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A pan-cancer analysis of SLC1A5 in human cancers

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ABSTRACT

Background: The alanine-serine-cysteine transporter 2, ASCT2 (solute carrier family 1 member 5, SLC1A5), is a major transporter of the amino acid, glutamine. Although SLC1A5 has been reported to be associated with some types of cancer, less pan-cancer analysis, which would give a comprehensive understanding of SLC1A5 across human cancers, has been carried out.
Methods: We used the TCGA and GEO databases to investigate the oncogenic role of SLC1A5. We examined gene and protein expression, survival, genetic mutations, protein phosphorylation, immunocyte infiltration and the related genes correlated pathways. In HCT116 cells, SLC1A5 was silenced by siRNAs and the mRNA and protein was checked by Q-PCR and WB, respectively and the cellular function was assessed by CCK8, cell cycle and apoptosis.
Results: We found that SLC1A5 was over-expressed in multiple types of cancer and that elevated expression of SLC1A5 was associated with poor survival in many cancers. The missense mutation of R330 H/C was associated with poor survival, especially in uterine carcinosarcoma. Furthermore, we found enhanced phosphorylation of S503 in uterine corpus endometrial carcinoma and lung adenocarcinoma. In addition, elevated SLC1A5 expression was associated with immune cell infiltration in many cancers. KEGG and GO analysis showed that SLC1A5 and its related genes were involved in central carbon metabolism in cancer, due to their amino acid transport activity. The cellular function indicated that SLC1A5 may influence the cell proliferation by affecting DNA synthesis.
Conclusions: Our findings highlighted the important role of SLC1A5 in tumorigenesis and provided insights into potential cancer treatment strategies.

1. Introduction

Amino acids play a critical role in metabolism because they are involved in protein synthesis, signaling and energy production [1, 2]. Glutamine is the most abundant free amino acid in the human body, and has roles in metabolism, apoptosis and proliferation, protein synthesis and degradation. Furthermore, the plasma concentration of glutamine must be regulated and kept consistent (the average extracellular concentration is 0.7 mM, and the intracellular concentration is between 2–20 mM) [3]. SLC1A5 (solute carrier family 1 member 5) and SLC7A5 are two transporters for glutamine uptake. SLC1A5 participates in the sodium (Na^+)-coupled influx of glutamine, whereas SLC7A5 also mediates the influx of leucine [4]. In many human cancers, gene mutations and epigenetic

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modifications play an important role in the interactions between the tumor and the immune cells [5–8]. The majority of cells infiltrating tumors are T cells and the correlation between immune infiltration and prognosis has been demonstrated in many studies [9–11]. Myeloid-derived suppressor cells (MDSCs) can suppress immune cell function in the tumor microenvironment [12], and may therefore promote tumor cell survival, angiogenesis, invasion of healthy tissue, and metastases [13].

Our study used the TCGA and GEO databases to conduct a pan-cancer analysis of SLC1A5. We examined gene and protein expression, survival, genetic mutations, protein phosphorylation, immune cell infiltration and the related genes correlated pathways. Furthermore, the cellular function was showed that SLC1A5 may influence the cell proliferation by affecting DNA synthesis.

These findings may further the understanding of the molecular mechanisms of SLC1A5 and provide insights into the development of potential cancer therapeutic approaches.

2. Methods

2.1. Gene expression of SLC1A5

The online tool TIMER 2.0 (http://timer.comp-genomics.org/) was used to explore the differential expression of SLC1A5 between tumor and adjacent normal tissues in samples from TCGA database [14]. Distributions of gene expression levels are shown using box plots. The statistical significance computed by the Wilcoxon test is annotated by the number of stars (*: p-value <0.05; **: p-value <0.01; ***: p-value <0.001). The gray columns denote the data that were available for tumors and the normal tissues, while some tumors did not have adjacent normal tissues. We used the online tool GEPIA2 (http://gepia2.cancer-pku.cn/#index) to match TCGA normal and GTEx data using the "Expression DIY-Box Plot" function ($|Log2FC|$ cutoff >1), p-value cutoff <0.05 (*: p-value <0.05) [15].

2.2. Protein expression of SLC1A5

We used the CPTAC dataset of UALCAN (http://ualcan.path.uab.edu/) to analyze the protein expression of SLC1A5 [16]. The Human Protein Atlas (https://www.proteinatlas.org/) of Pathology was used to present the protein expression profiles of SLC1A5 in various cancers. In addition, we used GEPIA2 to investigate the relationship between the expression level of SLC1A5 and cancer stages using the "Stage Plot" function.

2.3. Prognostic analysis of SLC1A5

GEPIA2 was used to analyze TCGA tumor data for survival contribution (Overall Survival and Disease-Free Survival) of SLC1A5 gene expression levels by employing the "Survival Map" function. Both the upper and lower limits, which were used as cut-off values, were 50% of the mean.

2.4. Mutation analysis of SLC1A5

We used the cBioportal database (http://www.cbioportal.org/) to examine genetic mutations of SLC1A5 with "TCGA PanCancer Atlas Studies" [17,18]. The "Cancer Types Summary" function gave a map of SLC1A5 mutations in various cancers. The "Mutations" function gave a 3D structure of the mutation site. The "Modify Query" function of each TCGA tumor with "Comparison/Survival" showed the correlation between the SLC1A5 variants and survival.

2.5. The phosphorylation of SLC1A5 protein and the SLC1A5-related immune infiltration analysis

The CPTAC dataset of UALCAN was used and the "PhosphoProtein" database showed the phosphorylation site and level of phosphorylation in breast cancer, colon cancer, uterine corpus endometrial carcinoma and lung adenocarcinoma.

TIMER 2.0 ("Immune" function) was used to explore the correlation between the SLC1A5 expression level and immune infiltration. We analyzed the MDSCs by TIDE algorithms. Heat maps and scatter plots were used for visualizing data.

2.6. Enrichment analysis of SLC1A5-related genes

We used the STRING database (https://cn.string-db.org/) to screen the top 50 proteins that interacted with SLC1A5 [19]. Then, GEPIA2 ("Similar Genes Detection" function) was used to search for the top 100 genes that had a similar expression pattern with the SLC1A5 gene in different cancer types. Finally, the "Correlation Analysis" function of GEPIA2 was used to compute the correlation of SLC1A5 with similar gene expression patterns in multiple cancer types. We used the Spearman Correlation Coefficient, and used the non-log scale for calculation and the log-scale axis for visualization.

In addition, interacting genes and similar expression pattern genes were put into the DAVID database (https://david.ncifcrf.gov/) to determine their relationship with biological processes [20]. The enrichment dot bubble and Sankey dot pathway enrichment were plotted by a free online platform for data analysis and visualization (http://www.bioinformatics.com.cn/).

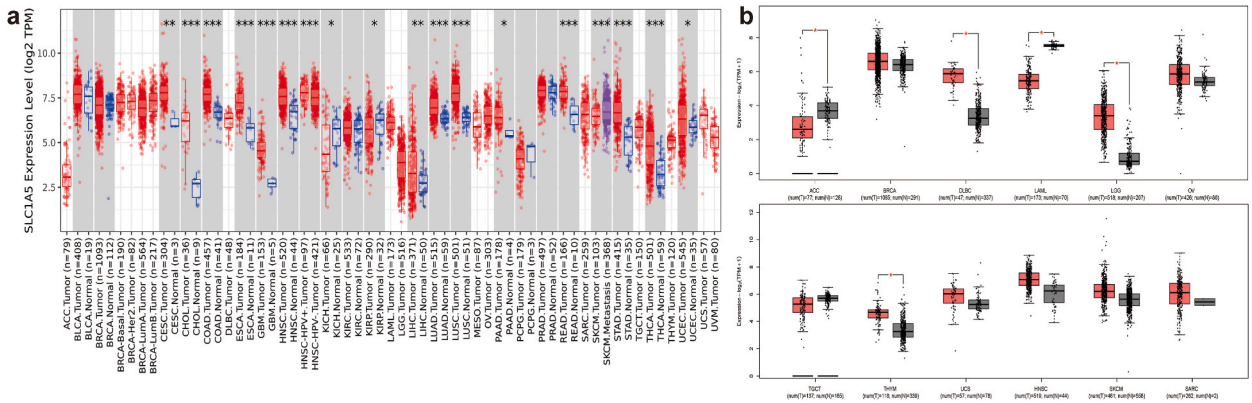

Fig. 1. The gene expression of SLC1A5 based on TCGA and GEO databases. (a) SLC1A5 expression levels were determined in different types of human cancer using the RNA-seq data in TCGA by TIMER 2.0. (b) Box plot data showing the differences in SLC1A5 expression from matching TCGA normal and GTEx data by GEPIA2. *: p-value <0.05; **: p-value <0.01; ***: p-value <0.001.

2.7. SLC1A5 siRNA synthesis and transfection

Three different siRNA sequences targeting SLC1A5 were synthesized and the sequences were as follows: siRNA-1 sense: 5′-GCCUGAGUUGAUACAAGUGAATT-3′, siRNA-1 antisense: 5′-UUCACUUGUAUCAACUCAGGCTT-3′, siRNA-2 sense: 5′-CUGGAUUAUGAGGAAUGGAUATT-3′, siRNA-2 antisense: 5′-UAUCCAUUCCUCAUAAUCCAGTT-3′, siRNA-3 sense: 5′-CCGCCUUGGCAAGUACAUUTT-3′, siRNA-3 antisense: 5′-AAUGUACUUGCCAAGGCGGTT-3’. The negative control sense sequences were 5′-UUCUCCGAACGUGUCACGUdTdT-3′, antisense sequences were 5′-ACGUGACACGUUCGGAGAAdTdT-3′ (Azenta). HCT116 cells were cultured in DMEM and RPMI-1640 supplemented with 10% fetal bovine serm (Procell), respectively. When the cells were at approximately 30% confluency, the NC, siRNA-1, siRNA-2, siRNA-3 were transfected according to the manufacturer’s instructions with INTERFERin® transfection reagent (Polyplus-transfection® Inc.).

2.8. Q-PCR and Western blot

The total RNA was extracted using the Eastep® SuperTotal RNA Extraction kit (Promega) and 1 μg total RNA was used for cDNA synthesis using Reverse Transcription Kit (Biosharp). The Taq Pro Universal SYBR qPCR Master Mix was used for cDNA amplify (Vazyme). The relative gene expression levels were normalized to actin with the $2^{-\Delta\Delta Ct}$ method and PCR conditions were 95 °C for 10 min, 95 °C for 15 s, and 60 °C for 30 s for 40 cycles. The primer sequences used for quantitative real-time PCR were: SLC1A5 sense: 5′-ACCGGAACCAGGGTGAAGGT-3′, antisense: 5′-CACATGATCCAGGAGACCAGAAC-3′, actin sense: 5′-TGGCACCCAGCACAATGAA-3′, antisense: 5′-CTAAGTCATAGTCCGCCTAGAAGCA-3’.

The total protein was collected after ice bath with RIPA lysis buffer contained 1 × cocktail (Servicebio) and phosphatase inhibitor for 0.5 h. The protein quantification was detected with BCA protein assay kit (Servicebio) and then boiled for 10 min by adding loading buffer. 50 μg total protein was used for 12% SDS-PAGE gel running and then transferred to 0.45 μm PVDF membranes. The membranes were blocked with 5% BSA for 2 h and then incubated overnight at 4 °C with SLC1A5 (1:5000, Proteintech) or ACTB (1:30,000, Huabio). The membranes were washed for 10 min three times with 1 × TBST at room temperature and incubated in HRP - goat anti-rabbit IgG, HRP - goat anti-mouse IgG (1:2000, Servicebio), for 1.5 h and then washed 10 min three times with 1 × TBST. Subsequently the membranes were exposed to chemiluminescence substrate (Servicebio).

2.9. Cell proliferation assay

CCK8 kit (APExBIO) was used to investigate cell proliferation. Cells were seeded into a 96-well plate with 5000 cells per well and then incubated at 37 °C, 5% CO_2 for 48 h. 10% of CCK8 regent was added into each well, and incubated at 37 °C for 2 h. Then absorbance values at $\lambda = 450$ nm were measured.

2.10. Cell cycle and apoptosis

The transfected cells were washed with PBS and harvested with trypsin. The cells were stained with PI according to the instructions (Elabscience) and the cell cycle was analyzed on flow cytometry.

The trypsin without EDTA was used to harvested the cells and then the cells were stained with PI and annexin V. The apoptosis was analyzed on flow cytometry.

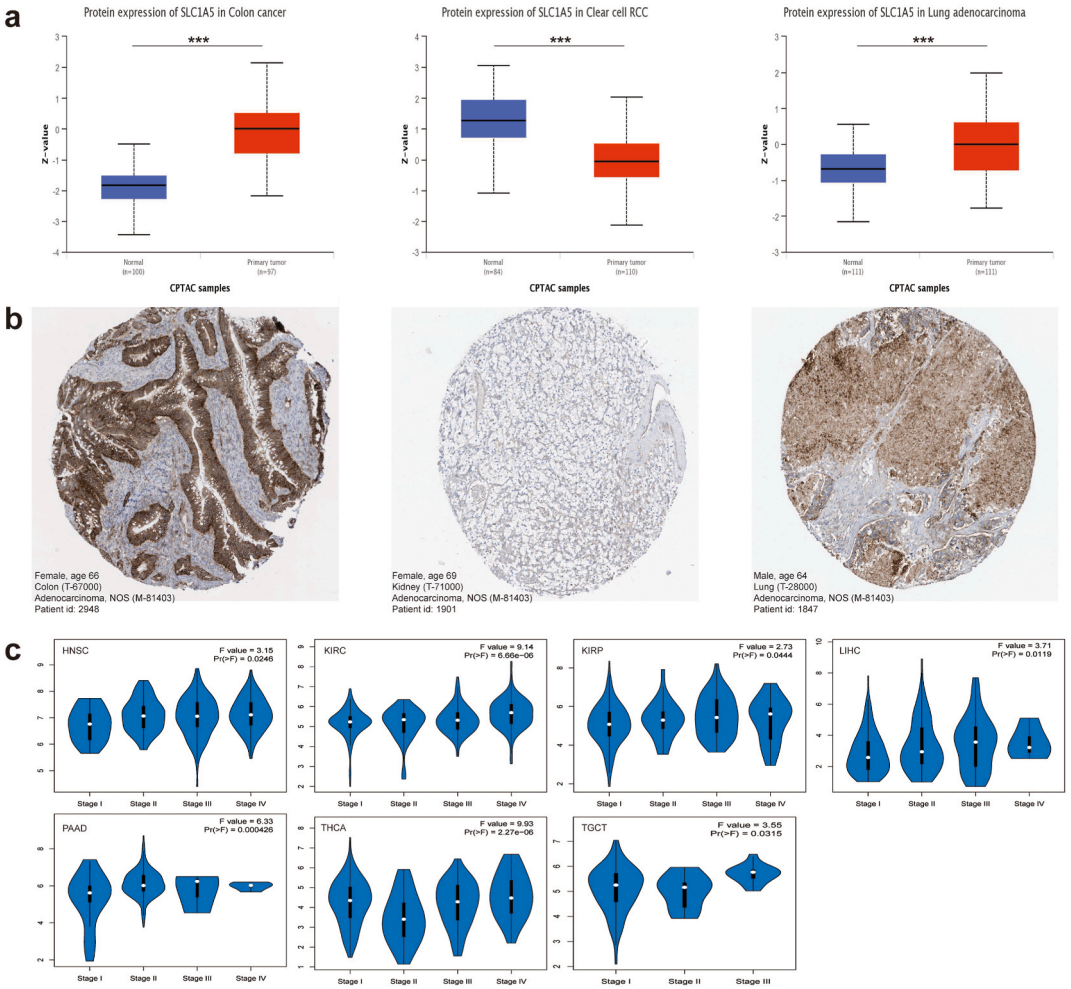

Fig. 2. The protein expression of SLC1A5 in various tumors. (a) The total protein expression of SLC1A5 was compared between normal and tumor tissues using UALCAN (***: p-value <0.001). (b) Immunohistochemically staining of SLC1A5 protein from The Human Protein Atlas. (c) Gene expression of SLC1A5 in different tumors at each stage.

3. Results

3.1. Gene expression of SLC1A5

TIMER 2.0 analysis revealed that the expression levels of SLC1A5 were significantly increased in CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), CHOL (cholangiocarcinoma), COAD (colon adenocarcinoma), ESCA (esophageal carcinoma), GBM (glioblastoma multiforme), HNSC (head and neck squamous cell carcinoma), LIHC (liver hepatocellular carcinoma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), PAAD (pancreatic adenocarcinoma), READ (rectum adenocarcinoma), STAD (stomach adenocarcinoma), THCA (thyroid carcinoma) and UCEC (uterine corpus endometrial carcinoma) compared to adjacent control samples. SLC1A5 was also overexpressed in BLCA (bladder urothelial carcinoma), KIRC (kidney renal clear cell carcinoma) and PRAD (prostate adenocarcinoma) compared to normal tissues. In contrast, SLC1A5 expression was significantly lower in KICH (kidney chromophobe) and KIRP (kidney renal papillary cell carcinoma) compared to normal controls. In PCPG (pheochromocytoma and paraganglioma), the expression of SLC1A5 was also found to be down-regulated compared to normal tissues (Fig. 1a).

The following tumor datasets, which were not found in the TCGA database, were analyzed by GEPIA2: ACC (adrenocortical carcinoma), BRCA (breast invasive carcinoma), DLBC (lymphoid neoplasm diffuse large B-cell lymphoma), LAML (acute myeloid leukemia), LGG (brain lower grade glioma), MESO (mesothelioma), OV (ovarian serous cystadenocarcinoma), SARC (sarcoma), TGCT (testicular germ cell tumors), THYM (thymoma), UCS (uterine carcinosarcoma), UVW (uveal melanoma), HNSC (head and neck squamous cell carcinoma) and SKCM (skin cutaneous melanoma). We found that the expression of SLC1A5 was significantly up-regulated in DLBC, LGG and THYM, but significantly down-regulated in ACC and LAML (p-value <0.05). Furthermore, SLC1A5

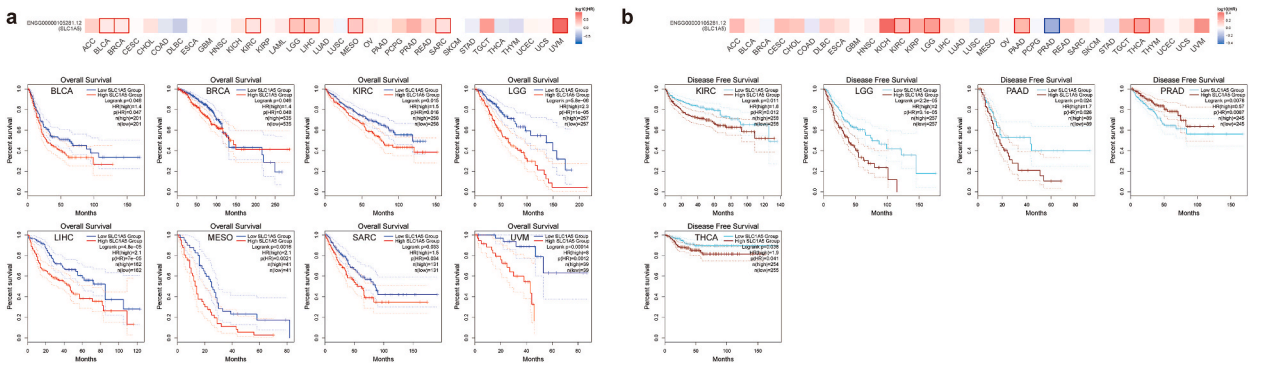

Fig. 3. Correlation between SLC1A5 gene expression and prognosis of tumors in TCGA. (a) Overall Survival. (b) Disease-free Survival.

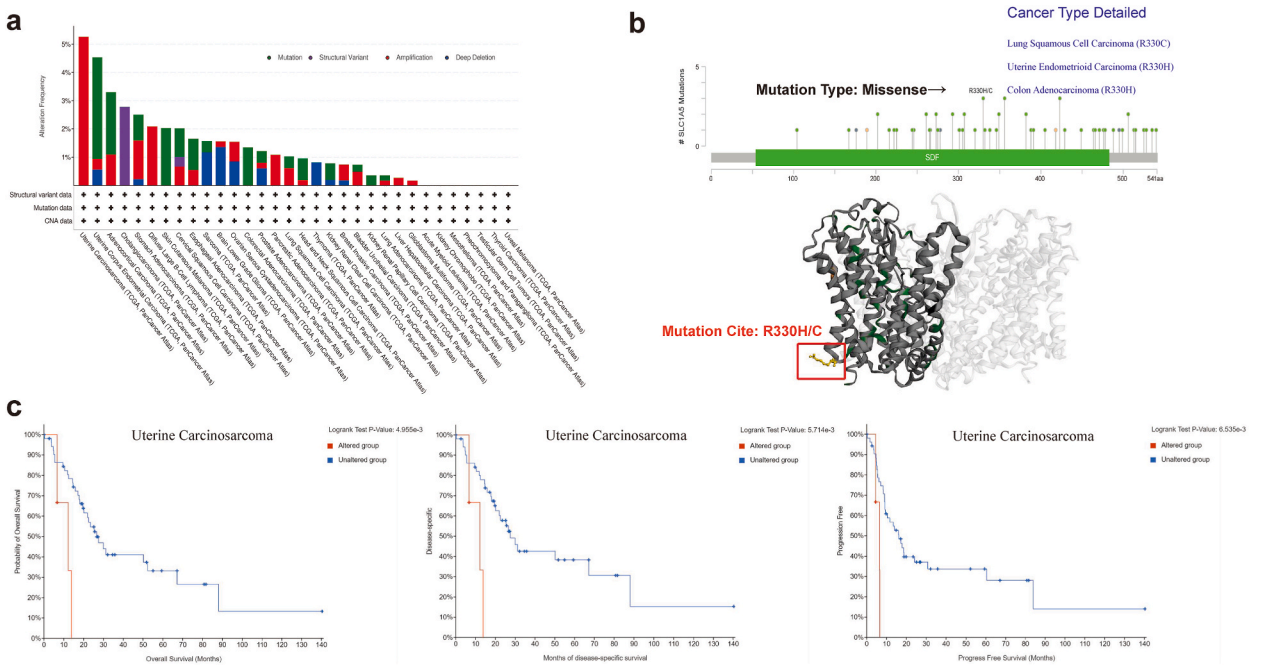

Fig. 4. The mutation of SLC1A5 in TCGA cancer. (a) The frequency of SLC1A5 mutations in TCGA cancer was determined by cBioPortal. (b) The SLC1A5 mutation site with the highest alteration frequency (R330 H/C) and the cancer types associated with this mutation. (c) Correlation between SLC1A5 mutation and overall survival, disease-specific survival and progression-free survival of uterine carcinosarcoma (log-rank p-value <0.05).

expression was increased in BRCA, OV, UCS, HNSC, SKCM and SARC, and decreased in TGCT compared to normal tissue (Fig. 1b).

3.2. SLC1A5 protein expression data

Based on CPTAC analysis, we found that SLC1A5 protein expression levels were significantly higher in some primary tumor tissues than normal tissues including colon cancer and LUAD, while significantly lower in KIRC (p-value <0.01, Fig. 2a). Immunohistochemically staining of colon cancer, LUAD and KIRC tumor samples confirmed the Human Protein Atlas database results (Fig. 2b). Furthermore, the correlation between SLC1A5 gene expression and different cancer stages was analyzed and we found a strong correlation in cases such as HNSC, KIRC, KIRP, LIHC, PAAD, THCA and TGCT (Pr (>F) < 0.05, Fig. 2c).

3.3. SLC1A5 prognostic analysis data

Using the "Survival Analysis" function of GEPIA2, we analyzed the correlation between SLC1A5 gene expression level and prognosis. Strong correlations were marked with a box. The overall survival and disease-free survival data are listed separately in Fig. 3a and b and are denoted as log-rank p-values <0.05. We found that high levels of SLC1A5 were associated with poor prognosis in BLCA, BRCA, KIRC, LGG, LIHC, MESO, SARC and UVM (Fig. 3a). Disease-free survival analysis showed that high SLC1A5 levels were

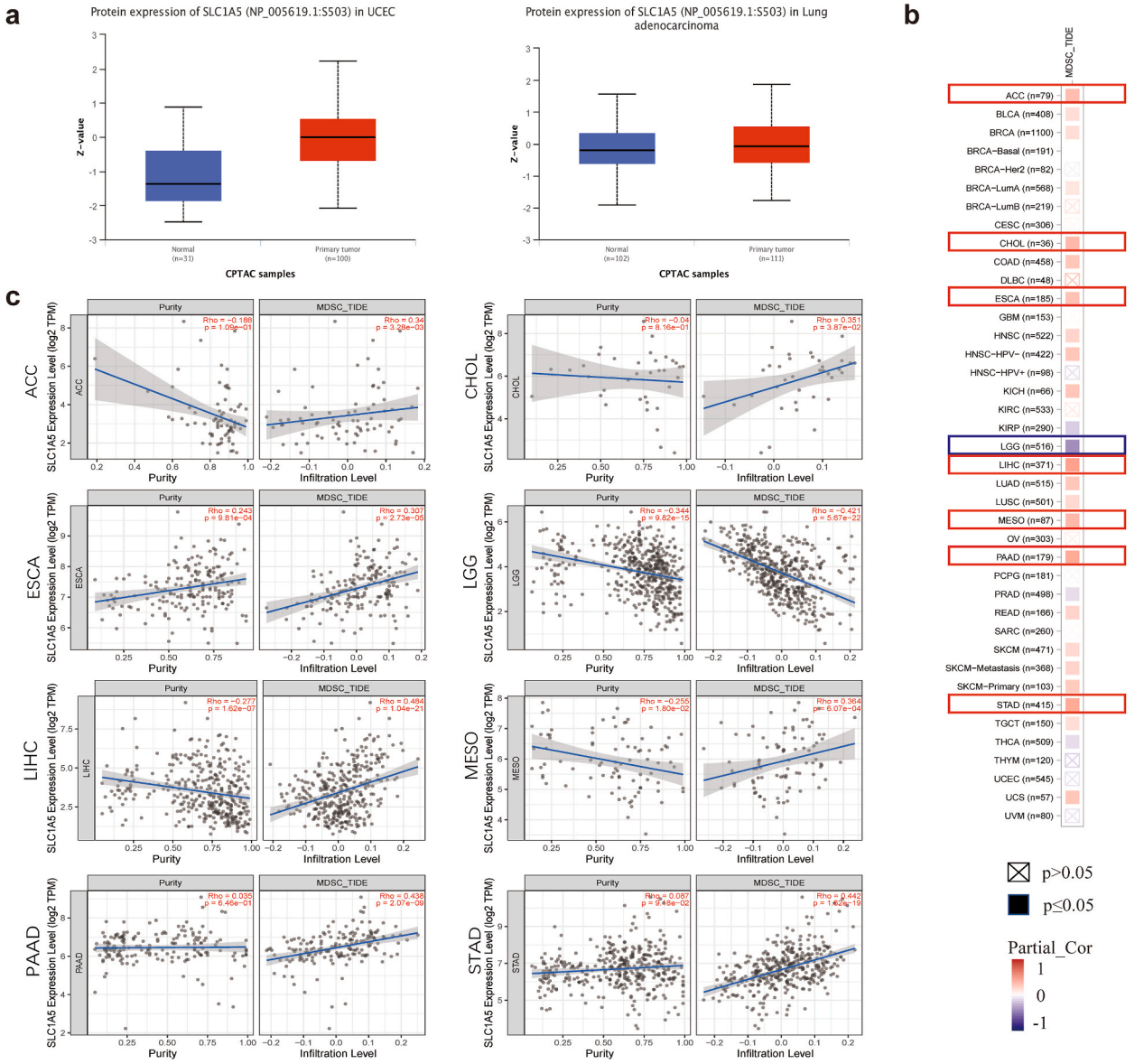

Fig. 5. Phosphorylation of SLC1A5 protein and the correlation between immune infiltrates and SLC1A5 gene expression levels in various tumors. (a) Phosphorylation analysis of SLC1A5 in uterine corpus endometrial carcinoma and lung adenocarcinoma. (b–c) Heat maps and scatter plots of the correlation analysis between SLC1A5 expression levels and immune infiltrates of MDSCs in TCGA tumors.

associated with poor prognosis in KIRC, LGG, PAAD and THCA, while in PRAD, overexpression of SLC1A5 was found to be associated with increased disease-free survival (Fig. 3b).

3.4. *Mutation analysis data of SLC1A5*

The gene mutations of SLC1A5 in TCGA cancers are shown. The highest frequency mutations were point mutations, structural variants, amplifications and deep deletions. The tumors with a low alteration in frequency were deleted (Fig. 4a). The primary mutation of SLC1A5 was a missense mutation at position 330 (R330 H/C). The mutation was found in LUSC (R330C), UCEC (R330H) and COAD (R330H) (Fig. 4b). Furthermore, we found that this mutation could lead to poor prognosis in UCS (log-rank p-value <0.05, Fig. 4c).

3.5. *Phosphorylation of SLC1A5 protein and SLC1A5-related immune infiltration analysis*

Using the CPTAC dataset of UALCAN, we found that the S503 site of SLC1A5 protein showed a high phosphorylation level in UCEC

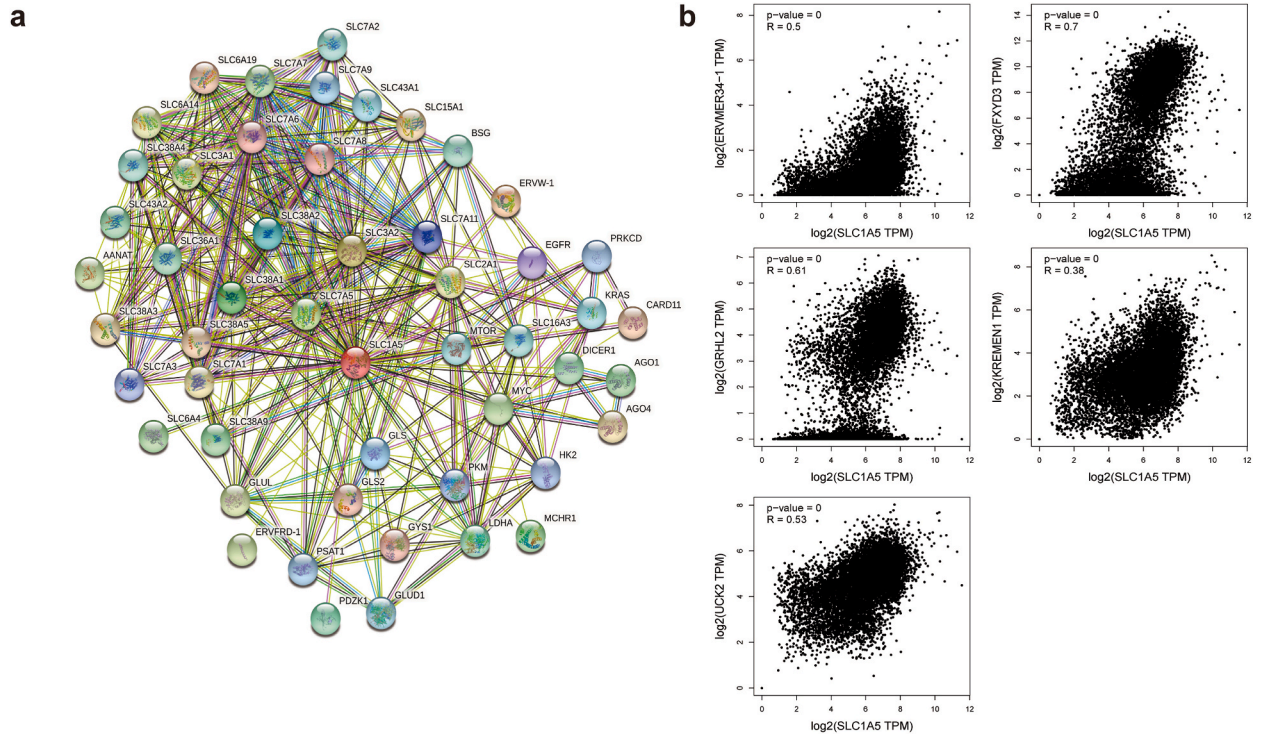

Fig. 6. Enrichment of SLC1A5-related genes. (a) Gene interaction network by STRING. (b) Correlation between SLC1A5 expression levels and five genes from the top 100 co-expression genes.

and LUAD (respectively p = 0.004 and p = 0.040, Fig. 5a). TIMER 2.0 analysis revealed that SLC1A5 expression levels positively correlated with immune infiltrates of MDSCs in ACC, CHOL, ESCA, LIHC, MESO, PAAD and STAD. However, LGG showed a negative correlation between the SLC1A5 expression level and immune infiltration level (|Rho| > 0.3, p-value <0.05, Fig. 5b and c).

3.6. Enrichment analysis data of SLC1A5-related genes

Using STRING, we identified 50 SLC1A5-binding proteins and generated a network (Fig. 6a). GEPIA2 was used to identify genes with similar expression patterns, and the top 100 genes are listed in Table 1. Correlation analysis found that the top five genes, ERVMER34-1 (R = 0.5, p-value <0.01), FXYD3 (R = 0.7, p-value <0.01), GRHL2 (R = 0.61, p-value <0.01), KREMEN1 (R = 0.38, p-value <0.01) and UCK2 (R = 0.53, p-value <0.01) were positively associated with SLC1A5 expression levels (Fig. 6b).

KEGG analysis revealed that SLC1A5-related genes play an important role in central carbon metabolism in cancer (Gene. Ratio = 0.156, p-value <0.01, Fig. 7a). GO analysis of biological processes indicated that these genes were involved in amino acid transport. Cellular component analysis showed that 23 of these related genes were correlated with the integral component of plasma membrane. Generally, SLC1A5-related genes possess the function of amino acid transmembrane transporter activity (Fig. 7b).

3.7. SLC1A5 plays an important role in the proliferation of COAD

As mentioned in the previous results in Fig. 2b, the expression of SLC1A5 was significantly increased in colon adenocarcinoma. To explore the function of SLC1A5, we silenced SLC1A5 in HCT116 cells. The mRNA and protein level were detected after transfection 48 h by Q-PCR and Western blot and we found that the expression of mRNA and protein were downregulated by siRNAs (Fig. 8a and b) and the siSLC1A5#1 was the most effective siRNA and the following cellular function used the sequence of siSLC1A5#1. We found that the reduced SLC1A5 suppressed the vitality of tumor cells (Fig. 8c) and influenced the proliferation of HCT116 by decreasing the S-phase (Fig. 8d). Total apoptosis, which included early apoptosis and late apoptosis, observed unchanged after SLC1A5 knockdown in HCT116 (S1).

4. Discussion

Amino acids are fundamental metabolites. The acquisition of amino acids is as important as the maintenance of their concentration inside and outside of the cell [21]. One factor regulating the stable concentration of amino acids is the cellular entry and exit of amino acids, a process that is dependent upon amino acid transporters [22]. SLC1A5, also known as ASCT2, is a neutral amino acid

Table 1
The top 100 genes presenting with similar expression patterns to SLC1A5.

Gene Symbol	Gene ID	PCC
CTB-147N14.6	ENSG00000275719.1	0.47
ERVMER34-1	ENSG00000226887.7	0.41
FXYD3	ENSG00000089356.16	0.4
GRHL2	ENSG00000083307.10	0.39
KREMEN1	ENSG00000183762.12	0.39
UCK2	ENSG00000143179.12	0.39
PYCR1	ENSG00000183010.16	0.39
EIF4G1	ENSG00000114867.19	0.38
METAP1	ENSG00000164024.11	0.38
CENPN	ENSG00000166451.13	0.38
PSMD7	ENSG00000103035.10	0.38
ZDHHC12	ENSG00000160446.18	0.37
FAM83H-AS1	ENSG00000203499.10	0.37
FGF20	ENSG00000078579.8	0.37
BCL2L12	ENSG00000126453.9	0.37
HDAC1	ENSG00000116478.11	0.37
KDF1	ENSG00000175707.8	0.37
ESRP1	ENSG00000104413.15	0.36
CD3EAP	ENSG00000117877.10	0.36
TK1	ENSG00000167900.11	0.36
S100A11	ENSG00000163191.5	0.36
EIF2S2	ENSG00000125977.6	0.36
NUP37	ENSG00000075188.8	0.36
DTX2	ENSG00000091073.19	0.36
RCC1	ENSG00000180198.15	0.36
CCDC86	ENSG00000110104.11	0.36
SLC52A2	ENSG00000185803.8	0.36
TMEM189	ENSG00000240849.10	0.35
FAM83F	ENSG00000133477.16	0.35
CORO2A	ENSG00000106789.12	0.35
STRA13	ENSG00000169689.14	0.35
Z83851.4	ENSG00000233903.2	0.35
SYNGR2	ENSG00000108639.7	0.35
GMPS	ENSG00000163655.15	0.35
STYK1	ENSG00000060140.8	0.35
NUDT19	ENSG00000213965.3	0.35
DKC1	ENSG00000130826.15	0.35
GRWD1	ENSG00000105447.12	0.35
AP2S1	ENSG00000042753.11	0.35
ESRP2	ENSG00000103067.11	0.35
FAM110A	ENSG00000125898.12	0.34
PSMA4	ENSG00000041357.15	0.34
EIF5A	ENSG00000132507.17	0.34
MOGS	ENSG00000115275.11	0.34
APEX2	ENSG00000169188.4	0.34
CCT5	ENSG00000150753.11	0.34
REEP4	ENSG00000168476.11	0.34
ACTL6A	ENSG00000136518.16	0.34
PKP3	ENSG00000184363.9	0.34
DPP3	ENSG00000254986.7	0.34

Supplement 1. Cell apoptosis was detected by PI/annexin V staining. (a) The apoptosis of siContol in HCT116. (b) The apoptosis of siSLC1A5#1 in HCT116.

transporter belonging to the SLC1 family. When biosynthesis and metabolism in an organism increases, the cellular demand for glutamine also increases. This process requires increased expression of transporters to increase the intake of amino acids [23,24]. One of the characteristics of cancer is enhanced proliferation [25]. In our study, many types of cancers have increased gene and protein expression of SLC1A5 (Figs. 1 and 2), which is consistent with previous studies [26,27]. During tumor progression, increased SLC1A5 expression was observed in HNSC, KIRC, KIRP, LIHC, PAAD, THCA and TGCT (Fig. 2c), which may be related to the rapid proliferation of cancer cells. Furthermore, overexpression of SLC1A5 was associated with poor prognosis (Fig. 3), a finding that deserves further study. Studies have shown that amino acids can activate the mTORC1 and mTOR signaling pathways, which regulate numerous biological functions [22,24,28].

Many types of mutations have been found in the SLC1A5 gene. The highest mutation frequency was found at site R330 H/C, and was detected in COAD, UCEC and LUAD. In UCEC, the mutation of R330H was correlated with poor survival (Fig. 4b). Studies have shown that the S481 and C482 residues of SLC1A5 are crucial for transporting L-glutamine to SLC1A5 [29].

Protein post-translational modifications such as *N*-glycosylation and phosphorylation can also influence protein function. The *N*-glycosylation of SLC1A5 at N163 and N212 was found to be crucial for trafficking [30]. However, there are few studies on the

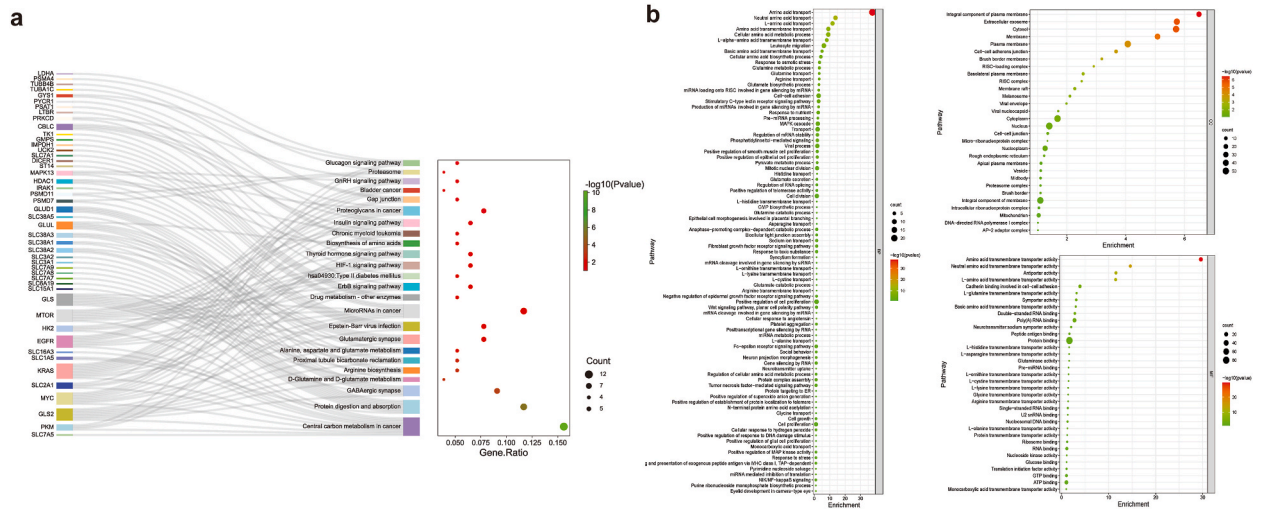

Fig. 7. Biological function analysis of SLC1A5-related genes. (a) KEGG pathway analysis of SLC1A5 and interacting genes. (b) Biological process (BP), cellular component (CC) and molecular function (MF) analyses.

phosphorylation of SLC1A5. In our study, we found that phosphorylation of SLC1A5 was higher in UCEC and LUAD than normal tissues (Fig. 5a). Thus, further studies are required to understand the role of SLC1A5 phosphorylation in tumor progression.

In many solid tumors, tumor-infiltrating immune cells are correlated with tumor progression and disease survival [31]. MDSCs are generated in the bone marrow, and migrate to the peripheral lymphoid organs and tumor when needed. They play an important role in chronic infectious diseases, inflammation, autoimmune diseases, trauma, sepsis and cancer. In cancer, MDSCs can promote tumor cell invasion and metastases [13,32–34]. In our study, we found that the SLC1A5 expression levels were associated with MDSC levels in many cancers. Furthermore, these findings were consistent with poor survival when SLC1A5 was over-expressed in LIHC, MESO and PAAD (Figs. 3, 5b and c).

In addition, studies have showed that antagonistic mAbs against SLC1A5 possess an anti-tumor effect on KRAS-mutated human colorectal cancer [35], indicating that SLC1A5 is an excellent therapeutic target for KRAS-mutated cancers. In summary, SLC1A5 is a potential tumor marker and immunotherapy target in a wide range of cancers.

The limitation of our article is that, we gave a summary relationship of the expression level, the mutation, the post-translational modifications of SLC1A5 with the survival, and SLC1A5 was very important in cell proliferation, further research is needed for how these aspects influence the cancer progression and the mechanism is waiting for reveal.

5. Conclusions

In this study, we focused on the pan-cancer analysis of SLC1A5 and investigated the association between SLC1A5 expression alterations, genomic mutations and protein phosphorylation with survival and immune infiltration. Our results provided a novel insight into tumorigenesis and indicated that SLC1A5 may be a beneficial tool for cancer treatment.

Author contribution statement

Juan Ni: Performed the experiments; Wrote the paper. Wumin Dai: Conceived and designed the experiments. Chun Liu: Analyzed and interpreted the data. Yutian Ling: Performed the experiments. Hanzhou Mou: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

Additional information

Supplementary content related to this article has been published online at [URL].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

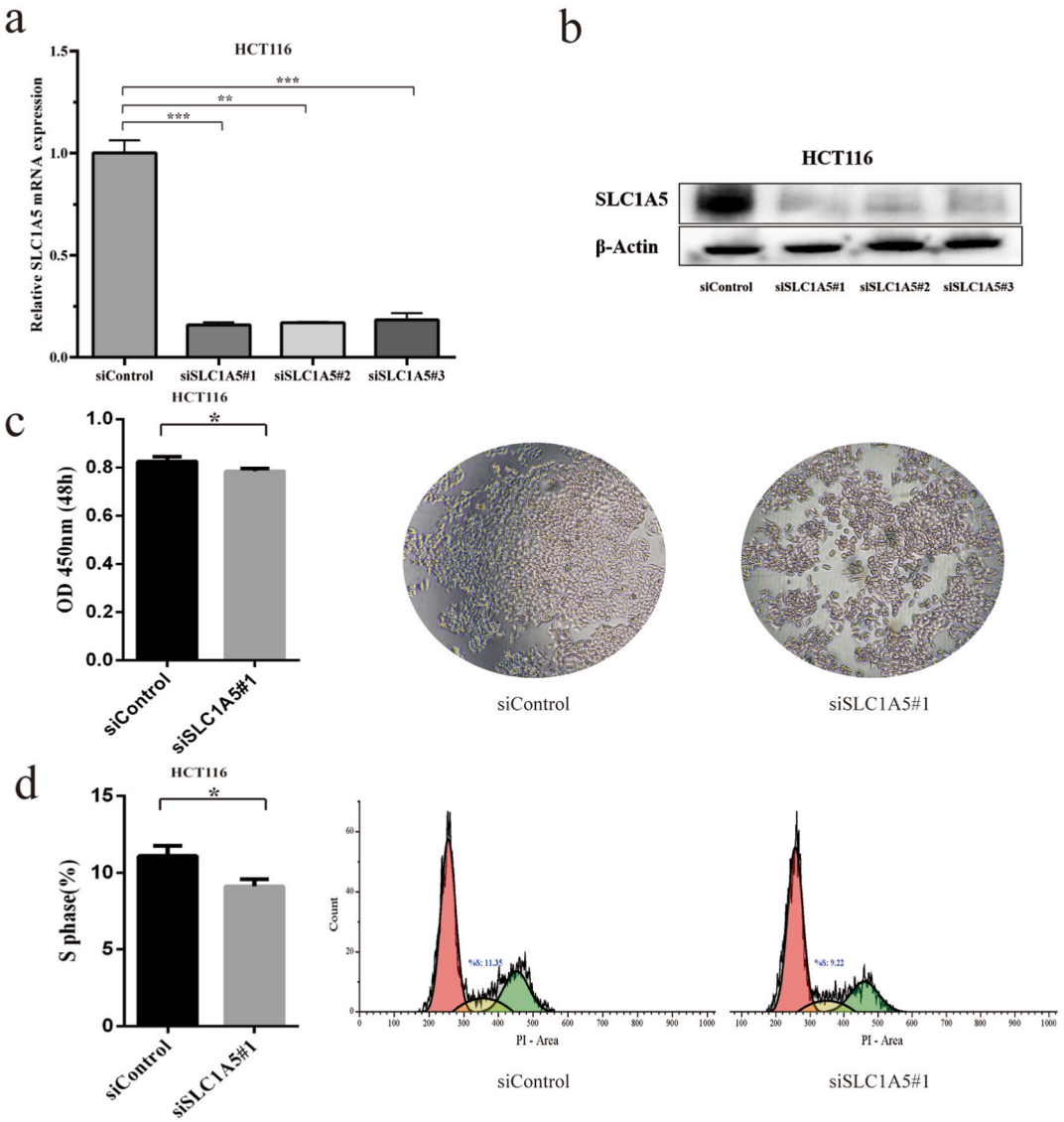

Fig. 8. SLC1A5 knockdown inhibited COAD progression. (a, b) Q-PCR and Western blot were used to verify the knockdown efficiency of SLC1A5. (c) CCK-8 assay was applied to detect the vitality. (d) Flow cytometry was performed to characterize the cell cycle. *: p-value <0.05; **: p-value <0.01; ***: p-value <0.001.

influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e17598.

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