Identification and Characterization of a Diamine Exporter in Colon Epithelial Cells

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SLC3A2, a member of the solute carrier family, was identified by proteomics methods as a component of a transporter capable of exporting the diamine putrescine in the Chinese hamster ovary (CHO) cells selected for resistance to growth inhibition by high exogenous concentrations of putrescine. Putrescine transport was increased in inverted plasma membrane vesicles prepared from cells resistant to growth inhibition by putrescine compared with transport in inverted vesicles prepared from non-selected cells. Knockdown of SLC3A2 in human cells, using short hairpin RNA, caused an increase in putrescine uptake and a decrease in arginine uptake activity. SLC3A2 knocked down cells accumulated higher polyamine levels and grew faster than control cells. The growth of SLC3A2 knocked down cells was inhibited by high concentrations of putrescine. Knockdown of SLC3A2 reduced export of polyamines from cells. Expression of SLC3A2 was suppressed in human HCT116 colon cancer cells, which have an activated K-RAS, compared with their isogenic clone, Hkh2 cells, which lack an activated K-RAS allele. Spermidine/spermine N\textsubscript{4}-acetyltransferase (SAT1) was co-immunoprecipitated by an anti-SLC3A2 antibody as was SAT1 with an anti-SAT1 antibody. SLC3A2 and SAT1 colocalized on the plasma membrane. These data provide the first molecular characterization of a polyamine exporter in animal cells and indicate that the diamine putrescine is exported by an arginine transporter containing SLC3A2, whose expression is negatively regulated by K-RAS. The interaction between SLC3A2 and SAT1 suggests that these proteins may facilitate excretion of acetylated polyamines.

Polyamines are essential for normal cellular functions (1, 2). They bind to intracellular polyamines such as nucleic acids and ATP and modulate their functions (3). Intracellular polyamine content is increased in response to growth stimuli (4) and regulated by biosynthesis and degradation (5). Uptake and export also play important roles in the regulation of cellular polyamine levels (5).

In recent years, polyamine transporters have been identified in bacteria, yeast, and protozoa, and their properties have been studied. In Escherichia coli, polyamine uptake is mediated by three systems, the spermidine-preferential uptake system PotABCD (6, 7), the putrescine-specific uptake system PotFGHI (8), and PuuP (9). Export of polyamines is mediated by PotE (10), CadB (11), and MdtH1 (12) in E. coli. Blt is a polyamine exporter in Bacillus subtilis (13). In Saccharomyces cerevisiae, uptake of polyamines is mediated by DUR3, SAM3, GAP1 (14, 15), and AGP2 (16) on the plasma membrane and UGA4 on vacuolar membranes (17). The four transporters TPO1–4 on the plasma membrane (18–20) and TPO5 on the post-Golgi secretory vesicles (21) are polyamine exporters in yeast. A plasma membrane polyamine transporter, LmPot1, in protozoan parasite Leishmania major (22) has been described. In these unicellular organisms, polyamine transport involves protein channels.

In animal cells, polyamine uptake is mediated, at least in part, by a caveolar-dependent endocytic mechanism (23) and is positively regulated by K-RAS through phosphorylation of caveolin-1 protein (24). Export of the diamines putrescine and cadaverine have been characterized in several cells (25–28). However, export of polyamines from animal cells has not been characterized at the molecular level.

We have described the biochemical properties of a diamine exporter (DAX)\textsuperscript{2} in Chinese hamster ovary (CHO) cells (29) and isolated putrescine-tolerant CHO (CHO-T) cells that appear to export putrescine at a higher rate than sensitive cells (30). To address the molecular mechanism of polyamine export, we compared membrane proteins of CHO-T with normal, putrescine-sensitive CHO (CHO-S) cells and found SLC3A2, a member of the solute carrier family (31), as one of the proteins highly expressed in CHO-T cells. In this study, we evaluated the role of SLC3A2 in polyamine transport in a human colon cancer cell line.

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The on-line version of this article (available at www.jbc.org) contains supplemental Table S1 and sequence data.

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2 The abbreviations used are: DAX, diamine exporter; CHO, Chinese hamster ovary; RNA, short hairpin RNA; SAT, spermidine/spermine acetyltransferase; CHO-T, putrescine-tolerant CHO; CHO-S, putrescine-sensitive CHO; FBS, fetal bovine serum; MES, 4-morpholineethanesulfonic acid; MS, mass spectrometry; HPLC, high performance liquid chromatography; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ODC, ornithine decarboxylase; NO, nitric oxide.
EXPERIMENTAL PROCEDURES

Cell Culture—The human colorectal carcinoma cell line HCT116, which has an activating K-RAS mutation (G13V) in one of the K-RAS alleles (32), and CHO cells were purchased from the American Type Culture Collection. CHO-T cells were isolated previously (30). The Hkh2 cell line, an isogenic clone of HCT116 that lacks the activated K-RAS allele, was kindly provided by Dr. Shirasawa, Research Institute, International Medical Center of Japan (33). Dulbecco’s modified essential medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin was used for culturing HCT116 and Hkh2 cells. G418 (0.6 mg/ml) was supplemented for Hkh2 cells. 1% penicillin/streptomycin was used for culturing HCT116 that lacks the activated K-RAS allele, was kindly provided by Dr. Shirasawa, Research Institute, International Medical Center of Japan (33). Dulbecco’s modified essential medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin was used for culturing HCT116 and Hkh2 cells. G418 (0.6 mg/ml) was supplemented for Hkh2 cells. Modified essential medium supplemented with 10% FBS and 1% penicillin/streptomycin was used for growth of CHO cells. CHO-T cells were grown in medium supplemented with 15 mM putrescine. Cells were maintained in a humidified incubator at 37 °C with 5% CO₂.

Preparation of Membrane Vesicles—Inside-out membrane vesicles were prepared by the method of Schaub et al. (34) and Saxena and Henderson (35). One to 2 g of cells were suspended in hypotonic buffer (0.5 mM sodium phosphate, pH 7.0, 0.1 mM Tris-HCl, pH 7.4, 250 mM sucrose, and 50 mM NaCl) and homogenized by placing the reaction mixture on ice and diluting to 3500 × g for 5 min at 4 °C, and the supernatant was subjected to centrifugation at 100,000 × g for 45 min at 4 °C. White fluffy material was collected and homogenized in hypotonic buffer with a 15-ml Potter-Elvehjem homogenizer. The homogenate was layered onto 14 ml of a 38% sucrose solution and centrifuged at 100,000 × g for 30 min at 4 °C. The turbid layer at the interface was collected into 25 ml of TS buffer (10 mM Tris-HCl, pH 7.4, 250 mM sucrose, and 50 mM NaCl) and pelleted by centrifugation at 100,000 × g for 30 min at 4 °C. After suspending in 5 ml of TS buffer, vesicles were formed by passing the suspension through a 27-gauge needle with a syringe. Inside-out vesicles were enriched by applying to a column of wheat germ agglutinin-linked CNBr-activated Sepharose 4B equilibrated with TS buffer. Unbound inside-out vesicles were resuspended in TS buffer and stored at −70 °C.

Putrescine Uptake by Inside-out Membrane Vesicles—Assays were performed as described by Xie et al. (29). The reaction mixture containing 100 µg of protein of membrane vesicles, 10 mM ATP, 10 mM MgCl₂, 0.2 mM CaCl₂, 10 mM creatine phosphate, 100 µg/ml creatine kinase, 250 mM sucrose, 10 mM Tris-HCl, pH 7.4, 10 mM dithiothreitol, and 1 µM [³H]putrescine (37 MBq/mmol; GE Healthcare) was prepared on ice. The reaction mixture was incubated at 37 °C, and putrescine uptake was terminated by placing the reaction mixture on ice and diluting with 1 ml of ice-cold stop buffer (250 mM sucrose, 150 mM NaCl, and 10 mM MES, pH 5.5). The vesicles were collected by rapid filtration onto premoistened Millipore HAWP 0.45- µm filters and washed four times with stop buffer. Radioactivity on the filters was measured in a liquid scintillation counter.

Liquid Chromatography Coupled to Tandem Mass Spectrometry (MS/MS) Analysis—CHO-T and CHO-S membrane proteins (100 µg) were run on an 11-cm immobilized pH gradient strip, pH 5–8, then resolved on a 8.5% SDS-polyacrylamide gel, and stained using silver solution (2.5% silver nitrate and 37% formaldehyde). Bands highly expressed in CHO-T membrane were excised and digested with trypsin for 16 h (36). The peptides extracted from the gel following digestion were analyzed by a ThermoFinnigan (San Jose, CA) LCQ DECA XP PLUS ion trap mass spectrometer equipped with a Michrom (Auburn, CA) Paradigm MS4 high performance liquid chromatography (HPLC) instrument and a nanoelectrospray source. Peptides were eluted from a 15-cm pulled tip capillary column (100-µm inner diameter × 360-µm outer diameter; 3–5-µm tip opening) packed with 7 cm of Vydac (Hesperia, CA) C₁₈ material (5 µm, 300-Å pore size) using a gradient of 0–65% solvent B (98% methanol, 2% water, 0.5% formic acid, and 0.01% trifluoroacetic acid) over a 60-min period at a flow rate of 350 nl/min. The LCQ electrospray positive mode spray voltage was set at 1.6 kV, and the capillary temperature was set at 180 °C. Dependent data scanning was performed by Xcalibur software (37) with a default charge of 2, an isolation width of 1.5 atomic mass units, an activation amplitude of 35%, an activation time of 30 ms, and a minimal signal of 1000 ion counts (arbitrary units based on the mass spectrometer used). Global dependent data settings were as follows: reject mass width of 1.5 atomic mass units, dynamic exclusion enabled, exclusion mass width of 1.5 atomic mass units, repeat count of 1, repeat duration of 1 min, and exclusion duration of 5 min. Scan event series included one full scan with mass range 350–2000 Da followed by three dependent MS/MS scans of the most intense ion. Tandem MS spectra of peptides were analyzed with TurboSEQUEST™, a program that allows the correlation of experimental tandem MS data with theoretical spectra generated from known protein sequences (38). Parent peptide mass error tolerance was set at 1.5 atomic mass units, and fragment ion mass tolerance was set at 0.5 atomic mass unit during the search. The criteria that were used for a preliminary positive peptide identification are the same as described previously (39), namely peptide precursor ions with a +1 charge having an Xcorr > 1.8, +2 charge having an Xcorr > 2.5, and +3 charge having an Xcorr > 3.5. A ΔCn score >0.08 and a fragment ion ratio of experimental/theoretical >50% were also used as filtering criteria for reliable matched peptide identification (39). All spectra were searched against the latest version of the non-redundant protein data base downloaded July 7, 2006 from the National Center for Biotechnology Information (NCBI).

Transfection—SureSilencing shRNA plasmid for human SLC3A2 (Bioscience Corp.) was transfected to Hkh2 cells using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s protocol. Transfected cells were selected by the resistance to 2 µg/ml puromycin. Knockdown of SLC3A2 was confirmed by semiquantitative PCR.

Semiquantitative PCR Analysis—Total RNA was isolated using the Qiagen RNaseasy kit according to the manufacturer’s protocol. One microgram of total RNA was treated with RNase-free deoxyribonuclease I (Fermentas Life Science) and reverse transcribed into cDNA using the Moloney murine leukemia virus reverse transcriptase (Fermentas Life Science). The DNA fragments of SLC3A2, SAT1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were amplified using primer sets of SLC3A2_F (5’-TTTTCAGTACGGGGATGAGAT-3’) and SLC3A2_R (5’-GCAGAAAACACCCTATTTGG-3’) for
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SLC3A2, SAT1_F (5'-TTTACCACTGCTGGTGTG-3') and SAT1_R (5'-AACAGAAAATCTAAGTACGATGTC-3') for SAT1, and GAPDH_F (5'-TTGTATGGTTGAAAAGGACTCATGAC-3') and GAPDH_R (5'-AGAGTTCCAGTGCTTCCGTTGAC-3') for GAPDH.

Western Blotting—Cells were washed with buffered saline and lysed in 10 mM Tris-HCl, pH 8.0, containing 10 μg/ml aprotinin, 500 μM sodium orthovanadate, and 10 μg/ml phenylmethylsulfonyl fluoride. Forty micrograms of protein were separated on a 10% polyacrylamide gel. Proteins were transferred electrophoretically to a HyBond-C nitrocellulose membrane (Amersham Biosciences). Blots were blocked in 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) for 30 min at room temperature. SLC3A2, SAT1, and β-tubulin were detected by the ECL Western blotting detection system (GE Healthcare) using anti-SLC3A2 (1:1000 dilution; Santa Cruz Biotechnology), anti-SAT1 (1:1000 dilution; Sigma), and anti-β-tubulin (1:10,000 dilution; Santa Cruz Biotechnology) as primary antibodies.

Transport Assay in Cells—Assays were performed as described by Tahara et al. (40) with modification. One million cells were plated in a 6-well plate and cultured for 2 days. After the medium was aspirated, cells were washed twice with assay buffer containing 5 mM Hepes-NaOH, pH 7.4, 145 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, and 5 mM glucose and incubated for 5 min at 37 °C in the same buffer. Uptake was started by the addition of 2 mM [3H]putrescine (37 MBq/mmol; GE Healthcare), 2 mM [14C]arginine (37 MBq/mmol; GE Healthcare), or 2 mM [14C]ornithine (37 MBq/mmol; GE Healthcare). After incubation for varying times, cells were washed twice with ice-cold assay buffer containing 20 mM putrescine, arginine, or ornithine. Cells were lysed in 0.5N NaOH, and radioactivity was counted using a Beckman LS 5000TD scintillation counter. Total cellular protein content was determined by the bicinchoninic acid (BCA) protein assay solution (Pierce).

Measurement of Polyamine Content in Cells—Three million cells were homogenized in 0.2 N HClO₄. An acid-soluble fraction was separated by reverse-phase ion pair HPLC, and polyamines were detected as described elsewhere (41). An acid-insoluble fraction was used for determination of protein amount by the BCA protein assay (Pierce).

Diamine Export Assay in Cells—One million cells were plated in a 6-well plate and cultured for 1 day. Cells were washed three times and incubated in 1 ml of Dulbecco’s modified essential medium without FBS at 37 °C. After 8 h, the medium was removed, and diamines exported into the medium were measured by HPLC. Cells were washed with buffered saline and counted.
Immunoprecipitation—Immunoprecipitation was performed using the method of Torrents et al. (42) with modifications. Hkh2 cells were detached from culture flasks mechanically and washed with buffered saline. Cells were lysed in lysis buffer containing 50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40, and proteinase inhibitor mixture. One hundred micrograms of cell lysate were incubated with anti-SLC3A2, anti-SAT1, or anti-ornithine decarboxylase (ODC) at 4 °C for 16 h. Lysates were incubated with protein G-Sepharose beads (Santa Cruz Biotechnology) for 16 h at 4 °C and washed five times with the same buffer and incubated with fluorophore-labeled secondary antibody (1:1000 dilution of Alexa 633-conjugated anti-goat IgG and Alexa 488-conjugated anti-rabbit IgG (both from Molecular probes)) for 16 h at 37 °C. Cells were washed five times and mounted in Prolong Gold Mounting Solution (Clontech). Fluorescence was visualized using a Nikon confocal microscope.

RESULTS
Identification of Proteins Highly Expressed in CHO-T Cells—To identify and characterize the polyamine exporter, we isolated CHO-T cells (30). These cells showed resistance to the high exogenous concentrations of putrescine, whereas the growth of non-selected CHO-S cells was inhibited by more than 10 mM putrescine (Fig. 1A). Putrescine uptake by inside-out membrane vesicles prepared from CHO-T cells was significantly higher than the same vesicles prepared from CHO-S cells (Fig. 1B). This result indicates that the resistance of CHO-T cells to the high concentrations of putrescine was associated with elevated putrescine export. We looked for proteins highly expressed in CHO-T cells, compared with CHO-S cells, by proteomics analysis. Membrane proteins of CHO-T and CHO-S cells were separated by two-dimensional gel electrophoresis, and bands highly expressed in the plasma membrane of CHO-T were subjected to peptide sequencing by liquid chromatography followed by tandem mass spectrometry. Data base searching revealed the sequence of several proteins that were highly expressed in the plasma membrane of CHO-T cells (supplemental information). Among these, there were two transport proteins, SLC3A2, a glycosylated heavy chain of a cationic amino acid transporter, and SLCA6A19, a Na+/H+-dependent neutral amino acid transporter. Because polyamines and diamines are positively charged in physiological conditions, we examined whether the SLC3A2-containing transporter was involved in the export of diamines.
SLC3A2-dependent Putrescine, Arginine, and Ornithine Transport—We tested putrescine transport activity of the SLC3A2-containing transporter in colon cancer cells because polyamine transport plays an important role in colon carcinogenesis (43). We used the Hkh2 cell line, which is an isogenic clone of HCT116 colon carcinoma cells lacking its activated K-RAS allele. Stable transfection of a plasmid encoding shRNA for SLC3A2 reduced both mRNA and protein levels of SLC3A2 to 50% (Fig. 2A). Fig. 2B shows putrescine, arginine, and ornithine uptake by SLC3A2 knockdown cells and control plasmid-transfected cells. Putrescine uptake was higher in SLC3A2 knockdown cells than control cells, whereas arginine uptake was lower. Ornithine uptake was not changed.

Effect of SLC3A2 on Cell Growth—The growth rate of SLC3A2 knockdown cells was determined. SLC3A2 knockdown cells grew faster than control cells (Fig. 3A). The sensitivity of these cells to exogenous putrescine was examined. SLC3A2 knockdown cells were more sensitive to high concentrations of putrescine than control cells (Fig. 3B). The polyamine contents of these cells cultured in the presence or absence of 20 mM putrescine were determined (Fig. 3C). Putrescine and N<sup>1</sup>-acetylputrescine contents were elevated in SLC3A2 knockdown cells grown in medium lacking supplemented putrescine. N<sup>8</sup>-Acetylputrescine was not detected. Intracellular levels of putrescine were further increased when these cells were cultured in the presence of 20 mM putrescine, whereas spermidine and spermine levels were decreased.

SLC3A2-dependent Polyamine Export—To confirm that SLC3A2 was involved in export of polyamines, the effect of SLC3A2 knockdown on polyamine export from cells was examined. SLC3A2 knockdown and control cells were incubated in medium without FBS for 8 h, and polyamines exported into the medium were measured. As shown in Fig. 4, putrescine, spermidine, spermine, and acetylated spermidine were exported at lower levels into the medium of SLC3A2 knockdown cells compared with control cells.

Regulation of SLC3A2 by K-RAS—Uptake of polyamines is mediated by caveolae-dependent endocytosis in colon epithelial cells, and K-RAS positively regulates polyamine uptake through phosphorylation of caveolin-1 protein (24). K-RAS also contributes to the regulation of intracellular polyamine content by up-regulating ODC (44). Therefore, we examined whether SLC3A2 was regulated by K-RAS. The polyamine content in HCT116, a colon cancer cell line expressing a mutant K-RAS, and its isogenic clone, Hkh2, which lacks the activated K-RAS allele, was determined (Fig. 5). Putrescine, spermidine, and N<sup>1</sup>-acetylputrescine were significantly decreased in Hkh2 cells. Spermine and cadaverine showed similar levels in the two cell line clones. HCT116 cells grew faster than Hkh2 cells (data not shown). The protein and mRNA levels of SLC3A2 were determined (Fig. 6). Both the protein level (Fig. 6A) and mRNA level (Fig. 6B) were also higher in Hkh2 compared with the

FIGURE 4. SLC3A2-dependent polyamine export. Cells transfected with control plasmid (white column) or plasmid encoding shRNA for SLC3A2 (black column) were cultured for 1 day. Cells were washed with Dulbecco’s modified essential medium without FBS and incubated 8 h in the same medium. Polyamines exported into the medium were determined by HPLC. Values are expressed as nmol in medium/mg of protein and shown as means ± S.E. of triplicate determinations, *, p < 0.05.

FIGURE 5. Polyamine content in K-RAS-activated HCT116 and its isogenic K-RAS-inactivated clone Hkh2. HCT116 cells (white column) and Hkh2 cells (black column) were cultured, and polyamine and diamine content was determined as described under “Experimental Procedures.” Values are presented as means ± S.E. of triplicate determinations. *, p < 0.05.

A. Western blotting  B. RT-PCR

SLC3A2 uptake was negatively regulated by K-RAS. The protein and mRNA levels of SLC3A2 and SAT1 in HCT116 and Hkh2 cells were determined by Western blotting (A) and reverse transcription (RT)-PCR (B) as described under “Experimental Procedures.” β-Tubulin and GAPDH levels are shown as loading control.
isogenic HCT116 cells. These results indicate that the expression of SLC3A2 was negatively regulated by K-RAS. SAT1, the polyamine-catabolizing enzyme that catalyzes spermine and spermidine acetylation, is also regulated by K-RAS. The protein levels of SAT1 were higher in Hkh2 cells, whereas the mRNA levels were not changed (Fig. 6). This result indicates that SAT1 expression is regulated by K-RAS at the post-transcriptional level in these cells.

**SLC3A2 Interaction with SAT1**—It has been reported that SLC3A2 interacts with and mediates integrin signaling (45) and that SAT1 interacts with a cytoplasmic domain of specific integrins (46). These reports led us to hypothesize that SLC3A2 and SAT1 may form a complex at the plasma membrane. To test this possibility, the interaction of SLC3A2 and SAT1 was assessed by immunofluorescence microscopy and immunoprecipitation (Fig. 7). SLC3A2 and SAT1 were colocalized on the plasma membrane (Fig. 7A). SAT1 was co-immunoprecipitated by anti-SLC3A2 antibody as was SLC3A2 with anti-SAT1 antibody. A low amount of SAT1 was co-immunoprecipitated by anti-ODC1 antibody (Fig. 7B). These results indicate that SLC3A2 and SAT1 form a complex on the plasma membrane and suggest a functional consequence for this complex.

**DISCUSSION**

In this study, we identified and characterized SLC3A2 as a part of DAX. SLC3A2 was one of the proteins that were highly expressed in CHO-T cells compared with CHO-S cells. Knockdown of SLC3A2 using shRNA increased putrescine uptake and decreased arginine uptake in colon cancer cells. Polyamine contents in SLC3A2 knockdown cells were increased, and cell growth was stimulated. High concentrations of exogenous putrescine inhibited cell growth of SLC3A2 knockdown cells more than in control cells. Supplementation of culture medium with putrescine caused an increase in both putrescine and monoacetylated spermidine in the cells, both substrates for export by DAX (29). Knockdown of SLC3A2 reduced polyamine export from cells. These results indicate that SLC3A2 is involved in the export of the diamine putrescine and monoacetylated spermidine. Immunofluorescence and immunoprecipitation studies indicated that SLC3A2 and SAT1 form a protein complex on the plasma membrane. The proximity between SLC3A2 and SAT1 may facilitate the export of acetylated polyamines, the products of SAT1 activity, by the adjacent exporter containing SLC3A2 when cellular polyamine levels reached high levels. Our result also suggests that ODC may interact with SAT1 (Fig. 7B). ODC has been reported to translocate to the plasma membrane (47). The interaction of SAT1 and ODC support the association of ODC to the plasma membrane and may suggest a mechanism for metabolic channeling of biosynthesis and acetylation of polyamines. Experiments in Hkh2 cells (Fig. 2) showed that the exporter that contains the SLC3A2 catalyzes putrescine export and arginine uptake, suggesting a putrescine/arginine exchange reaction. Arginine is metabolized to ornithine, a precursor of putrescine biosynthesis. Arginine also is a substrate for nitric-oxide (NO) synthase in the production of NO. NO is required for polyamine uptake (23). DAX containing SLC3A2 may play an important role in regulation of cellular polyamine level not only by export of polyamines but also by modulating cellular arginine and the NO level to enhance polyamine uptake. This hypothesis is currently under examination.

Putrescine accumulated in cells when they were cultured in the presence of 20 mM putrescine. Spermidine and spermine were decreased in these cells (Fig. 3C). This result indicated that biosynthesis of spermidine and spermine was down-regulated by high levels of intracellular putrescine. Biosynthesis of spermidine and spermine requires supplementation of a methyl group from decarboxylated S-adenosylmethionine. Putrescine accumulation can deplete S-adenosylmethionine due to the excess usage for biosynthesis of...
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![Diagram](image)

**FIGURE 8. The model of DAX in colon epithelial cells.** SLC3A2 is involved in diamine export by an arginine/diamine exchange mechanism. SAT1 forms a complex with SLC3A2 to couple acetylation and export of polyamines. The expression of SLC3A2 and SAT1 is negatively regulated by K-RAS. Orn, ornithine; PUT, putrescine; SPD, spermidine; SPM, spermine.

spemidine and spermine (48), and this depletion might be the mechanism for reduced spermidine and spermine pools seen in these experiments.

Knockdown of SLC3A2 decreased the export of putrescine and monoacetylated spermidine as well as spermidine and spermine (Fig. 4). Because spermidine and spermine are not substrates for DAX (29), SLC3A2 may influence other exporters associated with spermidine and spermine export. The detection of relatively high levels of $N^\alpha$-acetylspemidine in Fig. 4 could be due to the growth of cultures in serum-free medium. We used medium without FBS for the export assay because FBS contains amine oxidase (49). The SLC3A2-dependent putrescine export rate (0.02 nmol/h/mg of protein) was comparable with the difference in putrescine accumulation in SLC3A2 knockdown and control cells (1 nmol/48 h/mg of protein) cultured with 20 nM putrescine (Fig. 2). FBS did not appear to influence the activity of DAX.

We found that K-RAS negatively regulated SLC3A2 and SAT1 expression. K-RAS acts to elevate intracellular polyamine contents by increasing polyamine uptake (24) and biosynthesis (44, 50) and suppressing degradation (51). Our results show that K-RAS also can down-regulate polyamine export to increase cellular polyamine content.

Polyamine uptake and export have significant roles in colon carcinogenesis as dietary and intestinal luminal polyamines influence this process (52, 53). The tumor suppression effect of the non-steroidal anti-inflammatory drug sulindac is reduced by dietary polyamines (54). Understanding polyamine transport mechanisms may be important for the development of new strategies for cancer treatment and chemoprevention. Our results provide the first evidence of molecular characterization of polyamine export in colon epithelial cells.

A model depicting DAX is summarized in Fig. 8. DAX is a heterodimer composed of SLC3A2 and a y+ LAT light chain. The SLC3A2-containing transporter catalyzes export of putrescine and acetylpolyamines via arginine exchange activity. SAT1 may interact with SLC3A2 to couple acetylation and export of the longer chain polyamines, spermidine and spermine. K-RAS negatively regulates SLC3A2 and SAT1 expression.

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