SLC38A2 promotes cell proliferation and invasion by promoting glutamine metabolism in adenomyosis

KUI WU*, WEI GONG*, JIA-CHANG HU, YING-CHUN DUAN, HUI-HUI KE and LI CHEN

Department of Obstetrics and Gynecology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai 201399, P.R. China

Received April 3, 2023; Accepted February 6, 2024

DOI: 10.3892/etm.2024.12506

Abstract. Adenomyosis is a benign uterine disorder that is associated with female infertility, a reduced clinical pregnancy rate and a high risk of miscarriage. Solute carrier family 38 member a2 (SLC38A2) is a glutamine (Gln) transporter that serves roles in various medical conditions. The present study aimed to reveal the role of SLC38A2 in adenomyosis. The mRNA expression levels of SLC38A2 in eutopic endometrial (EU) and ectopic endometrial (EC) tissues from adenomyotic patients were examined by reverse transcription-quantitative PCR. EU and EC cell proliferation and invasion were analyzed by Cell Counting Kit-8 and Transwell assays. Changes in the oxygen consumption rate (OCR) were determined to indicate the mitochondrial respiratory function and observed using a Seahorse analyzer. SLC38A2 expression in EC tissues was upregulated compared with that in normal endometrial tissues. SLC38A2 knockdown repressed EC cell proliferation and invasion. In addition, the Gln content and OCR were decreased in EC cells transfected with SLC38A2-knockdown lentivirus, whereas SLC38A2 overexpression had the opposite effect in EU cells. Furthermore, the increased proliferation and invasion rates and Gln level induced by SLC38A2 overexpression in EU cells were alleviated by CB-839, a glutaminase inhibitor. SLC38A2 overexpression promoted Gln metabolism and oxygen consumption rate, resulting in an increase in cell proliferation and invasion in the adenomyosis context. The present study indicated that reduction of SLC38A2 expression could be a novel target for adenomyosis therapy, and SLC38A2 may be a valuable clinical diagnostic molecule for adenomyosis.

Correspondence to: Mrs. Li Chen or Mrs. Hui-Hui Ke, Department of Obstetrics and Gynecology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, 2800 Gongwei Road, Shanghai 201399, P.R. China
E-mail: chenli8369@163.com
E-mail: melody1881_hh@sina.com

*Contributed equally

Abbreviations: SLC38A2, solute carrier family 38 member a2; EU, eutopic endometrial; EC, ectopic endometrial; CE, normal endometrial; OCR, oxygen consumption rate

Key words: adenomyosis, SLC38A2, glutamine, OCR

Introduction

Adenomyosis is a benign uterine disorder characterized by the presence of abnormal endometrial glands and stroma in the myometrium (1). Women with adenomyosis may suffer from heavy menstrual bleeding, dysmenorrhea, reproductive failure and infertility (2). Hysterectomy is the most effective treatment for adenomyosis (3). To increase desired fertility, preserve sexual function and attenuate stress in younger patients, uterus-sparing therapy tends to be a popular treatment for adenomyosis (3). Therefore, it is important to explore the mechanism underlying adenomyosis and develop novel treatments. Some studies (4-6) have reported that inflammatory molecules, sex steroid hormone receptors, growth factors and extracellular matrix enzymes serve important roles in the pathogenesis of adenomyosis. However, the underlying etiology remains unclear.

Amino acids are the substrates needed for protein synthesis, serving critical roles in metabolism as sources of energy, carbon and nitrogen. Amino acids, including glutamine (Gln), have been reported to regulate cell proliferation, apoptosis and metabolism (7). Although Gln is a non-essential amino acid, it is of great importance in intermediary metabolism (8). Gln is a substrate for protein and nucleotide synthesis (9). Furthermore, Gln can be a nitrogen and carbon donor that provides energy for cell proliferation (10). Gln transporters are the main regulators of the intracellular Gln level (11). The solute carrier (SLC) superfamily constitutes the largest group of transporters for transporting large numbers of substances, including drugs and ions (11,12). SLC dysfunction can induce a diverse range of diseases, such as heart disease, Alzheimer's disease, diabetes and cancer (13-15). SLC family 38 member a2 (SLC38A2) belongs to the system A (alanine preferential) transporters and contributes to a high Gln concentration (10). Given the critical function of SLC38A2 in amino acid transport, SLC38A2 serves an important role in numerous signaling pathways and medical conditions, including diabetes, neurological diseases and cancer (10). SLC38A2, a Gln transporter, is a major regulator of intracellular Gln levels and can lead to excessive Gln concentrations (10). In immune disorders and other chronic

inflammatory diseases, such as endometriosis, activation of the immune system consumes a large amount of energy, of which glucose and Gln are the main sources (10). An increased level of Gln has been observed in patients with endometriosis (16,17). As adenomyosis is considered internal endometriosis (16) and as elevated SLC38A2 leads to the increased uptake of Gln (17), it is reasonable to hypothesize that SLC38A2 serves a role in adenomyosis. However, to the best of our knowledge, the Gln content and the function of SLC38A2 in adenomyosis remain largely unclear. The purpose of the present study was to explore the function of SLC38A2 and determine whether SLC38A2 increases the cell proliferation rate by increasing mitochondrial respiratory function in adenomyosis.

Materials and methods

Samples. Between January 2023 and February 2023, 10 paired eutopic endometrial (EU) and ectopic endometrial (EC) tissues were derived from 10 adenomyotic patients who received a partial resection of adenomyotic tissues at Shanghai Pudong Hospital (Shanghai, China). The last tissue was collected on February 16, 2023. The preoperative diagnosis of adenomyosis was based on the typical clinical symptoms of dysmenorrhea and/or menorrhagia and pelvic pain, physical examination findings and imaging results, including transvaginal ultrasound and MRI scans. EC tissue, referring to the adenomyosis nidus formed locally by the invasion of the endometrium and stroma into the muscle layer, was collected during laparoscopic surgical procedures, and the paired EU tissue was collected through scraping during surgery. The age of the patients included in the present study ranged between 35 and 45 years, with a mean age of 38.96±5.01 years. All women in the control and adenomyosis groups had regular menstrual cycles (28-35 days). The exclusion criteria were: i) Patients who were diagnosed with uterine fibroids and subsequently received hormonal therapy within the previous 6 months; ii) patients who had reproductive tract infections, immune system disease or endocrine diseases; iii) patients whose preoperative hysteroscopy excluded the diagnosis of endometrial lesions; and iv) patients with comorbidities or major organ dysfunction. Samples were obtained when the women were in the early proliferative phase of the menstrual cycle. Normal endometrial (CE) tissues were obtained between January 2023 and February 2023 from 10 women (age range, 27-41 years; mean age, 34.27±4.62 years) who voluntarily sought placement of an intrauterine device at Shanghai Pudong Hospital (Shanghai, China) and who had no diseases and had not used hormone drugs in the previous 3 months; these patients were considered controls. Written informed consent was obtained from all participating patients. The present study was approved by the Ethics Committee of Shanghai Pudong Hospital (approval no. 2023-WZ-04) and performed in accordance with the tenets and guidelines of the Declaration of Helsinki.

Cells. The three types of fresh tissues (CE, EU and EC tissues) were washed three times in Hank's Balanced Salt Solution (Gibco; Thermo Fisher Scientific, Inc.) containing 1,000 U/ml penicillin and 1,000 μg/ml streptomycin. The 10 CE, EU or EC samples were digested together using 0.4% collagenase and 0.05% pancreatic enzyme plus 20 μg/ml DNase I at 37˚C with shaking. After 2-3 h, the digestive solution was replaced with DMEM-F12 (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C with 5% CO_2. CE, EU and EC cells were obtained using a method established in a previous study (18). The primary cells were passaged at a fusion rate of 80% after digestion using 0.25% trypsin. The cells were passaged every 3 days. At 8 days after tissue digestion, CE, EU and EC cells (third passage) were used for the subsequent experiments.

Lentivirus construction. Lentiviruses (including SLC38A2 overexpression and knockdown lentiviruses, constructed into the pLVX-Puro plasmid and pLKO.1 plasmid, respectively) were custom synthesized from Shanghai GeneChem Co., Ltd. Briefly, the SLC38A2 coding sequence was cloned into the pLVX-Puro vector (Clontech; Takara Bio USA, Inc.), and the SLC38A2 short hairpin RNA (shRNA/sh) was cloned into the pLKO.1 vector (Clontech; Takara Bio USA, Inc.). The 293T cells (The Cell Bank of Type Culture Collection of The Chinese Academy of Science) were transfected with the constructed vector, lentiviral packaging and envelope plasmids (psPAX2 and pMD2G; at the ratio of 10:9:1 μg; Addgene, Inc.) using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.). The 293T cells were cultured with DMEM-F12 containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C with 5% CO_2 for 48 h. The lentiviral supernatants were then harvested by centrifugation at 3,000 x g at 4˚C for 10 min. and filtered using a 0.45-μm filter. A MOI of 10 was used to infect cells. The EU/EC cells were infected with lentiviral supernatant, incubated at 37˚C overnight using DMEM-F12 containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C with 5% CO_2, selected with 1.5 μg/ml puromycin (Merck KGaA) for 14 days and maintained with 0.625 μg/ml puromycin for the subsequent experiments. The following three knockdown lentiviruses and a non-targeting control (shNC) were used: shSLC38A2-1, 5'-GCTTTGTTC TTCCTGTTAA-3'; shSLC38A2-2, 5'-GAAGAAGTATGA AACAGAA-3'; shSLC38A2-3, 5'-GCATCTGGATCAATT ACAA-3'; and shNC, 5'-GGACGAGCTGTACAAGTAA-3'. The empty pLVX-Puro vector was used as the NC for overexpression, and the cells in the control group were not infected.

Cell treatment. The cell experiment was divided into three sections. In section 1, we have assessed the effects of Gln exposure on the cell viability of EU and EC cells. The EU and EC cells were treated with Gln (1.5 mM; G3126; Merck KGaA) and the cells were cultured with DMEM-F12 containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C with 5% CO_2 for 48 h. Finally, cell viability was assessed using a Cell Counting Kit-8 assay. In section 2, the effects of SLC38A2 knockdown on EC cells and SLC38A2 overexpression on EU cells were evaluated. The effectiveness of the vector was detected by RT-qPCR and western blotting. The stable transgenic EC cells and EU cells were cultured with DMEM-F12 containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C with 5% CO_2 for 48 h. The cell viability was assessed at 0, 12, 24 and 48 h. The cellular oxygen consumption rate (OCR), invasion, Gln content and relative protein levels were finally evaluated. In section 3, the

SLC38A2-overexpressing EU cells were treated with 10 μM CB-839 (glutaminase inhibitor; Merck KGaA) and cultured with DMEM-F12 containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C with 5% CO_2 for 48 h. The cell viability was assessed at 0, 12, 24 and 48 h. The Gln content and cell invasion were finally evaluated. Three independent experiments were performed in duplicate (six duplications for the OCR assay).

Reverse transcription-quantitative PCR. Total RNA was isolated from tissues or treated cells ($2x10^7$) using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed into cDNA using a High Capacity cDNA RT kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. The fluorophore used in qPCR was 2x SYBR Green PCR Master mix (Thermo Fisher Scientific, Inc.), and PCR was performed on an ABI7500 real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions were as follows: Denaturation 95˚C for 5 min, followed by 40 cycles of denaturation at 95˚C for 10 sec, annealing at 60˚C for 20 sec and extension at 72˚C for 20 sec. Relative gene expression was analyzed using the $2^{-\Delta\Delta Cq}$ method with GAPDH as the internal control (19). The primer sequences are listed in Table I. Three independent experiments were performed in duplicate.

Cell proliferation assay. Cells ($2x10^3$ cells per well) were seeded in a 96-well plate with six replicates per group and cultured at 37˚C overnight. Then, cells in each group were treated as indicated. At each time point, the culture medium was replaced with 90 μl DMEM-F12 without FBS and 10 μl Cell Counting Kit-8 (Beyotime Institute of Biotechnology) for 1 h of culture. Finally, cell proliferation was analyzed by measuring the absorbance value at 450 nm using a microplate reader (Bio-Rad Laboratories, Inc.).

Transwell assay. The Transwell assay was performed to evaluated the invasion of cells. The Transwell chamber (Corning, Inc.) was pre-coated with 80 μl Matrigel (Corning, Inc.) at 37˚C for 30 min. Subsequently, cells ($1x10^5$ cells/ml; 200 μl) were added into the upper chamber of the insert with DMEM-F12 without FBS, and DMEM-F12 with 10% FBS was added to the lower chamber. After 48 h of incubation at 37˚C, the cells in the lower chamber were fixed with 4% formaldehyde solution for 10 min at room temperature and stained with 0.5% crystal violet for 30 min at room temperature. Finally, unmigrated cells in the upper chamber were removed and the migrated cells in the lower chamber were imaged using a light microscope (CX33; Olympus Corporation).

Seahorse assay. The cellular OCR was analyzed using a Seahorse XF24 analyzer (Agilent Technologies, Inc.) as described in a previous study (20). The OCR experiments were performed in six parallel replicates.

Gln content. Cells were seeded in a 24-well plate at a density of $1x10^4$ cells/well and treated as indicated. The concentration of Gln was measured using a Glutamine Assay Kit (Colorimetric) (cat. no. ab197011; Abcam) according to the manufacturer's instructions.

Western blotting. The total protein of the cell samples was extracted using protein extraction reagent (RIPA buffer; cat. no. 89901; Thermo Fisher Scientific, Inc.) and the protein concentration was initially measured using the BCA method according to the instructions of the Pierce Rapid Gold BCA assay kit (cat. no. A53226; Thermo Fisher Scientific, Inc.). Thereafter, total protein (25 μg/lane) was fractionated via 10% SDS-PAGE and transferred onto nitrocellulose membranes (MilliporeSigma), which were then blocked with 5% nonfat dry milk at room temperature for 1 h, and incubated with primary antibodies at 4˚C overnight. After washing three times using TBS with Tween-20 (0.1%), the membranes were incubated with horseradish peroxidase-labelled secondary antibody anti-IgG (cat. no. A0208/A0192; 1:1,000; Beyotime Institute of Biotechnology) at 37˚C for 1 h. An enhanced chemiluminescence system (Ranon GIS-2008; Tanon Science and Technology Co., Ltd.) was used to determine the protein levels using ECL-PLUS reagent (Thermo Fisher Scientific, Inc.). The protein bands were analyzed using ImageJ 1.8 (National Institutes of Health). The primary antibodies were: Anti-SLC38A2 (cat. no. bs-12125R; 1:1,000; BIOSS), anti-NADH-ubiquinone oxidoreductase subunit B8 (NDUFB8; cat. no. ab192878; 1:1,000; Abcam), anti-ubiquinol-cytochrome *c* reductase core protein 2 (UQCRC2; cat. no. ab203832; 1:1,000; Abcam) and anti-β-actin (cat. no. 81115-1-RR; 1:5,000; Proteintech Group, Inc.). β-actin served as the loading control.

Statistical analysis. The results are presented as the mean ± standard deviation of three or more independent experiments performed in duplicate. GraphPad Prism 7 (Dotmatics) was used for statistical analysis and graph generation. Statistical comparisons between groups were performed using an unpaired Student's t-test, or a one-way ANOVA followed by Tukey's multiple comparisons test, or a two-way ANOVA with Bonferroni as the post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

SLC38A2 expression is increased in EC samples. The mRNA expression levels of the SLC38A family, including SLC38A1-11, were examined in CE, EU and EC tissues. As shown in Fig. 1A-C, SLC38A2, SLC38A3, SLC38A4, SLC38A5, SLC38A8, SLC38A10 and SLC38A11 expression was increased in EU and EC tissues. However, only SLC38A2 expression was significantly upregulated in EC samples compared with that in CE samples.

EC cells exhibit more dependence on Gln. Gln was applied to the cells derived from EU and EC tissues. A cell proliferation assay demonstrated that Gln significantly promoted EC cell proliferation at 48 h after treatment (Fig. 1D). However, no significant increase was observed in EU cells with or without Gln treatment. EC cells with or without Gln treatment exhibited enhanced viability compared with EU cells, indicating that only EC cell proliferation was dependent on Gln.

Silencing of SLC38A2 inhibits cell proliferation, invasion, Gln content and the OCR in EC cells. To explore the function of SLC38A2, lentiviruses targeting SLC38A2 were

Table I. Primers used for reverse transcription-quantitative PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
SLC38A1	GCCATTATGGGCAGTGGGAT	TACGAACTTCCCTGTGGTGC
SLC38A2	GACCGCAGCCGTAGAAGAAT	ACAGCCAGACGGACAATGAG
SLC38A3	AGTCTGAGCTGCCACTTGTC	AGAGAGAAGCCGCTGGAGTA
SLC38A4	ATGAACACCATCCCGGAACC	CCCTCCTTCCTTGGCTGTTT
SLC38A5	TGTGAGGCCCAGATGTTCAC	AGGTTGCTGTGAGCCCATAC
SLC38A6	TGTCCAGCAGCCTGAAGAAG	CAGGAGAGCAACTGTCAGCA
SLC38A7	CCCCAGGGAGATTGGTTTCC	GGTCTTCACTTCAGGCTGCT
SLC38A8	CTCAGCGAGATCGTCAGCAT	AAGATGAAGGTGCCGACCAG
SLC38A9	TGATAATCCTATTTTCATTCGCACA	CCCTGAGCATTCAAGTCAGC
SLC38A10	TACGCCGGCCTGGCATTC	ATGGAGGCCATCATGTTCCG
SLC38A11	TGTAGCCACGCTTGTGTCAT	CCATGGGTGCAGTCTTGAGT
GAPDH	AATCCCATCACCATCTTC	AGGCTGTTGTCATACTTC

SLC38A, solute carrier family 38 member a.

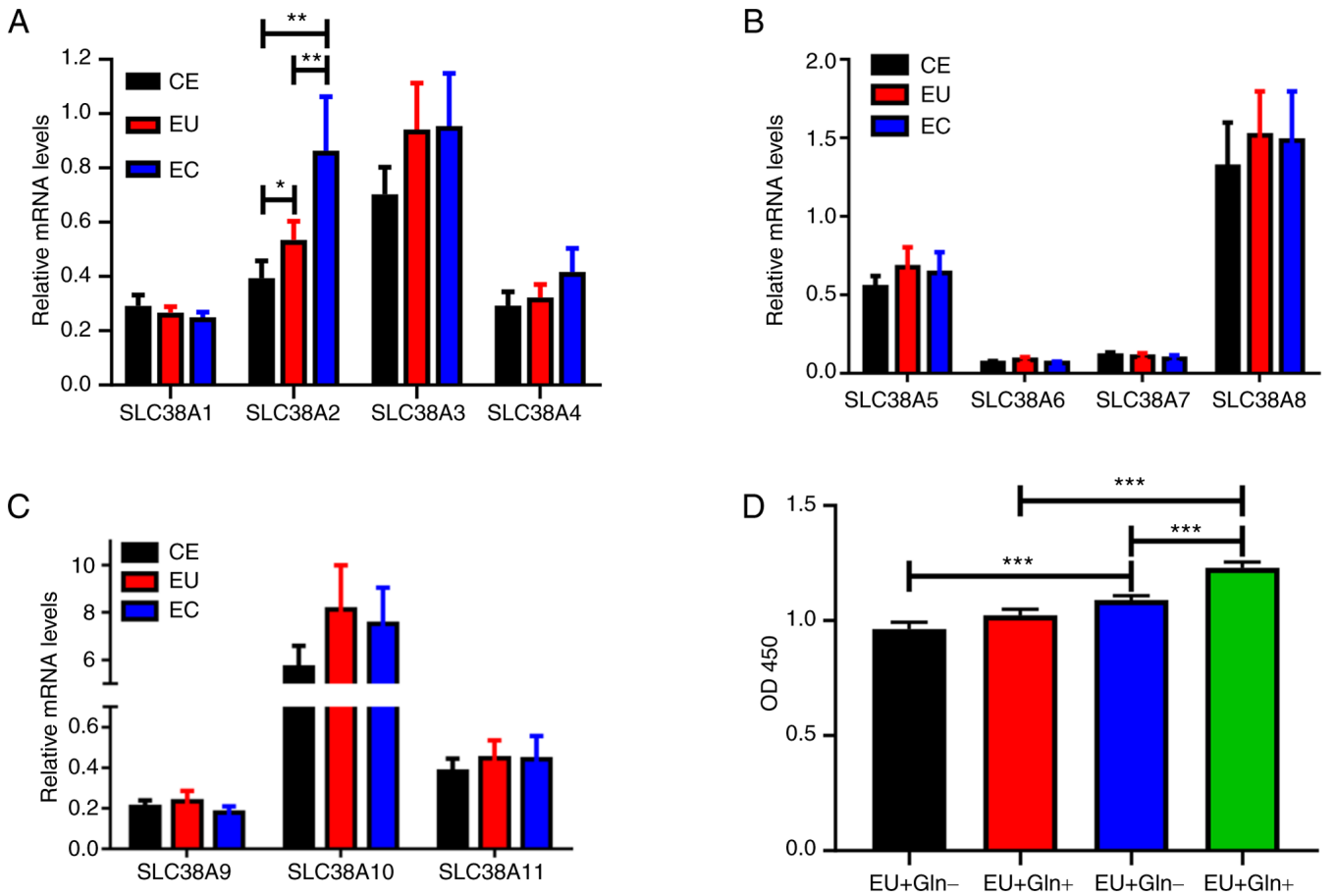

Figure 1. SLC38A2 expression is upregulated in EC samples. (A) mRNA expression levels of the SLC38A family, (A) SLC38A1-4, (B) SLC38A5-8 and (C) SLC38A9-11, were detected using reverse transcription-quantitative PCR in CE, EU and EC tissues. (D) Cell proliferation was measured in EU and EC cells treated with or without Gln. *P<0.05, **P<0.01 and ***P<0.001. CE, normal endometrial; EC, ectopic endometrial; EU, eutopic endometrial; Gln, glutamine; OD, optical density; SLC38A, solute carrier family 38 member a.

constructed to knock down SLC38A2 expression. As shown in Fig. 2A, both the protein and mRNA levels of SLC38A2 were markedly decreased following transfection with shSLC38A2 lentiviruses. Cell proliferation was examined and the results showed that SLC38A2 knockdown decreased the proliferation of EC cells at 48 h (Fig. 2B). Similarly, the number of invading cells was significantly decreased in the SLC38A2 knockdown groups compared with that in the NC

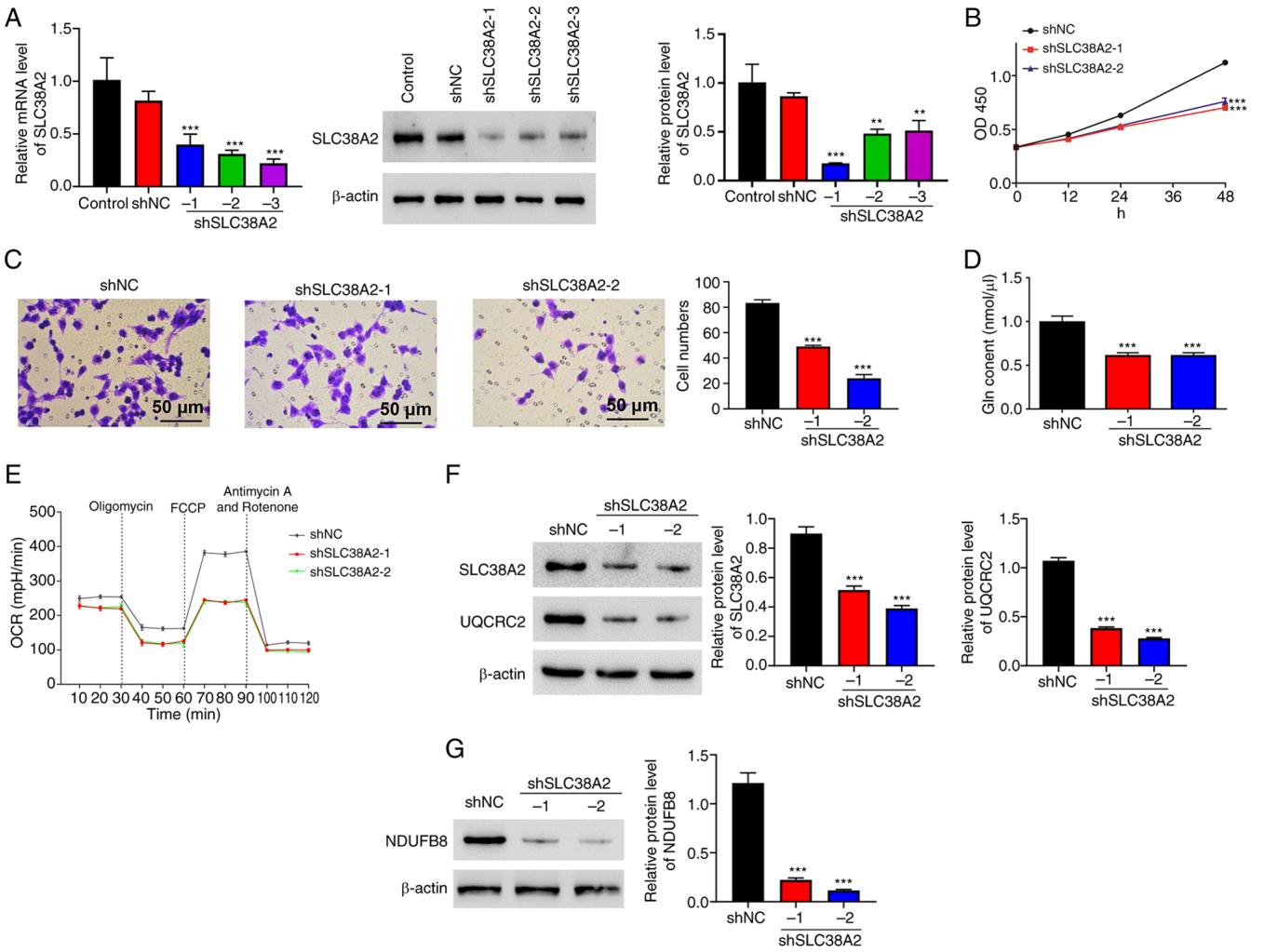

Figure 2. Silencing of SLC38A2 inhibits cell proliferation, invasion, Gln content and the OCR in EC cells. SLC38A2 knockdown lentiviruses were applied to EC cells. (A) mRNA and protein expression levels of SLC38A2 were detected using reverse transcription-quantitative PCR and western blotting. (B) Cell proliferation, (C) invasion (scale bar, 50 μm), (D) Gln content and (E) OCR level, and protein levels of (F) UQCRC2 and (G) NDUFB8 were examined. **P<0.01 and ***P<0.001 vs. shNC. EC, ectopic endometrial; FCCP, carbonylcyanide-p-trifluoromethoxyphenylhydrazone; Gln, glutamine; NC, negative control; NDUFB8, NADH-ubiquinone oxidoreductase subunit B8; OCR, oxygen consumption rate; OD, optical density; sh, short hairpin RNA; SLC38A2, solute carrier family 38 member a2; UQCRC2, ubiquinol-cytochrome *c* reductase core protein 2.

group, indicating that silencing of SLC38A2 significantly inhibited the invasion of EC cells (Fig. 2C). Furthermore, SLC38A2 knockdown decreased the Gln content in EC cells (Fig. 2D). SLC38A2 knockdown reduced the OCR, and downregulated the protein levels of NDUFB8 and UQCRC2, which are markers of mitochondrial respiration (21) (Fig. 2E-G).

SLC38A2 overexpression increases cell proliferation and invasion rates, Gln content, and the OCR in EU cells. SLC38A2 was overexpressed in EU cells following transfection with SLC38A2 overexpression lentivirus (Fig. 3A). Cell proliferation and invasion were analyzed after SLC38A2 overexpression. As shown in Fig. 3B and C, SLC38A2 overexpression significantly increased the proliferation and invasion of EU cells at 48 h. In addition, SLC38A2 overexpression increased the Gln content in EU cells (Fig. 3D), implying that the amount of Gln was increased. SLC38A2 overexpression increased the OCR, and increased the protein levels of NDUFB8 and UQCRC2 (Fig. 3E-G).

Effects of SLC38A2 overexpression are attenuated by a glutaminase inhibitor. The present study then explored whether the function of SLC38A2 was dependent on the Gln content. Accordingly, 10 μM CB-839, a glutaminase inhibitor, was used to treat EU cells after SLC38A2 was overexpressed. The increase in cell proliferation induced by SLC38A2 overexpression was inhibited by CB-839 at 48 h (Fig. 4A). A higher Gln content was observed in the SLC38A2 overexpression group compared with the vector group. Compared with that in the SLC38A2 overexpression group, the Gln content in the group treated with SLC38A2 overexpression lentivirus and CB-839 was significantly decreased (Fig. 4B). As shown in Fig. 4C, the number of invading cells was increased by SLC38A2 overexpression but decreased by CB-839 treatment compared with the vector group, and CB-839 abolished the effect of SLC38A2 overexpression, indicating that the promoting effect of SLC38A2 overexpression on cell invasion was blocked by CB-839. Additionally, CB-839 treatment alone decreased the cell proliferation at 48 h and invasion rates, and Gln

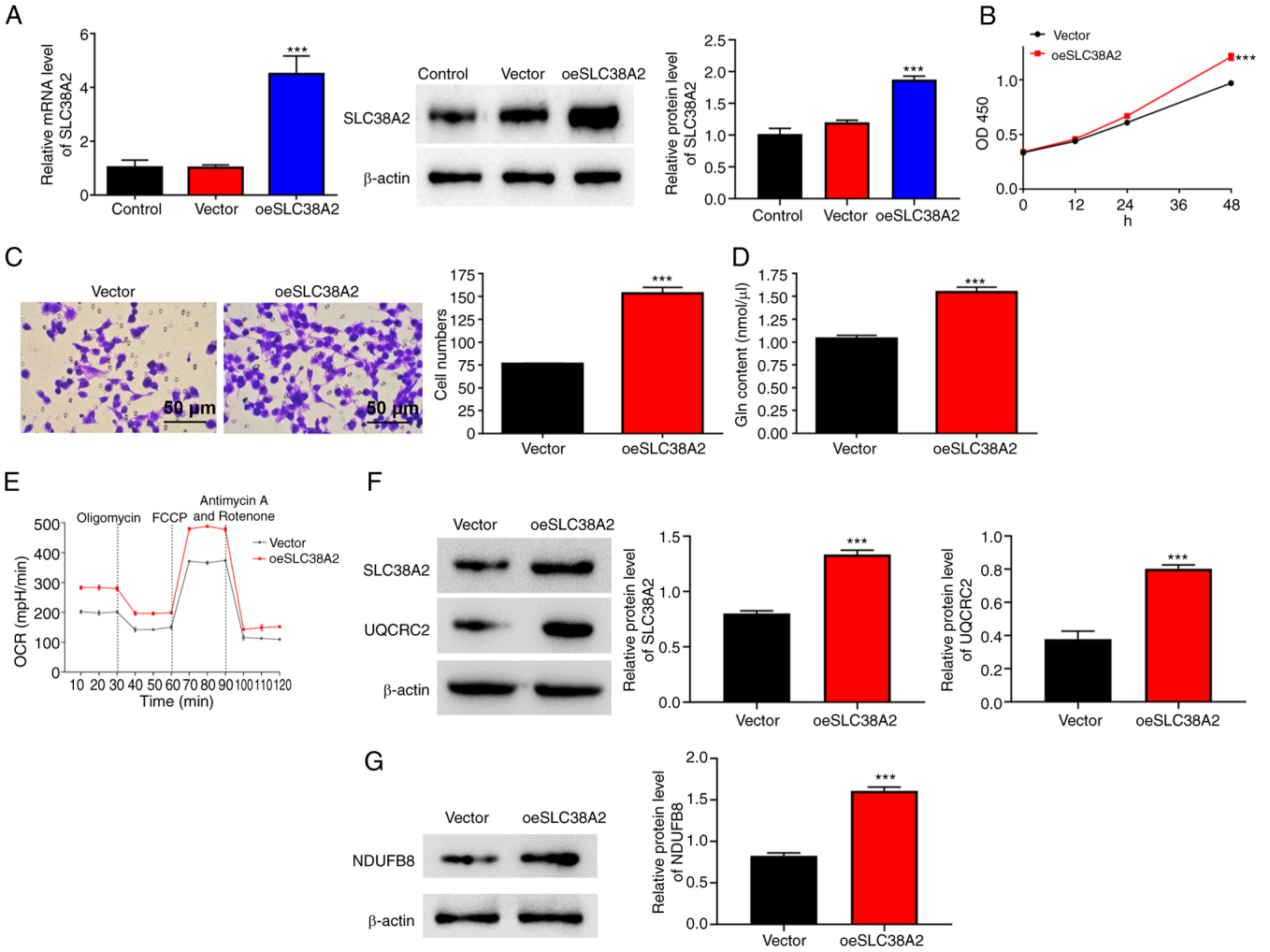

Figure 3. SLC38A2 overexpression increases cell proliferation, invasion, Gln content and the OCR in EU cells. SLC38A2 overexpression lentivirus was applied to EU cells. (A) mRNA and protein expression levels of SLC38A2 were detected using reverse transcription-quantitative PCR and western blotting. (B) Cell proliferation, (C) invasion (scale bar, 50 μm), (D) Gln content and (E) OCR level, and protein levels of (F) UQCRC2 and (G) NDUFB8 were examined. ***P<0.001 vs. vector. EU, eutopic endometrial; FCCP, carbonylcyanide-p-trifluoromethoxyphenylhydrazone; Gln, glutamine; NDUFB8, NADH-ubiquinone oxidoreductase subunit B8; OCR, oxygen consumption rate; OD, optical density; SLC38A2, solute carrier family 38 member a2; oeSLC38A2, SLC38A2 overexpression; UQCRC2, ubiquinol-cytochrome *c* reductase core protein 2.

content in EU cells (Fig. 4A-C). Taken together, these results indicated that SLC38A2 functioned by regulating Gln metabolism.

Discussion

Adenomyosis has been extensively studied; however, to the best of our knowledge, how metabolic pathways are affected in this disease remains largely unknown. Bourdon *et al* (22) reported a higher level of valine in patients with diffuse inner adenomyosis compared with that in control individuals. Valine is an essential amino acid and a precursor of the tricarboxylic acid cycle intermediate succinyl-coenzyme A (22). However, whether and how other amino acids, such as Gln, regulate adenomyosis remains to be elucidated.

To the best of our knowledge, the present study was the first to use CE, EU and EC tissues and primary cells from these tissue sources to explore the roles of Gln in adenomyosis. The results demonstrated that SLC38A2 was upregulated in EC tissues compared with paired EU tissues and CE tissues. Furthermore, SLC38A2 knockdown has been demonstrated to inhibit cell proliferation, decrease Gln content and induce reactive oxygen species production in Gln-sensitive breast cancer cell lines (23). Genetic ablation of SLC38A2 decreased the proliferation of skeletal stem and progenitor cells, thereby reducing the number of osteoblasts and bone-forming activity (24). The present study demonstrated that manipulation of the SLC38A2 level interfered with cell phenotype acquisition (cell proliferation, invasion and Gln content) in EU and EC cells. The SLC38A2 level was positively associated with the malignant phenotype of adenomyosis *in vitro*.

Gln is the most abundant amino acid in the circulatory system, serving an essential role in energy generation, especially in cancer cells (25). Alterations in Gln and glutamate levels are observed early in the course of endometriosis in patients, indicating that Gln serves a role in endometriosis (17). Altered Gln and glutamate levels have been found to be associated with endometriosis-associated pelvic pain (26). Additionally, some studies showed that SLC1A5 knockdown decreased Gln content (27) and an anti-SLC1A5 monoclonal

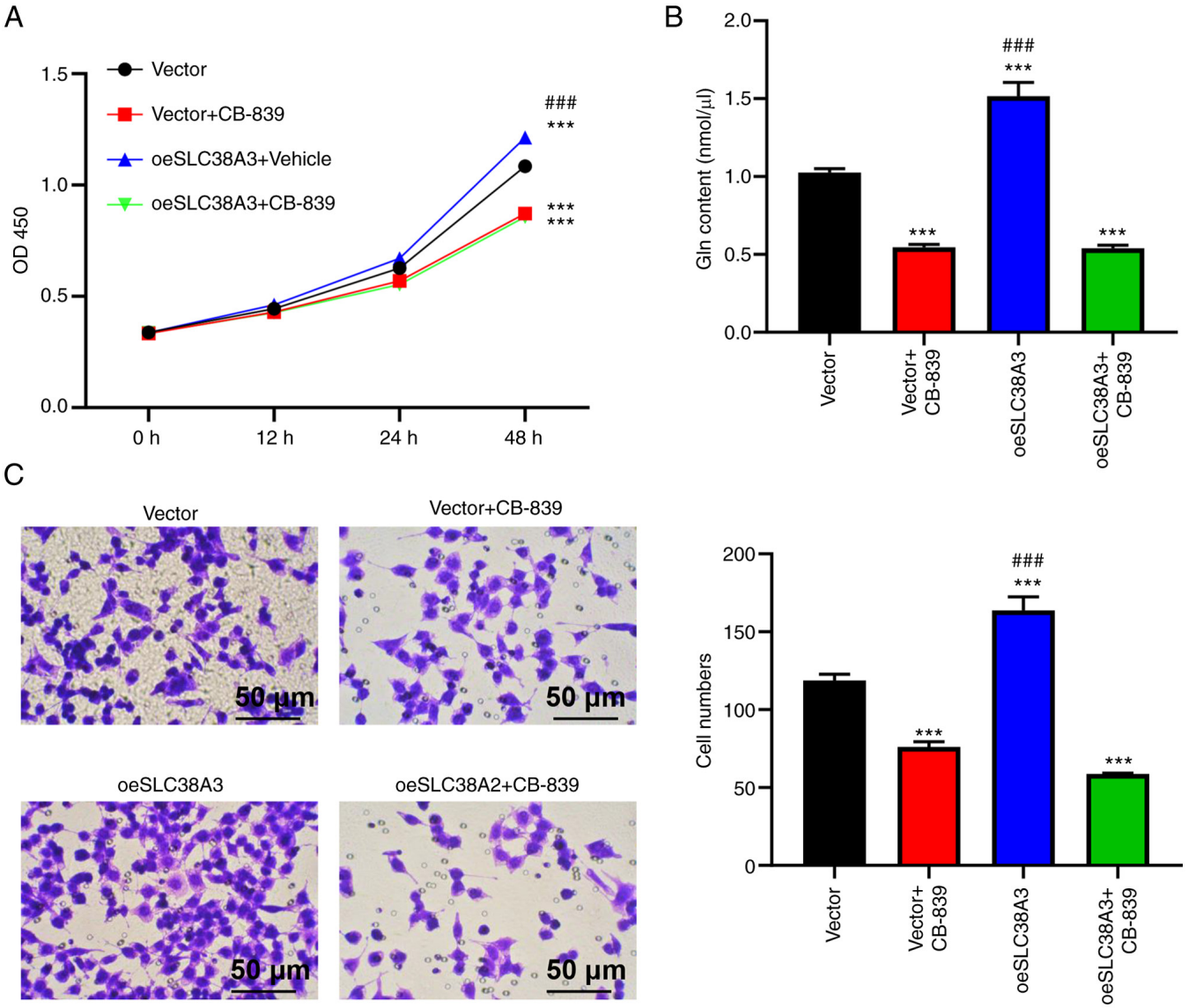

Figure 4. Effect of SLC38A2 overexpression is attenuated by the Gln inhibitor. Eutopic endometrial cells were treated with SLC38A2 overexpression lentivirus and/or CB-839 (10 μM). (A) Cell proliferation, (B) Gln content and (C) invasion (scale bar, 50 μm) were examined. ***P<0.001 vs. vector; $^{###}$P<0.001 vs. oeSLC38A2 + CB-839. Gln, glutamine; OD, optical density; oeSLC38A2, SLC38A2 overexpression; SLC38A2, solute carrier family 38 member a2.

antibody inhibited tumor growth (28). In the present study, silencing of SLC38A2 decreased Gln content and inhibited proliferation in EC cells. Silencing of SLC38A2 was shown to decrease the OCR and the protein levels of NDUFB8 and UQCRC2, indicating that mitochondrial respiration was reduced. Furthermore, SLC38A2 knockdown decreased cell proliferation, probably by mediating mitochondrial impairment.

SLC38A2 is a Gln transporter that serves a key role in the rapid division of T cells by mediating net Gln uptake (29). In immune disorders and other chronic inflammatory diseases, such as endometriosis, activation of the immune system consumes large amounts of energy, with glucose and Gln being the main sources of abundant energy (30). Elevated Gln levels have been observed in endometriosis. Elevated SLC38A2 expression leads to increased Gln uptake (31). Therefore, it was hypothesized that SLC38A2 serves an important role in the pathogenesis of endometriosis/adenomyosis. A previous study has confirmed that both glutamate glutathione and oxidized glutathione are increased in the myometrium of adenomyosis (32). The present study examined the metabolic function of Gln in adenomyosis and found that SLC38A2 promoted adenomyosis in a Gln-dependent manner. However, how Gln promotes the acquisition of specific cell phenotypes remains unclear and more studies are needed to explore the mechanism underlying the effect of SLC38A2 on mitochondrial respiration in adenomyosis. The limitation of the present study was that the role of cell death was not further investigated. The role of cell death may be further investigated in future studies. In addition to *in vitro* studies, transgenic mouse models are also needed to further study the role of SLC38A2 in the pathogenesis of adenomyosis. SLC38A2 may be a useful target for adenomyosis. In conclusion, the present study provided evidence to support future investigation of SLC38A2 as a potential target for adenomyosis.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Key Specialty Construction Project of Pudong Health and Family Planning Commission of Shanghai (grant no. PWZzk2022-21) and Talents Training Program of Pudong Hospital Affiliated to Fudan University (grant no. PJ201902).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LC and HHK confirm the authenticity of all the raw data. LC and HHK conceived and designed the study. JCH and YCD performed the experiments. KW and WG analyzed and interpreted the data. KW and HKK performed the statistical analysis. KW and WG drafted the manuscript. HHK revised the manuscript for important intellectual content. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shanghai Pudong Hospital (approval no. 2023-WZ-04; Shanghai, China) and all patients participating in the study provided written informed consent prior to participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zhai J, Vannuccini S, Petraglia F and Giudice LC: Adenomyosis: Mechanisms and pathogenesis. Semin Reprod Med 38: 129-143, 2020.
2. Vannuccini S and Petraglia F: Recent advances in understanding and managing adenomyosis. F1000Res 8: F1000 Faculty Rev-283, 2019.
3. Jiang L, Han Y, Song Z and Li Y: Pregnancy outcomes after uterus-sparing operative treatment for adenomyosis: A systematic review and meta-analysis. J Minim Invasive Gynecol 30: 543-554, 2023.
4. Carrarelli P, Yen CF, Funghi L, Arcuri F, Tosti C, Bifulco G, Luddi A, Lee CL and Petraglia F: Expression of inflammatory and neurogenic mediators in adenomyosis. Reprod Sci 24: 369-375, 2017.
5. Carrarelli P, Yen CF, Arcuri F, Funghi L, Tosti C, Wang TH, Huang JS and Petraglia F: Myostatin, follistatin and activin type II receptors are highly expressed in adenomyosis. Fertil Steril 104: 744-752.e1, 2015.
6. Vannuccini S, Tosti C, Carmona F, Huang SJ, Chapron C, Guo SW and Petraglia F: Pathogenesis of adenomyosis: An update on molecular mechanisms. Reprod Biomed Online 35: 592-601, 2017.
7. Wu G: Amino acids: Metabolism, functions, and nutrition. Amino Acids 37: 1-17, 2009.
8. Yang Y, Chen Q, Fan S, Lu Y, Huang Q, Liu X and Peng X: Glutamine sustains energy metabolism and alleviates liver injury in burn sepsis by promoting the assembly of mitochondrial HSP60-HSP10 complex via SIRT4 dependent protein deacetylation. Redox Rep 29: 2312320, 2024.
9. Zhang D, Hua Z and Li Z: The role of glutamate and glutamine metabolism and related transporters in nerve cells. CNS Neurosci Ther 30: e14617, 2024.
10. Menchini RJ and Chaudhry FA: Multifaceted regulation of the system A transporter Slc38a2 suggests nanoscale regulation of amino acid metabolism and cellular signaling. Neuropharmacology 161: 107789, 2019.
11. Garibsingh RA and Schlessinger A: Advances and challenges in rational drug design for SLCs. Trends Pharmacol Sci 40: 790-800, 2019.
12. Colas C, Ung PM and Schlessinger A: SLC transporters: Structure, function, and drug discovery. Medchemcomm 7: 1069-1081, 2016.
13. Li YY, Lu XZ, Wang H, Yang XX, Geng HY, Gong G, Zhan YY, Kim HJ and Yang ZJ: Solute carrier family 30 member 8 gene 807C/T polymorphism and type 2 diabetes mellitus in the Chinese population: A meta-analysis including 6,942 subjects. Front Endocrinol (Lausanne) 9: 263, 2018.
14. Li L, He M, Zhou L, Miao X, Wu F, Huang S, Dai X, Wang T and Wu T: A solute carrier family 22 member 3 variant rs3088442 G→A associated with coronary heart disease inhibits lipopolysaccharide-induced inflammatory response. J Biol Chem 290: 5328-5340, 2015.
15. Sun T, Bi F, Liu Z and Yang Q: SLC7A2 serves as a potential biomarker and therapeutic target for ovarian cancer. Aging (Albany NY) 12: 13281-13296, 2020.
16. Murgia F, Angioni S, D'Alterio MN, Pirarba S, Noto A, Santoru ML, Tronci L, Fanos V, Atzori L and Congiu F: Metabolic profile of patients with severe endometriosis: A prospective experimental study. Reprod Sci 28: 728-735, 2021.
17. Dutta M, Singh B, Joshi M, Das D, Subramani E, Maan M, Jana SK, Sharma U, Das S, Dasgupta S, *et al*: Metabolomics reveals perturbations in endometrium and serum of minimal and mild endometriosis. Sci Rep 8: 6466, 2018.
18. Chen L, Li C, Guo J, Luo N, Qu X, Kang L, Liu M and Cheng Z: Eutopic/ectopic endometrial apoptosis initiated by bilateral uterine artery occlusion: A new therapeutic mechanism for uterus-sparing surgery in adenomyosis. PLoS One 12: e0175511, 2017.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
20. Lee DE, Yoo JE, Kim J, Kim S, Kim S, Lee H and Cheong H: NEDD4L downregulates autophagy and cell growth by modulating ULK1 and a glutamine transporter. Cell Death Dis 11: 38, 2020.
21. Burska D, Stiburek L, Krizova J, Vanisova M, Martinek V, Sladkova J, Zamecnik J, Honzik T, Zeman J, Hansikova H and Tesarova M: Homozygous missense mutation in UQCRC2 associated with severe encephalomyopathy, mitochondrial complex III assembly defect and activation of mitochondrial protein quality control. Biochim Biophys Acta Mol Basis Dis 1867: 166147, 2021.
22. Bourdon M, Santulli P, Kateb F, Pocate-Cheriet K, Batteux F, Maignien C, Chouzenoux S, Bordonne C, Marcellin L, Bertho G and Chapron C: Adenomyosis is associated with specific proton nuclear magnetic resonance (^{1}H-NMR) serum metabolic profiles. Fertil Steril 116: 243-254, 2021.
23. Morotti M, Zois CE, El-Ansari R, Craze ML, Rakha EA, Fan SJ, Valli A, Haider S, Goberdhan DCI, Green AR and Harris AL: Increased expression of glutamine transporter SNAT2/SLC38A2 promotes glutamine dependence and oxidative stress resistance, and is associated with worse prognosis in triple-negative breast cancer. Br J Cancer 124: 494-505, 2021.
24. Shen L, Yu Y and Karner CM: SLC38A2 provides proline and alanine to regulate postnatal bone mass accrual in mice. Front Physiol 13: 992679, 2022.
25. Mayers JR and Vander Heiden MG: Famine versus feast: Understanding the metabolism of tumors in vivo. Trends Biochem Sci 40: 130-140, 2015.
26. As-Sanie S, Kim J, Schmidt-Wilcke T, Sundgren PC, Clauw DJ, Napadow V and Harris RE: Functional connectivity is associated with altered brain chemistry in women with endometriosis-associated chronic pelvic pain. J Pain 17: 1-13, 2016.

27. Zhao X, Petrashen AP, Sanders JA, Peterson AL and Sedivy JM: SLC1A5 glutamine transporter is a target of MYC and mediates reduced mTORC1 signaling and increased fatty acid oxidation in long-lived Myc hypomorphic mice. Aging Cell 18: e12947, 2019.
28. Osanai-Sasakawa A, Hosomi K, Sumitomo Y, Takizawa T, Tomura-Suruki S and Imaizumi M, Kasai N, Poh TW, Yamano K, Yong WP, *et al*: An anti-ASCT2 monoclonal antibody suppresses gastric cancer growth by inducing oxidative stress and antibody dependent cellular toxicity in preclinical models. Am J Cancer Res 8: 1499-1513, 2018.
29. Carr EL, Kelman A, Wu GS, Gopaul R, Senkevitch E, Aghvanyan A, Turay AM and Frauwirth KA: Glutamine uptake and metabolism are coordinately regulated by ERK/MAPK during T lymphocyte activation. J Immunol 185: 1037-1044, 2010.
30. Wik JA, Chowdhury A, Kolan S, Bastani NE, Li G, Alam K, Grimolizzi F and Skålhegg BS: Endogenous glutamine is rate-limiting for anti-CD3 and anti-CD28 induced CD4+ T-cell proliferation and glycolytic activity under hypoxia and normoxia. Biochem J 479: 1221-1235, 2022.
31. Bau DT, Hsieh YY, Wan L, Wang RF, Liao CC, Lee CC, Lin CC, Tsai CH and Tsai FJ: Polymorphism of XRCC1 codon arg 399 Gln is associated with higher susceptibility to endometriosis. Chin J Physiol 50: 326-329, 2007.
32. Song W, Zhang Z, Jiang Y, Cao Y, Zhang B, Wang Y, Shi H and Zhu L: Integrative metabolomic profiling reveals aberrations in myometrium associated with adenomyosis: A pilot study. Reprod Biol Endocrinol 20: 49, 2022.