Shared Epigenetic Mechanisms in Human and Mouse Gliomas Inactivate Expression of the Growth Suppressor SLC5A8

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Abstract

Tumors arise in part from the deleterious effects of genetic and epigenetic mechanisms on gene expression. In several mouse models of human tumors, the tumorigenic phenotype is reversible, suggesting that epigenetic mechanisms also contribute significantly to tumorigenesis in mice. It is not known whether these are the same epigenetic mechanisms in human and mouse tumors or whether they affect homologous genes. Using an integrated approach for genome-wide methylation and copy number analyses, we identified SLC5A8 on chromosome 12q23.1 that was affected frequently by aberrant methylation in human astrocytomas and oligodendrogliomas. SLC5A8 encodes a sodium monocarboxylate cotransporter that was highly expressed in normal brain but was significant down-regulated in primary gliomas. Bisulfite sequencing analysis showed that the CpG island was unmethylated in normal brain but frequently localized methylated in brain tumors, consistent with the tumor-specific loss of gene expression. In glioma cell lines, SLC5A8 expression was also suppressed but could be reactivated with a methylation inhibitor. Expression of exogenous SLC5A8 in LN229 and LN443 glioma cells inhibited colony formation, suggesting that it may function as a growth suppressor in normal brain cells. Remarkably, 9 of 10 murine oligodendrogial tumors (from p53+/− or ink4a/arf +/- animals transgenic for S100β-v-erbB) showed a similar tumor-specific down-regulation of mSLC5A8, the highly conserved mouse homologue. Taken together, these data suggest that SLC5A8 functions as a growth suppressor gene in vitro and that it is silenced frequently by epigenetic mechanisms in primary gliomas. The shared epigenetic inactivation of mSLC5A8 in mouse gliomas indicates an additional degree of commonality in the origin and/or pathway to tumorigenesis between primary human tumors and these mouse models of gliomas. (Cancer Res 2005; 65(9): 3617-23)

Introduction

Both genetic and epigenetic alterations contribute to tumorigenesis. Tumorigenic epigenetic changes, such as aberrant methylation of normally unmethylated CpG islands, lead to widespread inactivation of genes, including tumor suppressors (1–3). However, current models of tumor development are mainly derived from genetic approaches that detect gains, losses, mutations, and rearrangements of chromosome regions. An integrated genetic and epigenetic approach is therefore necessary to determine the full complement of genes involved in tumorigenesis and to expose hidden targets for cancer therapy.

The methylation status of thousands of CpG islands can be assessed by restriction landmark genome scanning (RLGS), a two-dimensional gel-based method (4). RLGS has been used to identify novel imprinted genes (5, 6) as well as aberrant methylation (7–12) and gene amplifications (13–15) in human and mouse cancers. With the development and validation of in silico methods for determining the sequence and chromosomal location of DNA fragments on RLGS profiles (16, 17), methylation data from RLGS can now be integrated directly with copy number data from high-resolution genomic methods, such as array comparative genome hybridization (array CGH; ref. 18). Array CGH in combination with RLGS provides a large-scale view of the contribution and interaction of aberrant genetic and epigenetic mechanisms in tumorigenesis. This integrated approach has been used to identify recurrent convergence of methylation and deletion at particular loci and more generally shows that the majority of aberrant CpG island methylation is independent of deletions (16, 17). These studies have allowed identification of putative cancer genes that are not detectable using conventional genomic or candidate gene approaches.

Using this integrated approach, we identified a RLGS fragment, 3D41 (16, 17), corresponding to a CpG island and expressed sequence tag (EST) on chromosome 12q23.1 that was frequently altered by methylation in astrocytomas and glioblastomas (16). The full-length gene corresponding to the EST was identified as SLC5A8 (19, 20), a member of the SLC5 family of sodium monocarboxylate transporters. The associated CpG island was frequently methylated in colon cancer cell lines and primary colon cancers, and overexpression of SLC5A8 in colon cancer cells inhibited colony formation (21). These studies suggest that SLC5A8 may be a tumor suppressor gene in colon and possibly brain cancer.

In addition to the functional analysis of candidate tumor suppressors in human tumor cell lines, mouse models of human cancer provide a complementary and manipulable route for analysis of dysregulated genes found in human tumors. An epigenetic contribution to tumorigenesis in mice is supported by studies in which the tumorigenic phenotype of tumor derived nuclei can be reversed by nuclear transplantation (22–25). Several models that recapitulate the invasive and morphologic features of human oligodendrogliomas have been produced (26–30). A mouse model of human oligodendrogioma was developed by overexpressing v-erbB, under the glial cell-specific S100β promoter. Tumors in this model arose at increased incidence and of higher grade in mice mutant at p53 or ink4a/arf (26). These models serve as an excellent in vivo system for determining if and how additional genes dysregulated in human oligodendrogliomas may contribute to tumorigenesis, especially...
because cell lines from human oligodendrogliomas have not yet been successfully produced. However, it is not yet known whether the same epigenetic mechanisms are operative in these human and mouse tumors or whether they affect homologous genes.

Here, we report the identification of SLC5A8 as a target of aberrant CpG island–related gene silencing in human gliomas. We used complementary analyses of primary human tumors, human tumor cell lines, and a mouse model of brain tumors to test whether epigenetic inactivation of SLC5A8 is functionally important to gliomagenesis.

### Materials and Methods

**Tissue and cell lines.** Human tumor samples were obtained from the Neurosurgery Tissue Bank at the University of California-San Francisco (San Francisco, CA), including 17 WHO grade II astrocytomas, 10 grade II oligodendrogliomas, and 13 grade III oligodendrogliomas. Nontumor brain tissues include four samples from different regions of one autopsy brain (anterior and posterior, across white matter and gray matter) and two surgical samples from individuals with epilepsy (NB4 and NB8). All samples were obtained with informed consent, and the usage was approved by the Committee on Human Research at the University of California-San Francisco.

Cell lines used include two human glioma cell lines LN229 and LN443 and a colon cancer cell line (RKO). Cells were plated at low density and maintained in DMEM containing 10% fetal bovine serum (for RKO cells) or DME H21 containing 10% fetal bovine serum (for glioma cells). Cells were plated at low density and maintained in DME H21 with 10% fetal bovine serum (for glioma cells) or DME H21 with 10% fetal bovine serum (for RKO cells). For reactivation experiments, the cells were treated with 5 μM 5-aza-2′-deoxycytidine (Sigma) for 3 days, changing the medium and drug every 24 hours.

Mouse oligodendrogliomas were obtained from p53+/− or ink4a/arf+/− mice transgenic for S100t-v-erbB (26). Founders were derived from C57Bl/6J (The Jackson Laboratory, Bar Harbor, ME) and subsequently backcrossed into FVB/N. Mice with heterozygous deletion of p53 were FVB/N. Mice deleted for ink4a/arf were outbred. The mouse and human brain tumor samples were subject to routine histologic analysis by a neuropathologist.

**Plasmid constructs and virus production.** SLC5A8-v5-his-TOPO expression vector and control vector were gifts from Dr. Sanford Markowitz (University Hospitals of Cleveland and Case Western Reserve University, Cleveland, OH). The SLC5A8-v5 retroviral plasmid was constructed by cloning the 1.7-kb HindIII-PmeI fragment containing SLC5A8 into pBabe retroviral plasmid (kindly provided by Dr. Gerard Evan, University of California-San Francisco). Phoenix amphi cells were used for virus production (31). Twenty-four hours after Phoenix cells were transduced with either pBabe or SLC5A8 retroviral plasmid using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA), fresh medium was added. After 24 hours, virus-containing medium was harvested, passed through a 0.45 μm filter, and then tested for viral titer on rat 208F cells before infecting target cells.

### Table 1. Frequency of SLC5A8 (3D41) methylation in gliomas relative to common genetic alterations (no. tumors with alterations/no. tumors analyzed)

<table>
<thead>
<tr>
<th>Alterations</th>
<th>Oligodendrogliomas grade II</th>
<th>Oligodendrogliomas grade III</th>
<th>Astrocytomas grade II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p−</td>
<td>9/10</td>
<td>13/13</td>
<td>3/17</td>
</tr>
<tr>
<td>19q−</td>
<td>9/10</td>
<td>13/13</td>
<td>4/17</td>
</tr>
<tr>
<td>7p+</td>
<td>1/10</td>
<td>5/13</td>
<td>2/17</td>
</tr>
<tr>
<td>12q23− (SLC5A8 locus)</td>
<td>0/10</td>
<td>0/13</td>
<td>3/17</td>
</tr>
<tr>
<td>SLC5A8 methylation (M or MM)</td>
<td>9/10</td>
<td>13/13</td>
<td>6/17</td>
</tr>
</tbody>
</table>

NOTE: Deletion (−) and gain (+) were defined by at least five consecutive BACs showing one copy loss or gain, respectively.

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**Restriction landmark genome scanning.** RLGS was conducted with the NotI/EcoRV/Hinfl combination as described previously (16). The profiles were analyzed by visual inspection of overlaid autoradiographs. Profiles from three normal brain samples were used as references.

**Array comparative genome hybridization.** Array CGH was conducted on 40 primary human brain tumors and 6 mouse brain tumors to identify genetic alterations. Briefly, for human array CGH, DNA from normal and tumor tissues were labeled with Cy5 and Cy3, respectively, and cohybridized to an array consisting of 2,463 mapped BAC clones containing human genomic DNA segments distributed at ~1 Mb intervals across the genome. For mouse arrays, the assay was carried out by cohybridizing Cy3- and Cy5-labeled test and reference DNA samples to the customized mouse array generated by printing 5-amino-linked degenerate oligonucleotide primer PCR-amplified BAC DNA onto amine-reactive slides (32, 33). The log2 ratios of signal intensities were used to score the proportion of normal copy number for each BAC, using a threshold of 2 for loss and <0.5 for gain.

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**Figure 1.** The CpG island corresponding to RLGS fragment 3D41 was affected frequently by aberrant methylation in primary human gliomas. **Left,** RLGS profiles with the 3D41 fragment (arrow); right, array CGH from the same sample showing copy number along chromosome 12, with the position corresponding to the 3D41 fragment (arrow). A RLGS fragment exhibiting an intensity between 30% and 70% of normal brain, with no genetic alteration by array CGH, was categorized as partially methylated (M). If the intensity of a RLGS fragment was <30% of normal and no deletion was detected, it was designated homozygous methylation (MM). If neither deletion nor methylation was detected, it was designated as no change (C; refs. 16, 17).
of test to reference signal were calculated based on the intensity of fluorescence, and the DNA copy number of each locus in tumor samples was plotted across the genome.

**RNA isolation, reverse transcription, and real-time reverse transcription-PCR.** Cell lines and tissues were lysed with TRIzol (Invitrogen) for RNA isolation. The reverse transcription reaction was done as described previously (17). We amplified \( SLC5A8 \) with both standard reverse transcription-PCR (RT-PCR) and real-time quantitative RT-PCR. The variables for standard RT-PCR of human \( SLC5A8 \) were 35 cycles of 95°C for 45 seconds, 54°C for 45 seconds, 72°C for 60 seconds, and 72°C for 10 minutes before cooling to 4°C. The primers for human \( SLC5A8 \) were 5'-TCCGAGGTCTACCGTTTTG-3' and 5'-GGGCAGGGGCATAAATAAC-3'. \( ACTB \) was used as a control. The real-time quantitative RT-PCR was done with an Assay-on-Demand for human \( SLC5A8 \) (Hs00377618_m1) and mouse \( SLC5A8 \) (Mm00520629_m1; Applied Biosystems, Foster City, CA) using an Opticon2 thermocycler (MJ Research, Waltham, MA) following the manufacturer's instructions. Human \( Gus \) and mouse \( Gus \) were used as controls for quantitation.

**Bisulfite sequencing analysis.** DNA was treated with sodium bisulfite as described (34, 35) with modifications (16). DNA (2 \( \mu \)g) was first digested with EcoRV, phenol/chloroform extracted, ethanol precipitated, and resuspended in water. DNA was treated with bisulfite at 55°C for 4 hours. After purification, DNA was dissolved in 50 \( \mu \)L, and 1 \( \mu \)L was used for PCR. Touchdown PCR was done for a total of 35 cycles starting at initial annealing temperature of 68°C and decreasing by 2°C every two cycles to a final annealing temperature of 58°C for the final 25 cycles. The product was cloned into the TOPO TA cloning/pCR2.1 vector (Invitrogen). Individual bacterial colonies were subject to PCR and the products were sequenced.

**Figure 2.** A, a CpG content plot of the chromosome locus corresponding to fragment 3D41 and the putative transcriptional start site of \( SLC5A8 \) (arrow). Short thick horizontal line, region of bisulfite sequencing analysis. B, aberrant methylation of the 3D41 CpG island in human glioma samples and cancer cell lines. The methylation status of the CpG island in normal brain, gliomas, and cancer cells was analyzed by sequencing subclones of PCR products from bisulfite-treated DNA. Each CpG is indicated by a circle, the fill pattern of which indicates the frequency of methylation at each CpG across all clones from an individual tumor. White circles, 0-33% methylation at the CpG; gray circles, 34-66% methylation; black circles, 67-100% methylation; n, number of clones analyzed for each sample. The position of the NotI site is indicated. The “genetic/epigenetic status” is derived from RLGS and array CGH as described in Fig. 1. Considering the individual clone data (Supplementary Fig. S1) and the fact that NotI is inhibited by methylation of either CpG in its recognition site, the bisulfite data are consistent with the RLGS analyses.

**Figure 3.** \( SLC5A8 \) mRNA levels are decreased in primary human gliomas and the gene is reactivated by a methylation inhibitor in glioma cell lines. A, expression of \( SLC5A8 \) in human glioma samples was determined by quantitative RT-PCR analysis and is expressed relative to a control gene, \( Gus \). Gene expression values were derived from the equation: \( \Delta C_t = (C_{t_SLC5A8} - C_{t_Gus}) \) followed by \( 2^{-\Delta C_t} * 100 \). Columns, average of triplicates; bars, SD. Two independent experiments yielded similar results. The “genetic/epigenetic status” is derived from RLGS and array CGH as described in Fig. 1. B, reactivation of \( SLC5A8 \) in two glioma cell lines and RKO colon cancer cells by treatment with 5-aza-2'-deoxycytidine as assessed by conventional RT-PCR. \( U \), untreated cells; \( A1 \) and \( A2 \), independent flasks of cells treated with 5 \( \mu \)mol/L 5-aza-2'-deoxycytidine for 3 days. \( ACTB \) was used as a control. C, quantitative RT-PCR analysis confirming the reactivation of \( SLC5A8 \) in three cell lines. Expression levels are calculated relative to a control gene, \( Gus \). ND, not detectable.
Results

The primers used for the human SLC5A8 CpG island are forward 5’- GAGGGGTTTGGATGATGGA-3’ and reverse 5’-CCCATACTAAATTAAACAC-3’. The primers used for the mouse SLC5A8 CpG island are forward 5’-TTTGGAGATTATTTGTAGGA-3’ and reverse 5’-CAAAAA-TACCCAAACATAACAAC-3’.

Colony formation assay. Cells (5,000 per well) were seeded and grown in six-well tissue culture plates for 24 hours and transfected with either SLC5A8-v5-his-TOPO expression plasmid or pCDNA control vector or infected with viral medium containing the SLC5A8-v5-his construct or empty plasmid control vector. Two days after transfection or infection, cells were exposed to G418 for plasmid-transfected cells or to puromycin for retrovirally infected cells for a total of 10 to 15 days. Cells were fixed in 20% methanol and stained with crystal violet, and colonies with >50 cells were counted and expressed as a percentage of empty vector controls for each cell line.

SLC5A8 was affected frequently by aberrant methylation in human astrocytomas and oligodendrogliomas. To determine the contribution of aberrant methylation and genetic alterations to gliomagenesis, we used RLGS and array CGH in primary human gliomas. A RLGS fragment exhibiting an intensity between 30% and 70% of normal brain, with no genetic alteration by array CGH, was considered as partially methylated. If the intensity of a RLGS fragment was <30% of normal and no deletion was detected, it was categorized as partially methylated. If the intensity of a RLGS fragment was >70% of normal and no methylation was detected, it was considered as methylated and deleted (16, 17). We did array CGH and RLGS on 40 primary human gliomas. The array CGH data showed chromosome 1p and 19q deletion to be quite common in these classic grade II and III oligodendrogliomas, whereas gain of chromosome 7 was more characteristic of grade II astrocytomas as reported previously (Table 1; refs. 36–39). Using the integrated approach, we identified a RLGS island fragment, 3D41, which was affected frequently by aberrant methylation in gliomas but rarely by large deletions (Fig. 1; Table 1). This CpG island is located on chromosome 12q23.1 and encompasses the putative transcription start site of SLC5A8 (Fig. 2A), a sodium monocarboxylate cotransporter (19–21). Among the 40 gliomas, 14 exhibited partial methylation, 14 were homozgyously methylated, but only 3 astrocytomas and no oligodendrogliomas exhibited deletion (Table 1).

To confirm methylation of the NotI site and to extend the analysis to additional CpGs of this CpG island, bisulfite sequencing was done on DNA from the primary glioma samples. We found that the CpG island was unmethylated in normal brain but exhibited frequent, localized methylation in brain tumors, consistent with the RLGS results (Fig. 2B; for the detailed bisulfite analysis of individual clones, see Supplementary Fig. S1). The fact that nearly all PCR alleles sequenced exhibited methylation strongly suggests homozygous methylation, although polymorphisms that distinguish alleles were not present within the sequenced region. These data suggest the CpG island encompassing the 5’ end of SLC5A8 was frequently aberrantly methylated often on both alleles and in a tumor-specific fashion.

SLC5A8 expression was significantly down-regulated in primary gliomas and demethylating treatment caused reactivation in glioma cell lines. To determine whether aberrant methylation of this CpG island affects SLC5A8 expression, real-time quantitative RT-PCR was done on a subset of the primary gliomas for which RNA was available. SLC5A8 was highly expressed in normal brain but was significantly down-regulated in 13 of 13 gliomas examined, particularly in those tumors with localized aberrant methylation (Fig. 3A). Two glioma cell lines, LN229 and LN443, also had little or no expression of SLC5A8 as assessed by both standard and quantitative RT-PCR. However, treatment of these cells with 5-aza-2’-deoxycytidine, a chemical inhibitor of methylation, reactivated SLC5A8 expression (Fig. 3B and C). As a positive control for reactivation, we showed that, in the colon cancer cell line RKO, SLC5A8 could also be reactivated by 5-aza-2’-deoxycytidine, consistent with a previous report (21). The reactivation of SLC5A8 in glioma cells was similar to or slightly higher than that of RKO. Consistent with the lack of gene expression, the CpG island was extensively methylated in all three cancer cell lines.
cell lines (Fig. 2B; see Supplementary Fig. S1 for the detailed results of individual clones). These data suggest that aberrant CpG island methylation contributes to the onset or maintenance of SLC5A8 inactivation in human gliomas.

Exogenous SLC5A8 expression inhibited colony formation by glioma cell lines. The frequent biallelic methylation and silencing of SLC5A8 suggests that these events provide a growth advantage to the glioma cells. To study the impact of SLC5A8 on proliferation, we infected tumor cells with SLC5A8 and then did colony formation assays for two glioma cell lines and for RKO cells as a positive control. Cells were retrovirally infected with SLC5A8 or empty vector and selected in puromycin. Compared with the empty vector, the colony number was dramatically decreased in cells infected with SLC5A8 (Fig. 4A). Expression of SLC5A8 caused a >90% decrease in colony number in LN229 cells, a 78% reduction in LN443 cells, and an 85% reduction in RKO cells (Fig. 4B; ref. 21). SLC5A8 expression was confirmed by immunocytochemical staining with antibodies against the V5 epitope tag (see Supplementary Fig. S2). In addition to this growth suppression by SLC5A8 viral infection, similar growth reductions were observed in these cell lines transfected with SLC5A8 expression plasmids (data not shown).

mSLC5A8, the murine homologue of SLC5A8, was frequently down-regulated in oligodendrogliomas derived from transgenic mice. Mouse models of human tumors provide a unique opportunity to investigate efficacy of new therapies and to understand the contribution of specific genes to tumorigenesis (27, 29, 40). By overexpressing a viral form of the EGFR, a gene that is frequently overexpressed in gliomas, in brain cells of mice heterozygous mutant for either p53 or Ink4a/Arf, tumors arise that appear morphologically similar to the human brain tumor they model (26). Whether the same epigenetic mechanisms in human tumors are operative in these and other mouse brain tumors or whether they affect homologous genes is unknown.

To determine if SLC5A8 also plays a role in the development of these murine tumors, we measured the expression level of murine SLC5A8 in normal mouse brain and in the murine brain tumors by real-time RT-PCR. Nine of 10 murine oligodendroglial tumors showed reduction in mSLC5A8 mRNA levels, including 3 tumors with little or no expression (Fig. 5A), similar to the primary human tumors. Bisulfite sequencing analysis showed that the methylation level increased in two of four tumor samples relative to normal brain, whereas in mT6, in which mSLC5A8 does not seem to be down-regulated, the CpG island was largely unmethylated (Fig. 5B). Furthermore, the copy number of this locus was not altered in any of six mouse tumors tested as assessed by array CGH on mouse BAC clone arrays (data not shown), suggesting that mainly epigenetic mechanisms, such as aberrant methylation, are involved in the decreased expression of mSLC5A8, although small deletions and point mutations would not be detected using these methods.

Taken together, our data show that SLC5A8 functions as a suppressor of glioma cell growth in vitro and that it is silenced frequently by aberrant methylation in several subtypes of primary human gliomas, including oligodendrogliomas and low-grade astrocytomas that have only small numbers of genetic alterations. The epigenetic inactivation of mSLC5A8 in mouse gliomas indicates a remarkable degree of commonality in the origin and/or pathway to tumorigenesis between primary human tumors and these mouse models of gliomas.

Discussion

Using our integrated genomic and epigenomic approach, we are able to identify novel genes that are inactivated in brain tumors and have been missed by classic nonintegrated methods. Because
RLGS and these array CGH experiments assess only a fraction of the human genome, our results raise the question of whether there exist many more cancer-related genes that are primarily inactivated by epigenetic mechanisms alone or primarily by a two-hit mechanism involving methylation and deletion. Here, we identify one such gene, SLC5A8, for which aberrant Cpg island methylation seems to be the most common mechanism of inactivation in oligodendrogliomas and low-grade astrocytomas. These in-depth experiments of SLC5A8 methylation are consistent with our previous analyses that suggested this locus (the Nat1 site) is primarily affected by methylation in a separate series of low and high-grade astrocytomas, although a low frequency of coincident methylation and deletion was also observed (16). The lack of deletion in the limited set of oligodendrogliomas and infrequent deletion in the low-grade astrocytomas studied here is not unexpected, because the frequency of chromosome 12q deletion in these tumor types is very low (38, 41, 42).

SLC5A8 was first identified by Rodriguez et al. (43) from an EST in a kidney cDNA library that has sequence similarity to SLC5A5, a sodium iodide symporter. SLC5A5 mediated iodide transport from thyrocytes into the colloid lumen through the apical membrane and was therefore originally designated hAIT for human apical iodide transporter. However, iodide transport was not observed when SLC5A8 was expressed in other cell types (19, 20). Subsequently it was renamed SLC5A8 by HUGO. In addition, SLC5A8 appears to transport sodium into Xenopus oocytes transfected with the full-length cDNA (21). Recently, SLC5A8 was identified as a putative tumor suppressor gene in colon cancer (21). SLC5A8 was expressed in normal colon mucosa but was decreased or absent in colon cancer cell lines. Expression of exogenous SLC5A8 inhibited colony-forming ability in the nonexpressing colon cancer cells.

In our study, SLC5A8 was expressed in normal brain but significantly decreased in all primary human gliomas and cell lines tested, particularly for those samples whose associated Cpg island was aberrantly methylated. The decreased SLC5A8 expression in the absence of aberrant methylation in several primary tumors suggests either that multiple epigenetic mechanisms may inactivate SLC5A8 or possibly that aberrant methylation is preceded and stimulated by transcriptional silencing, as has been suggested for glutathione S-transferase (44). Ectopic expression of SLC5A8 strongly suppressed colony formation in glioma cell lines, suggesting that it functions as a growth suppressor in glioma cells in vitro.

In addition to human tumor cell lines, we also tested a mouse model of human gliomas for SLC5A8 down-regulation. Although humans and mice are thought to have diverged nearly 75 million years ago (45), not only was mSLC5A8 consistently decreased in these tumors, but aberrant methylation also seemed to be important. The Cpg island in human SLC5A8 shows 88.6% identity to the mouse sequence. There are several conserved transcription factor binding site sequences within the aberrantly methylated portion of the Cpg island, including sites for AP-2, Sp1, and TCF-2a. It is not yet known if these conserved binding site sequences are functional in vivo or if the putative binding would be altered by aberrant methylation. To our knowledge, this is the first demonstration that aberrant methylation-related gene silencing occurs in murine brain tumors and further suggests that methylation of Cpg islands may be an important mechanism in the genesis and/or malignant progression of gliomas.

The mechanism by which SLC5A8 exerts growth suppression in glioma and colon cancer cell lines is unknown. Recent studies show that SLC5A8 also transports short-chain fatty acids and other monocarboxylic acids, such as pyruvate or butyrate, in a Na+-dependent manner (19, 20). One possibility then is that SLC5A8-mediated growth suppression involves the transport of antiproliferative molecules, such as butyrate, into the cells. The definitive function of this protein as a tumor suppressor underlying gliomagenesis requires further experimentation. The transport function of SLC5A8 is of particular interest in brain tumors, as butyrate is under investigation for therapy of human gliomas.

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