport activity also resulted in suppression of growth in cancer cells. Nonetheless, the observed effects were specific to SLC26A3; ectopic expression of SLC26A4 (pendrin), a protein most closely related in structure to SLC26A3, could not substitute for SLC26A3 for the tumour-suppressive function in cancer cells. Deletion of *Slc26a3* in mice supported the tumour-suppressive role of this transporter *in vivo* [82]. *Slc26a3*$^{-/-}$mice displayed chloride-losing diarrhoea similar to humans with congenital chloride diarrhoea resulting from loss-of-function mutations in SLC26A3; more importantly, these knockout mice showed increased proliferation of colonic crypt epithelium despite the fact that the transporter was not expressed in the crypts in normal colon. Thus, even though the exact mechanisms are not known, these studies do indicate a potential role for SLC26A3 in colon cancer progression.

In addition to colon cancer, down-regulation of *SLC26A3* may also have a role in the pathogenesis of ulcerative colitis [83,84]. Similar to what has been found in the case of

Slc5a8, the expression of Slc26a3 in colon is also controlled by colonic bacteria. Germ-free mice exhibit markedly reduced expression of the protein in colon compared with conventional mice, and recolonization of colon with bacteria brings back the expression of the protein to normal levels [48]. Furthermore, mouse strains that are more prone to colitis express lower levels of Slc26a3 in colon than mouse strains that are more resistant to colitis; colonization of the colon in mouse strains more prone to colitis with bacteria from the colon of the more resistant mouse strains normalizes the expression of the transporter [85]. Again, since chronic inflammation drives colon cancer, the findings relating the down-regulation of *SLC26A3* to colitis might be of relevance to the tumour-suppressive function of the transporter.

Based on the findings from the published reports described above, it can be concluded that SLC26A3 does function as a tumour suppressor in colon even though the molecular mechanisms underlying this function are not well established. We hypothesize that the tumour-suppressive function of SLC26A3 is related, at least in part, to its ability to export HCO_3^-, a process with a potential impact on intracellular pH. The observations that *Slc26a3*$^{-/-}$ mice exhibit up-regulation of SLC9A3 in colon [82] support this hypothesis. In normal colonic epithelial cells, the two transporters are functionally coupled with no real impact on cellular pH because SLC26A3-mediated acidification (i.e. exit of HCO_3^-) is compensated by SLC9A3-mediated alkalinization (i.e. exit of H^+). But, when *SLC26A3* is down-regulated and as a result *SLC9A3* is up-regulated as has been shown in *Slc26a3*$^{-/-}$ mouse colon, one might expect promotion of cellular alkalinization and facilitation of cell proliferation (Figure 3B). We posit that this might be the mechanism by which SLC26A3 functions as a tumour suppressor. In normal colon where the transporter is expressed, the transporter-mediated export of HCO_3^- functions against intracellular alkalinization. The transporter is silenced during carcinogenesis so as to maintain intracellular pH in the wake of tumour-associated lactic acid production. This complements the functions of MCT1 and MCT4, which also facilitate the efflux of H^+ along with lactate.

Analysis of TCGA database for the association between the expression levels of this transporter in colon cancer tissues and the survival probability of the patients indicated a positive trend, though not statistically significant, with lower level of the transporter expression correlating with decreased overall survival. Again, considering the fact that the expression of the transporter in the intestinal tract is controlled by the content of fibre in the diet [48], it might be difficult to draw any definitive conclusion from the lack of statistical significance for the association. However, there was a statistically significant positive correlation in breast cancer patients ($P = 0.02$); patients with lower level of the transporter expression had a decreased overall survival. This is interesting because virtually nothing is known on the expression and function of this transporter in mammary gland under normal or pathological conditions.

SLC39A1

Function

SLC39A1 is a transporter for the divalent cation Zn^{2+} [86]. There are two distinct families of Zn^{2+} transporters: SLC30 and SLC39. The members of the SLC30 family function primarily in the efflux of Zn^{2+} from cytoplasm into intracellular compartments. In contrast, many of the members of the SLC39 family mediate the influx of Zn^{2+} into cells from extracellular medium. SLC39A1 is also known as ZIP1 (Zrt and Irt-like protein; Zrt and Irt represent transporters for zinc and iron in non-mammalian organisms). Even though SLC39A1 is expressed widely in mammalian tissues, the tumour-suppressive role of this transporter has been demonstrated thus far only in the prostate [87,88]. Normal prostate is unique in citrate metabolism and citric acid cycle; acetyl CoA derived from glucose metabolism gets converted into citrate via citrate synthase and then does not go further through the citric acid cycle. As a result of the truncated citric acid cycle, normal prostate contains 25 times higher concentrations of citrate than other tissues. Citrate thus produced is secreted out, resulting in very high concentrations (~150 mM) of this metabolite in prostatic fluid. The physiological function of citrate in prostatic fluid remains unclear; most likely, it serves as a carbon source for sperm metabolism to generate energy necessary for motility. The principal reason for the truncated citric acid cycle in prostate is the Zn^{2+}-mediated inhibition of aconitase in the citric acid cycle, which converts citrate into isocitrate. Among various tissues in mammals, prostate contains highest concentration of Zn^{2+}, almost 10 times more than other tissues. SLC39A1 is the primary transporter in prostate epithelial cells responsible for this robust accumulation of Zn^{2+}.

SLC39A1 and prostate cancer

Citrate metabolism is markedly altered in prostate cancer. The citric acid cycle becomes active in the tumour tissue due to re-activation of aconitase, and consequently citrate levels in prostate cancer are markedly decreased and become almost comparable to those in other tissues. The re-activation of aconitase in tumour tissue is due to drastically reduced levels of Zn^{2+}. This occurs because of the down-regulation of the transporter SLC39A1. The underlying mechanism for the tumour-associated down-regulation of SLC39A1 is not direct methylation of its promoter but instead the silencing of the transcription factor AP-2α [89]. Overexpression of the Ras-responsive element binding protein-1 (RREB-1) in prostate cancer is also responsible for this phenomenon [90]. It is clear that the marked changes in citrate metabolism observed in prostate cancer result from decreased Zn^{2+} levels, but how this relates to the tumour-suppressive function of Zn^{2+} in normal tissue is less clear. One possibility is that citrate metabolism through the re-activated citric acid cycle in cancer cells increases ATP production to support tumour growth. Additional mechanisms are also possible. Ectopic expression of SLC39A1 in prostate cancer cells inhibits nuclear factor-κB (NF-κB) *in vitro* and *in vivo* [91] and consequently increases expression of pro-angiogenic and pro-metastatic cytokines [92].

SLC22A18

Function and potential relevance to cancer

SLC22A18 belongs to the *SLC22* gene family, which consists of transporters for anionic and cationic organic solutes. It is an imprinted gene, with preferential expression from the maternal allele. Heterologous expression studies have shown that SLC22A18 transports organic cations such as chloroquine and quinidine [93], but physiological substrates have not yet been identified. A recent study has established a novel link between the transporter and fat accumulation [94], but the molecular basis of this interesting link is not known. Several reports have described the silencing of this gene by DNA methylation in glioma and breast cancer [95–100], and evaluation of the relationship between SLC22A18 expression and disease status has revealed that low expression of the transporter in tumours correlates with tumour progression, recurrence and poor survival in both cancer types [95,96,97,100]. Mutations in SLC22A18 are associated with Wilms tumour, adrenocortical carcinoma, rhabdomyosarcoma and Beckwith–Wiedemann syndrome, all pointing to potential function of the transporter as a tumour suppressor. In contrast, studies in pancreatic cancer and lung cancer have indicated the opposite, suggesting a tumour-promoting role rather than a tumour suppressor role [101,102]. Lack of information on the *in vivo* function of the transporter and on the identity of its physiological substrates hinders progression in our understanding of the relevance of the transporter to cancer.

CONCLUSIONS

There has been a growing interest in plasma membrane transporters as they relate to cancer progression, both as tumour promoters and tumour suppressors. Understandably, transporters for essential nutrients often serve as tumour promoters, which are up-regulated in cancer to meet the increasing demands for such nutrients in cancer cells. In contrast, it was less clear as to how plasma membrane transporters could function as tumour suppressors. With the discovery of transporters for metabolites and ions that impact on epigenetics, intracellular pH and enzyme activity, it now seems logical that such transporters could potentially play a role as tumour suppressors. The transporters described in this review provide strong evidence in support of this logic, and we predict that more examples of plasma membrane transporters with tumour-suppressive function are likely to be discovered in coming years.

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Abbreviations

AOM	azoxymethane
CAII	carbonic anhydrase II

DC	dendritic cell
DCA	dichloroacetate
DR5	death receptor 5
DRA	down-regulated in adenoma
DSS	dextran sulfate sodium
GLUT1	glucose transporter SLC2A1
GPR81	G-protein-coupled receptor 81 (lactate receptor)
HDAC	histone deacetylases
LDH-A	lactate dehydrogenase isoform A
LDH-B	lactate dehydrogenase isoform B
MCT1	monocarboxylate transporter 1
MCT4	monocarboxylate transporter 4
NHE1	Na^+ /H^+ exchanger 1
NHE3	Na^+ /H^+ exchanger 3
PDK1	pyruvate dehydrogenase kinase 1
SCFA	short-chain fatty acid
TCGA	The Cancer Genome Atlas
TRAIL	tumour necrosis factor-related apoptosis-inducing ligand

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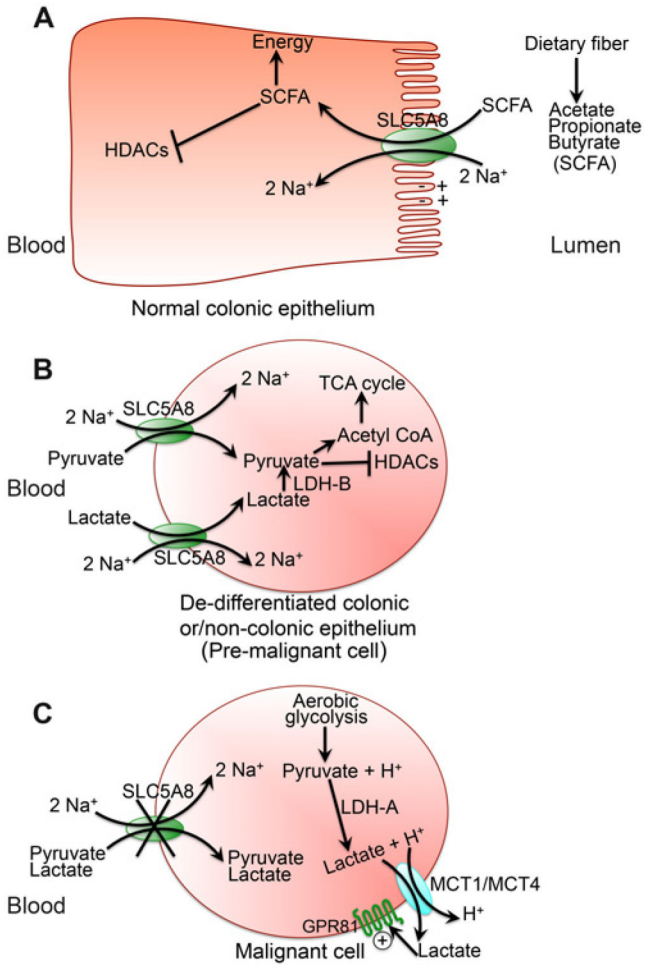

Figure 1. Role of SLC5A8 in colonic epithelium

(**A**) Normal colonic epithelium, (**B**) pre-malignant colonic cell and (**C**) malignant colonic cell.

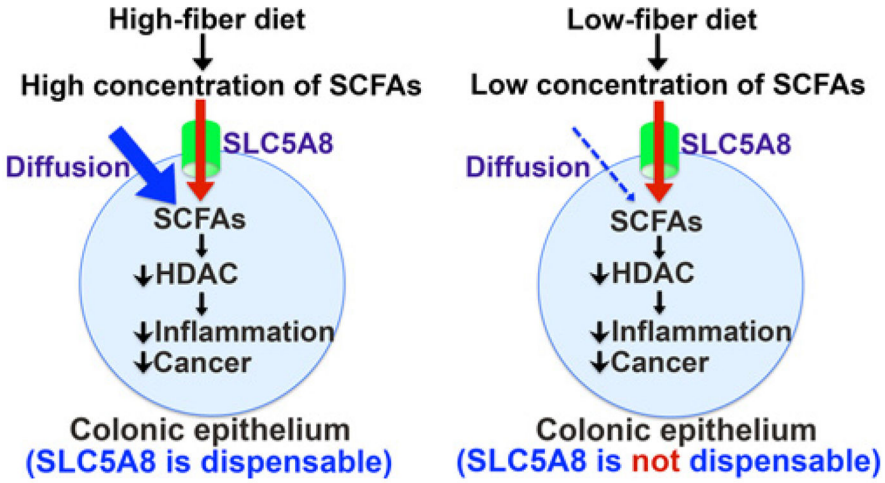

Figure 2.

Relationship of SLC5A8 to the entry of SCFA into colonic epithelial cells under high-fibre and low-fibre dietary conditions

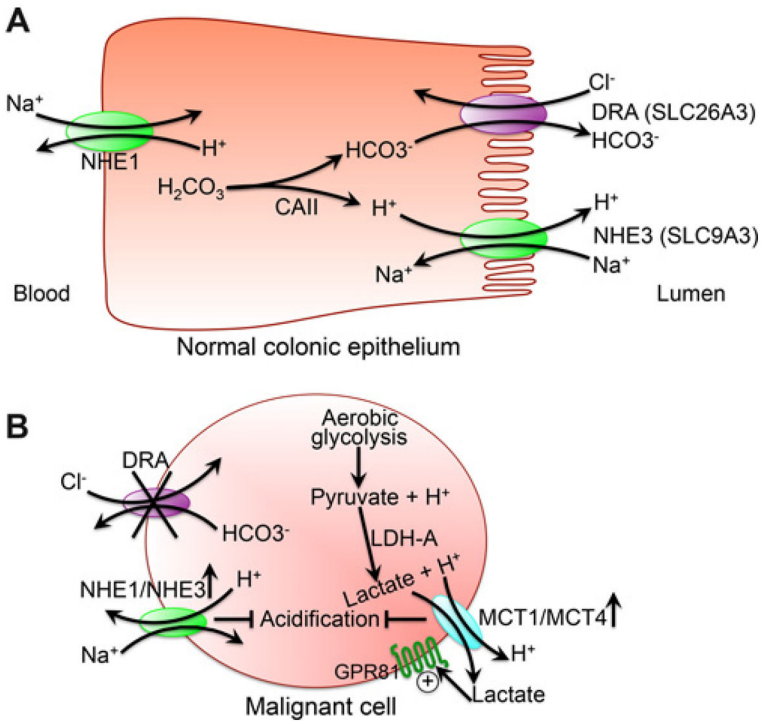

Figure 3. Role of SLC26A3 in colonic epithelium

(**A**) Normal colonic epithelium and (**B**) malignant colonic cell.