第51卷第2期 中国科学技术大学学报 Vol. 51, No. 2
2021年2月 JOURNAL OF UNIVERSITY OF SCIENCE AND TECHNOLOGY OF CHINA Feb. 2021
Received: 2021-01-28; Revised: 2021-02-20 doi:10.52396/JUST-2021-0033

Effects of alanine-serine-cysteine transporter 2 on proliferation and invasion of hepatocellular carcinoma

YU Shuqi[1], ZHANG Shitong[1], WANG Dezheng[1], SUN Yi[1], CHENG Meiling[1], Kan Chen[1*], Ni Fang[1,2,3*]

1. Department of Pathophysiology, School of Basic Medical Science, Anhui Medical University, Hefei 230032, China;
2. Department of Hematology, The First Affiliated Hospital of USTC(Anhui Provincial Hospital), Hefei 230001, China;
3. School of Basic Medicine, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230027, China
* Corresponding author. E-mail: ckanahmu@163.com; fangni@ustc.edu.cn

Abstract: Metabolic reprogramming is a major feature of tumors, and tumor cells adapt to their glutamine needs by up-regulating the alanine-serine-cysteine transporter 2(ASCT2). It was found that the ASCT2 expression in hepatocellular carcinoma (HCC) tissues was significantly higher than that in normal liver tissues. In addition, the higher expression of ASCT2 in HCC patients was closely associated with poor survival. The knockdown of ASCT2 inhibited the proliferation, clone formation, migration and invasion of HCC cells in vitro. Cell cycle analysis suggested that knockdown of ASCT2 inhibited the proportion of HCC cells in the S phase. In vivo tumorigenic assay confirmed that the knockdown of ASCT2 in HCC cells could significantly inhibit tumor growth. Further studies showed that the knockdown of ASCT2 significantly reduced mitochondrial oxidative phosphorylation(OXPHOS), ATP production, and the phosphorylation level of AKT/S6 in HCC cells. Overall, our results showed that knockdown of ASCT2 could inhibit the malignancy of HCC cells. In addition, the mitochondrial metabolism and phosphorylation level of the AKT/S6 signaling pathway of HCC cells were also inhibited following the ASCT2 inhibition, suggesting that the dysregulated mitochondrial metabolism and abnormal activation of AKT/S6 signaling pathway were closely associated with the HCC progression.

Keywords: ASCT2; hepatocellular carcinoma; proliferation; invasion; metabolism
CLC number: R735.7;R73-37 **Document code**: A

1 Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide and the fourth leading cause of cancer-related death[1]. Since the early clinical symptoms of HCC are not obvious, most patients are diagnosed at an advanced stage[2]. Surgical resection, liver transplantation, radiofrequency ablation, radiotherapy and chemotherapy are often ineffective, and patients who undergo these treatments are prone to recurrence and metastasis[3]. In recent years, it has been reported that the targeted regulation of the glutamine metabolism, glucose metabolism and lipid metabolism in HCC could effectively inhibit the growth of HCC[4]. Recent studies showed that changes of the glutamine, glucose and lipid metabolism were critical to liver carcinogenesis[5,6]. Targeted regulation of the glutamine metabolism in HCC could promote the progression and survival of HCC[4]. The glutamine metabolism has been shown to be involved in regulating the occurrence and development of HCC, but its underlying mechanism remains unclear.

Metabolic reprogramming is a major feature of tumors. After undergoing metabolic reprogramming, tumor cells will take a large amount of nutrients to meet their own needs[7]. Glutamine is one of the most abundant nonessential amino acids in the human body, and tumor growth depends on a large amount of glutamine[8]. Glutamine participates in a variety of metabolic pathways within the cell, including the synthesis of tricarboxylic acid cycle(TCA) metabolites, nonessential amino acids, nucleotides, fatty acids, antioxidants, and ATP energy[9]. Cancer cells are especially dependent on glutamine[10]. Glutamine is converted in turn into glutamate[11] and α-ketoglutarate (α-KG), and participates in the TCA, thereby supporting energy production[7] and providing intermediates for other biosynthetic pathways[12].

Citation: YU Shuqi, ZHANG Shitong, WANG Dezheng, et al. Effects of alanine-serine-cysteine transporter 2 on proliferation and invasion of hepatocellular carcinoma. J. Univ. Sci. Tech. China, 2021, 51(2): 129-139.

Recently, glutamine has been shown to be essential in promoting the mitochondrial metabolism to maintain the rapid growth of cancer cells[13-15]. The inhibition of the mitochondrial glutamine metabolism has been found to have a strong anticancer effect.

Alanine-serine-cysteine transporter 2 (ASCT2) is a transporter of glutamine present on cell membrane[16], and its expression is increased in highly proliferating cells such as inflammatory cells and stem cells to meet the increased glutamine demand[17]. Studies have shown that the ASCT2 expression was significantly increased in cancers, such as the colorectal cancer, prostate cancer, lung cancer, breast cancer, cervical cancer, kidney cancer and brain cancer[18]. This discovery has aroused the interest of scholars for ASCT2 to be a pharmacological target of new anticancer drugs[19]. Studies have shown that the knockdown of ASCT2 could inhibit neuroblastoma[20], breast cancer[21], and gastric cancer tumor growth[22]. Recently, ASCT2 has been reported to be closely associated with the prognosis of HCC[23]. However, the role of ASCT2 and its mechanisms in the progression of HCC have not been deeply studied.

In this study, we found that the expression level of ASCT2 in HCC tissues was negatively correlated with the prognosis and survival of patients. The knockdown of ASCT2 inhibited the capacity of proliferation, migration and invasion of HCC cells. In addition, the mitochondrial metabolism and associated AKT/S6 pathway of HCC cells following ASCT2 knockdown were also inhibited, suggesting that the dysregulated mitochondrial metabolism and the abnormal activation of AKT/S6 signaling pathway were closely associated with the HCC progression.

1 Materials and methods

1.1 Antibodies and reagents

All antibodies were purchased from Cell Signaling Technology (CST) Co., Ltd. The protein extraction kits were purchased from Beijing Solarbio Science & Technology Co., Ltd. The BCA protein content detection kit and MTT cell proliferation and the cytotoxicity detection kit were purchased from Kaiji Biotechnology Co., Ltd. The quantitative PCR kit was purchased from Takara Company. TRIzol reagent was purchased from Beijing Kangwei Century Biotechnology Co., Ltd. Protease inhibitor was purchased from Roche. Transwell chamber and matrigel matrix gels were purchased from BD. RPMI medium DMEM basic (1x) was purchased from Gibco. Cell cycle detection kit and ATP kit were purchased from Beyotime Biotechnology Co., Ltd. Dimethyl sulfoxide (DMSO) was purchased from Sigma. Imported FBS and the penicillin-streptomycin solution were purchased from HyClone. Lentivirus design and synthesis was performed by Shanghai Jikai-gene Co., Ltd. Primers were purchased from Sangon Biotech (Shanghai) Co., Ltd.

1.2 QPCR analysis

Total RNA was extracted from cells by the TRIzol method. Reverse transcription of RNA was performed using a reverse transcription kit and the PCR instrument (42 °C for 1 h; 70 °C for 5 min; 4 °C for 2 h). ASCT2 was amplified according to the instruction of the SYBR Q-PCR kit and completed on a LightCycler 480 system real-time quantitative PCR instrument. Relative quantitative mRNA levels were determined with the use of the $2^{-\Delta\Delta Ct}$ method, with β-actin (ACTB) as an internal control. Each reaction was repeated independently at least three times. Primers were purchased from Sangon Biotech (Shanghai) Co., Ltd, and the sequences were showed as follow:

ASCT2: 5'-GCTACCGGAATCATAATTCATTGTTAACCTCC-3' (forward);

5'-AGATCTGGAGTAGCGGTTACCAGCCAGAGAAAG-3' (reverse);

Human ACTB: 5'-GGCGGCACCACCATGTACCCT-3' (forward);

5'-AGGGGCCGGACTCGTCATACT-3' (reverse).

1.3 Western blotting

After the cells were collected, they were lysed with protease inhibitor and RIPA cell lysate. The BCA kit was used to detect the protein concentration of the sample. After proteins were denatured by heat, the same amount of proteins was added to the gel and separated, then transferred to the PVDF membrane. PVDF membranes were then incubated with the primary antibodies, such as AKT (CST, America/9272S), ASCT2 (CST, America/5345S), p-AKT (CST, America/4060S), S6 (CST, America/2317S), p-S6 (CST, America/4858S) and β-actin (CST, America/4970S) at 4 °C overnight. Next, PVDF membrane was incubated with secondary antibodies at room temperature for 1 h. Finally, ChemiDoc MP (Bio-Rad) was used to capture the positive signal. ImageJ software was responsible for the statistical analysis of western blot.

1.4 Cell culture and lentivirus transfection

All cell lines were provided by the Laboratory of Pathophysiology of the Basic Medical College of Anhui Medical University. All cells were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin, and incubated at 37 °C in the incubator with 5% CO_2. The titer of the negative control virus CONO77 was 1E+9 TU · mL^{-1}, and that of lv-slc1a5-rnai (43403-11) was 4E+8 TU · mL^{-1}. According to the manufacturer's instructions, the ASCT2 knockdown lentivirus and the negative-control lentivirus were transferred into SMMC-7721 and BEL-7404 cells. The following experimental cells were grouped into: Control: blank control group;

NC: negative control group; shASCT2: knockdown ASCT2 group.

1.5 Cell proliferation assay

Cells (1×10^3 well^{-1}) were seeded in the 96-well plate. A total of 4 plates were prepared. The cells were incubated at 37 °C in the incubator with 5% CO_2. The 96-well plates were labeled at 0 h and 20 μL solution of MTT was added to each well in a dark environment. Then, 150 μL solution of DMSO was added to each well to dissolve the precipitate at the bottom of the pore plate. The absorbance was detected at 490 nm by a microplate reader, and the subsequent 24-h, 48-h, 72-h plates were treated in the same way. At least 5 pores were analyzed for each group of cells, and the experiment was repeated 3 times to obtain the average value.

1.6 Clonal forming ability analysis

Cells (300 well^{-1}) were seeded in the 6-well plate. The cells were incubated at 37 °C in the incubator with 5% CO_2. The medium was removed and the cells were stained. Washing the wells with precooled PBS for twice. 0.1% crystal violet prepared with fixed solution (4% formaldehyde) was added to the wells for 2 h. The residual crystal violet was washed by PBS and dried at a room temperature. The number of clones in per well was counted with the ImageJ software, and statistical analyses were performed. The experiment was repeated 3 times to obtain the average values.

1.7 Cell cycle assays

Cells were collected and fixed at 4 °C with 70% ethanol. Washed with PBS, cells were incubated with 0.5 mL PI/RNase staining buffer at 4 °C in the dark for 30 min. The cell cycle analysis was examined by the flow cytometry. The FlowJo software was used to analyze the data.

1.8 Cell migration and invasion assay

For the migration test, 1.5×10^5 cells were plated in the upper chamber with 200 μL serum-free growth medium. In addition, 500 μL medium supplemented with 10 % FBS was added to the lower chamber. The chamber was incubated at 37 °C in incubator with 5% CO_2 for 24 h. Then, the membrane of the chamber was collected and dissolved in 150 μL 33% glacial acetic acid. The cell density was quantified by optical density (OD) at 490 nm with a microplate reader.

For the invasion assay, the Matrigel was thawed according to the instructions in the invasion test. 60 μL Matrigel was added on the upper chamber. Then, the culture plates with chamber was put in a humidified incubator at 37 °C for 60 min. 1.5×10^5 cells in 200 μL serum-free medium were added to the Matrigel-coating upper chamber. 500 μL medium containing 10 % FBS was added to the lower chamber. Then the 24-well plate was put in the incubator at 37 °C for 24 h. Photos were taken under the microscope. Five fields were randomly selected for each chamber to take photos and count.

Note: the above experiments were repeated 3 times to obtain the average value.

1.9 Tumorigenesis in nude mice

HCC cells expressing sh-NC and shASCT2 were subcutaneously injected into the 4-week-old female BALB/c nude mice ($n=6$/group). The tumor size was monitored every 3 days using a vernier caliper, and the mice were sacrificed after 4 weeks.

1.10 Energy metabolism measurement

The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured in real time using the Seahorse XF Extracellular Flux Analyzer (Seahorse Bioscience). The cells were seeded into the 24-well Seahorse microplates and then incubated at 37 °C. The calibrator plate was equilibrated overnight in a non-CO_2 incubator. The cells were washed with the XF cell mitochondrial pressure test solution and placed in the CO_2-free incubator at 37 °C for 1 h. The seahorse XF analyzer was used for metabolic analysis of HCC cells.

1.11 ATP detection

The cells were seeded in the 6-well plate. After 24 h incubation at 37 °C, the culture medium was removed and 200 μL solution of lysis buffer was added to each well. Then we collected the supernatant after centrifuging at 12000 g and 4 °C for 5 min. 100 μL solution of ATP reagent was added to the plate for 3 min at the room temperature. Finally, 20 μL sample or a standard solution was added to the plate. Chemiluminescence instrument was used to measure the fluorescence value.

1.12 Statistical analysis

All the data obtained in the experiment were represented as the Mean ± SEM. Each experiment was repeated for three times. SPSS16.0 statistical software was used to analyze the data, and independent *t*-test was used for the pair-wise comparison, while one-way ANOVA was used for comparison between multiple groups of data. $P<0.05$ indicates that the difference was statistically significant.

2 Results

2.1 The level of ASCT2 expression in HCC cells and its clinical correlation

To verify whether ASCT2 is up-regulated in HCC cell lines, we verified the expression of ASCT2 in HepG2, Hep3B, SMMC-7721, QGY-7701 and BEL-7404 HCC cells and normal liver cell lines. The qPCR assay showed that the mRNA level of ASCT2 in HepG2, Hep3B, SMMC-7721, QGY-7701, and BEL-7404 cells was significantly increased compared with normal liver cells (Figure 1(a)). Western blot analysis showed that

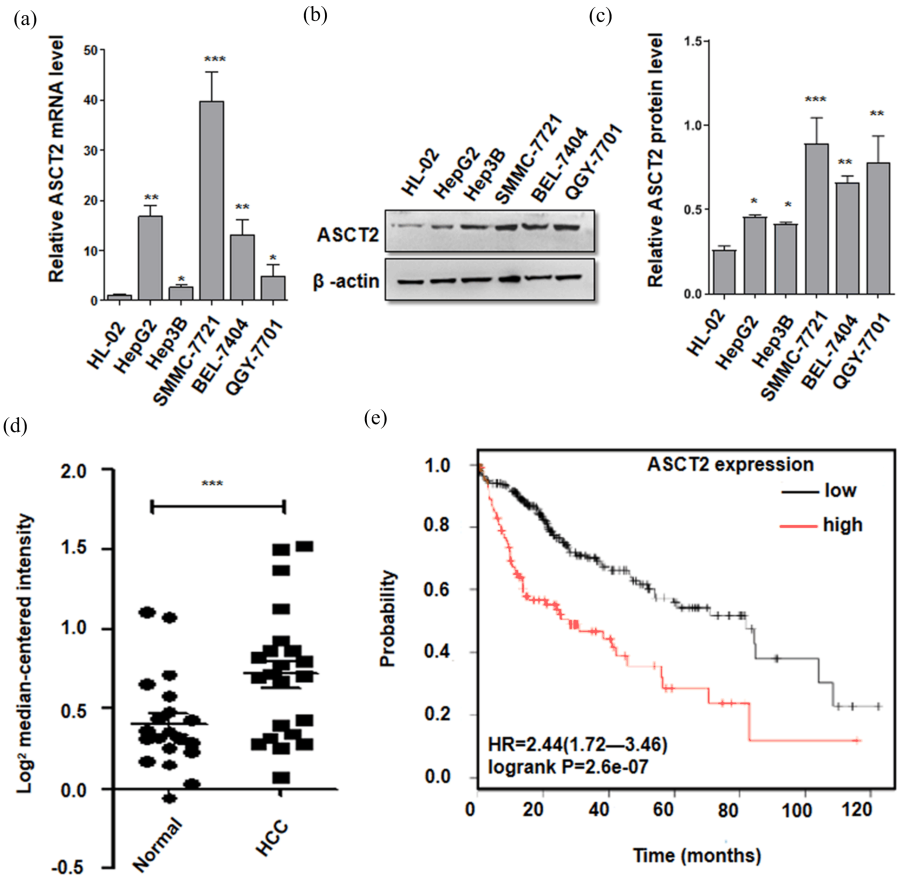

Figure 1. The level of ASCT2 expression in HCC cells and its clinical correlation. (a) ASCT2 mRNA expression level in HCC cells was detected. (b, c) ASCT2 expression level in HCC cells was detected. (d) ASCT2 expression level in HCC tissue ($n = 22$) compared with normal liver tissue ($n = 21$). (e) Relationship between ASCT2 expression level and long-term survival of patients. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

ASCT2 expression was also increased in HCC cell lines compared with normal liver cells (Figure1 (b, c)). Bioinformatic analysis using Oncomine database① showed that ASCT2 expression level in HCC tissue was also significantly increased compared with normal liver tissue (Figure 1 (d)). By Kaplan-Meier plotter database② analysis found that higher ASCT2 expression predicted poor survival in HCC patients (Figure 1(e)).

2.2 Effect of ASCT2 knockdown on the malignant biological behavior of HCC cells

To probe the role of ASCT2 in HCC progression, SMMC-7721 and BEL-7404 cell lines were selected and transfected with lentivirus to establish a stable negative control (NC) cell line and the ASCT2 knockdown (shASCT2) cell line. The transfection efficiency was observed by the fluorescence microscopy (Figure 2 (a)). Western Blot analysis showed that the expression level of ASCT2 in the knockdown group was significantly lower than that in the negative control group (Figure 2(b–d)). Due to the down-regulation of the expression of tumor suppressor genes, strong proliferative capacity is a major feature of tumor cells[24]. Compared with the blank control group and the negative control group, the MTT assay showed that the proliferation ability of cells in the knockdown group was inhibited (Figure 3(a, b)). The cell cycle is important for cell growth and the S phase ratio of cells reflects the degree of proliferative activity. The larger proportion of cells in the S phase, the more active they are in proliferation ability[25]. Cell cycle results showed that ASCT2 knockdown inhibited the proportion of HCC cells in S phase (Figure 3(c, d)). Clonal formation experiment showed that knockdown of ASCT2 inhibited the clonal ability of HCC cells (Figure 3(e, f)). The transwell experiment showed that the knockdown of ASCT2 inhibited the migration and invasion abilities of HCC cells (Figure 3 (g – j)). In conclusion, the knockdown of ASCT2 could inhibit the malignant biological behavior of HCC cells in vitro.

① https://www.oncomine.org.
② http://kmplot.com/analysis.

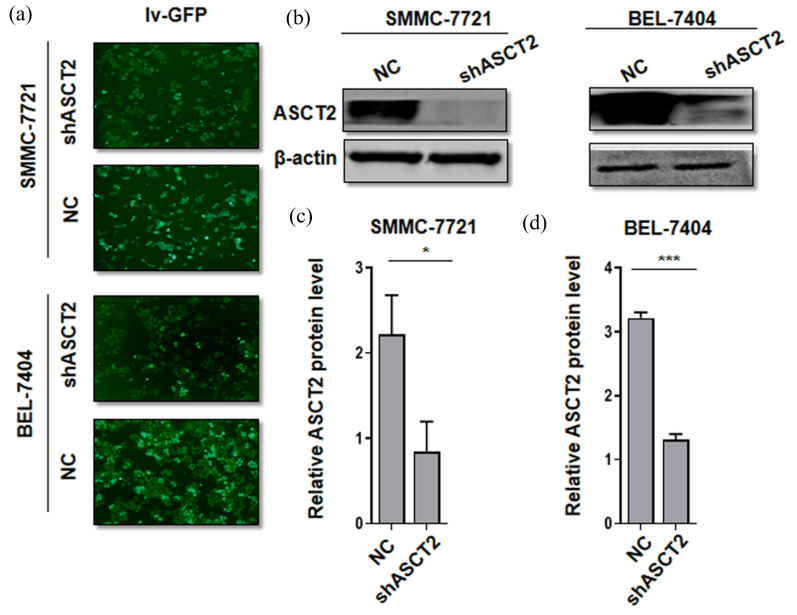

Figure 2. Transfection and knockdown effect of lentivirus in HCC cell lines. (a) Fluorescence observation of SMMC-7721 cells and BEL-7404 cells under the inverted fluorescence microscope (magnification 40 x). (b–d) ASCT2 knockdown detection in SMMC-7721 cells and BEL-7404 cells. $^{*}P<0.05$, $^{***}P<0.001$. NC, negative control. shASCT2, ASCT2 knockdown.

2.3 Effect of ASCT2 knockdown on tumor growth in vivo

HCC is a tumor with a high degree of malignancy. It has strong tumorigenesis[26]. In order to investigate the effect of ASCT2 knockdown on tumor development in vivo, BEL-7404 cells (control), lentiviral transfected negative control cells (NC) and ASCT2 knockdown cells (shASCT2) were injected into nude mice respectively. Tumor formation experiments in nude mice showed that compared with the blank control group and the negative control group, the tumor volume and weight of mice in the knockdown group were significantly reduced. There was no significant difference in the weight of the mouse between groups (Figure 4(a–e)). These results suggested that the knockdown of ASCT2 could inhibit the tumor growth.

2.4 Effects of ASCT2 knockdown on mitochondrial metabolism of HCC

To investigate whether ASCT2 knockdown has any effect on mitochondrial metabolism in HCC cells, oxidative phosphorylation (OXPHOS) level and glycolysis level were assessed by measuring the alteration of oxygen consumption rate (OCR, indicative of OXPHOS) and extracellular acidification rate (ECAR, indicative of glycolysis). The results showed that compared with the negative control group, the level of OCR was significantly inhibited in the ASCT2 knockdown group, while ECAR had no significant change between groups (Figure 5(a, b)). In addition, knockdown of ASCT2 in HCC cells decreased ATP levels (Figure 5(c)).

2.5 Effect of ASCT2 knockdown on the phosphorylation levels of AKT and S6

In order to explore the mechanism of ASCT2 knockdown affecting metabolism of HCC cells, the phosphorylation level of AKT/S6 signaling pathway related to energy metabolism was detected by western blot. The results showed that the phosphorylation level of AKT was significantly inhibited in the knockdown group compared with the blank and negative control groups (Figure 6(a, b)). Similarly, the phosphorylation level of S6 was also significantly inhibited (Figure 6(c, d)). These results showed that knockdown of ASCT2 could inhibit the activation level of AKT/S6 pathway.

3 Discussion

HCC is one of the most common malignant tumors in the world, with no significant symptoms in the early stage. Once diagnosed, most patients are in advanced stages, but no effective treatment has been found for advanced HCC[27]. Therefore, it is necessary to identify new tumor markers and find more effective detection and treatment methods to improve the diagnosis and treatment of HCC. Metabolic reprogramming is closely related to tumor proliferation, metastasis and poor prognosis[28]. With more profound knowledge of the tumor metabolism, the research has gradually shifted to amino acid metabolism, especially the glutamine metabolism pathway. It has been shown that the glutamine starvation inhibited the tumor metabolism, thereby alleviating tumor cells' proliferation and survival[29]. ASCT2 is a glutamine transporter located on

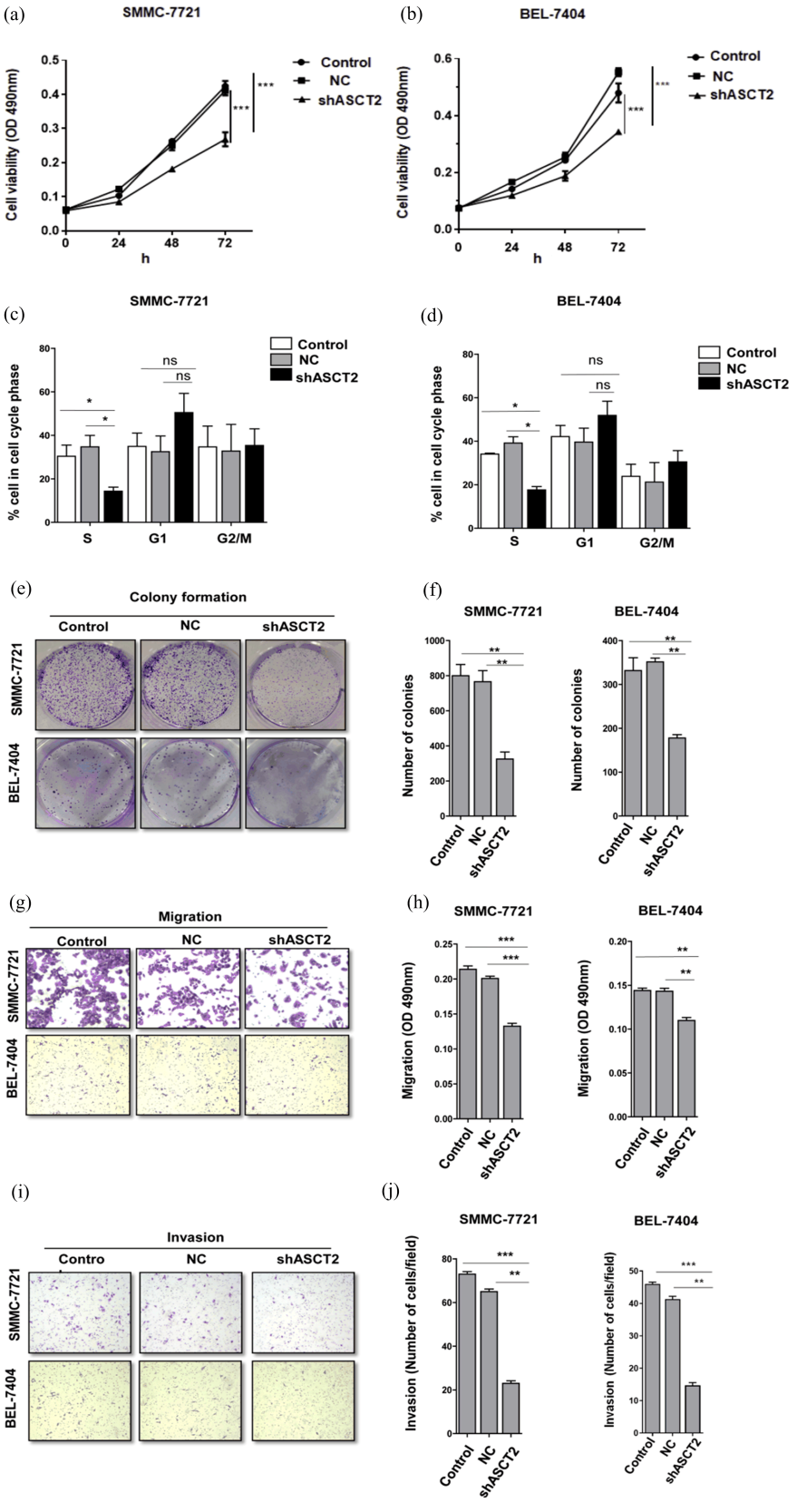

Figure 3. Effect of ASCT2 knockdown on the malignant biological behavior of HCC cells. (a, b) Cell proliferation ability test. (c, d) Cell cycle assay test. (e, f) Cell cloning ability test. (g, h) Cell migration ability test (magnification 40 x). (i, j) Cell invasion ability test (magnification 40 x). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. NC, negative control. shASCT2, ASCT2 knockdown.

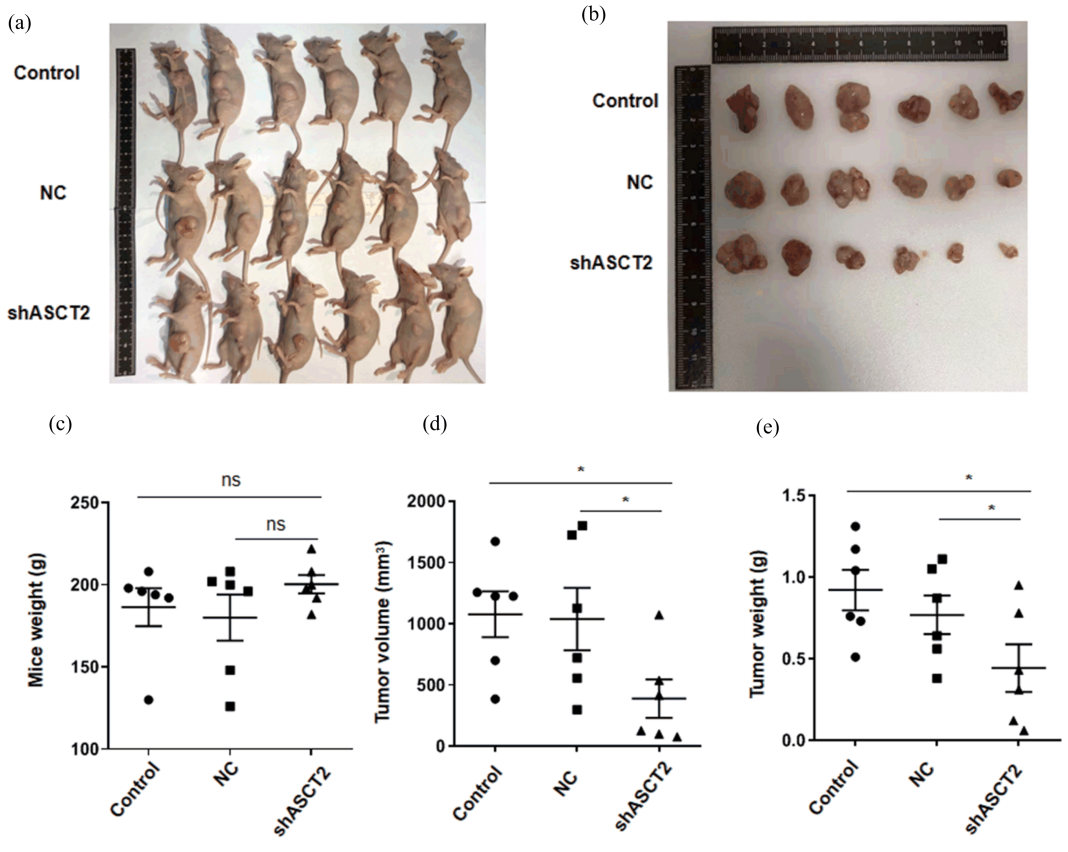

Figure 4. Effect of ASCT2 knockdown on tumor growth in vivo. (a,b) Comparison of tumor volume in each group. (c) Comparison of the body weight of mice in each group. (d) Comparison of tumor volume in each group. (e) Comparison of the tumor weight in each group. NS, nonsignificant, $^{*}P<0.05$. NC, negative control. shASCT2, ASCT2 knockdown.

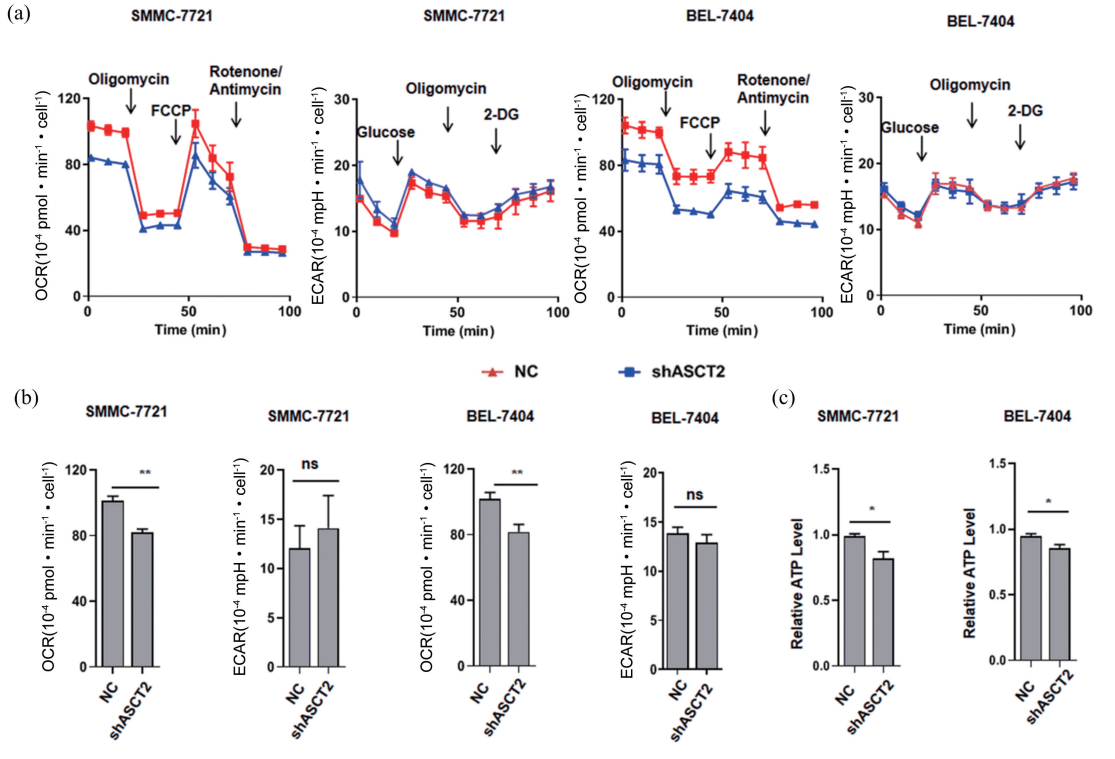

Figure 5. Effects of ASCT2 knockdown on mitochondrial metabolism of HCC. (a, b) Detection of the level of cells OCR change; detection of the level of cells ECAR change. (c) Detection of the level of ATP production. NS, nonsignificant, $^{*}P<0.05$, $^{**}P<0.01$. NC, negative control. shASCT2, ASCT2 knockdown.

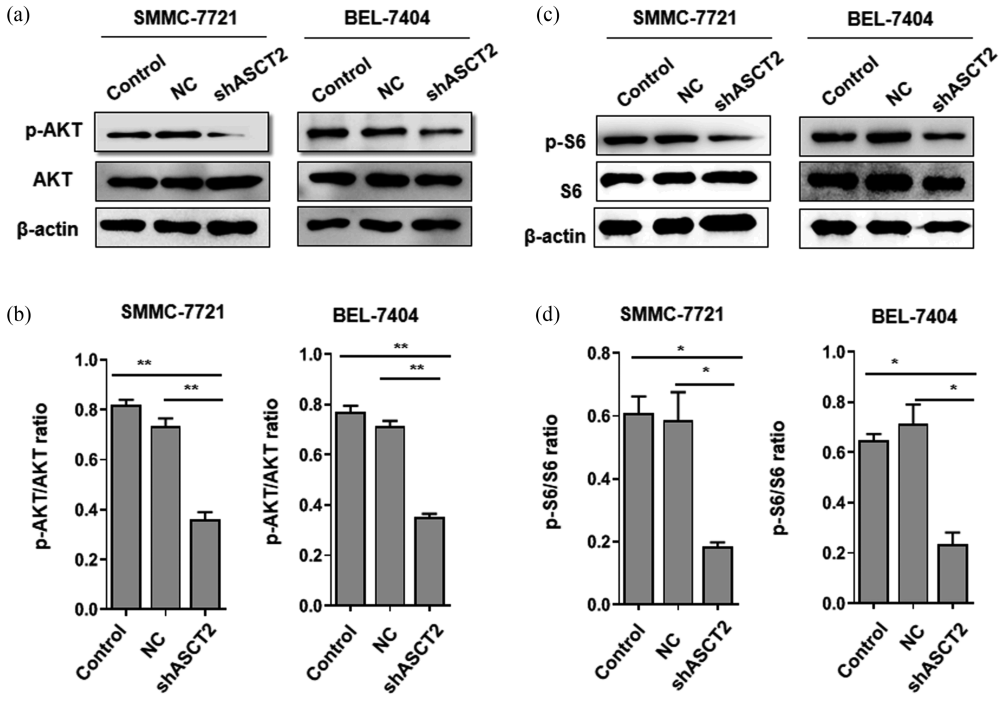

Figure 6. Effect of ASCT2 knockdown on the phosphorylation levels of AKT and S6. (a, b) Detection of the phosphorylation level of AKT. (c, d) Detection of the phosphorylation level of S6. $^{*}P<0.05$, $^{**}P<0.01$. NC, negative control. shASCT2, ASCT2 knockdown.

the cell membrane, which is closely related to the metabolism of glutamine. This study mainly explored the effect of knockdown of glutamine transporter ASCT2 on the progression of HCC and its possible mechanism.

The rapid proliferation and invasion and other life activities of tumor cells require a lot of energy support. Among all kinds of energy metabolism, the vigorous glutamine metabolism is an important characteristic of tumor[30]. The growth of tumor cells is dependent on glutamine, and the metabolism of glutamine in mitochondria plays an important role in the growth of tumor cells. Inhibition of glutamine transport in mitochondria can effectively inhibit the growth of many tumor cells[31]. ASCT2 is a glutamine transporter, which is closely related to the development of tumor, but the inhibitory effect of ASCT2 knockdown on different tumor cells is quite different. Research has reported that knockdown of ASCT2 could effectively inhibit the growth of breast cancer[21], neuroblastoma[20] and gastric cancer[22], but it cannot inhibit the growth of osteosarcoma[32] or liver epithelial cancer[33]. Numerous studies have shown that the survival of tumor cells depends on glutamine[34-37]. But ASCT2 is not the only transporter of glutamine uptake in tumor cells. Cancer cells take up glutamine through several glutamine transporter families: SLC1, 6, 7, and 38[38]. After ASCT2 knockdown, the compensatory effect of other transporters may enable tumor cells to still receive enough glutamine to maintain glutamine metabolism in tumor cells. Therefore, tumor cells with ASCT2 knockdown may obtain glutamine through compensatory uptake by other transporters to maintain the normal metabolism of glutamine. Selecting the SMMC-7721 and BEL-7404 cell lines, the knockdown of ASCT2 by shASCT2 lentivirus, compared with the blank control group and the negative control group, the proliferation assay and clonal formation assay showed that ASCT2 knockdown could inhibit cell proliferation. Further in vivo xenograft tumor assay showed ASCT2 knockdown could also inhibited the tumor formation. At the same time, transwell experiments showed that the migration and invasion abilities of cells were inhibited after ASCT2 knockdown. Overall, our results demonstrated that the knockdown of ASCT2 in HCC cells could inhibit the progression of HCC.

Considering that cancer cells require energy to support their survival, we speculated that the knockdown of ASCT2 affected the metabolism of HCC cells, evidenced by the decreased levels of OXPHOS and ATP. Previous studies showed that the activation of AKT signaling pathway promoted OXPHOS of cancer cells[39-44]. Consistently, our results showed that the knockdown of ASCT2 could inhibit the phosphorylation levels of AKT and S6. We hypothesized that ASCT2

might regulate OXPHOS and the production of ATP in HCC cells via the AKT/S6 signaling pathway. However, how ASCT2 regulates the signaling pathway and affects the phosphorylation level of downstream signal molecules still needs further research and exploration.

4 Conclusions

This study showed that the ASCT2 expression was up-regulated in HCC, and the knockdown of ASCT2 could inhibit the progression of HCC. In addition, the mitochondrial metabolism and the activation of AKT/S6 signaling pathway of HCC cells following ASCT2 knockdown were also inhibited, suggesting that the dysregulated mitochondrial metabolism and the abnormal activation of AKT/S6 signaling pathway are closely associated with HCC progression. This study reveals a new molecular mechanism of HCC and provides a potential target for the treatment of HCC.

Acknowledgments

The present study was supported by the National Natural Science Foundation of China (32070916, 8157152) and the Fundamental Research Funds for the Central Universities (WK3520000010).

Conflict of interest

The authors declare no conflict of interest.

Author information

YU Shuqi, ZHANG Shitong, and WANG Dezheng are co-first authors.

YU Shuqi is a graduate student under the supervision of Prof. Ni Fang at Anhui Medical University. Her current research is mainly focused on tumor metabolism.

ZHANG Shitong is a graduate student under the supervision of Prof. Ni Fang at Anhui Medical University.

WANG Dezheng is a graduate student under the supervision of Prof. Ni Fang at Anhui Medical University.

KAN Chen (corresponding author) received PhD degree in Anhui Medical University. He is currently an associate professor at Department of Pathophysiology of Anhui Medical University. He focuses on the study of stem cells biology in heterotopic ossification, especially for mesenchymal stem cells and hematopoietic stem cells.

NI Fang (corresponding author) received her PhD degree in Cell Biology from University of Science and Technology of China. She is currently a professor at University of Science and Technology of China. Her research interests include metabolic and biomechanical regulation of tumor cells, hematopoietic cells and natural killer cells.

References

[1] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin., 2018, 68(6): 394-424.

[2] Kulik L, El-Serag H B. Epidemiology and management of hepatocellular carcinoma. Gastroenterology, 2019, 156(2): 477-491.

[3] Yarchoan M, Agarwal P, Villanueva A, et al. Recent developments and therapeutic strategies against hepatocellular carcinoma. Cancer Res., 2019, 79(17): 4326-4330.

[4] Dai W, Xu L, Yu X, et al. OGDHL silencing promotes hepatocellular carcinoma by reprogramming glutamine metabolism. J. Hepatol., 2020, 72(5): 909-923.

[5] Yu L, Kim J, Jiang L, et al. MTR4 drives liver tumorigenesis by promoting cancer metabolic switch through alternative splicing. Nat. Commun., 2020, 11(1): 708.

[6] Gu L, Zhu Y, Lin X, et al. The IKKβ-USP30-ACLY axis controls lipogenesis and tumorigenesis. Hepatology, 2021, 73(1):160-174.

[7] Deberardinis R J, Lum J J, Hatzivassiliou G, et al. The biology of cancer: Metabolic reprogramming fuels cell growth and proliferation. Cell Metab., 2008, 7(1): 11-20.

[8] Geck R C, Toker A. Nonessential amino acid metabolism in breast cancer. Adv. Biol. Regul., 2016, 62: 11-17.

[9] Yoo H C, Park S J, Nam M, et al. A variant of SLC1A5 is a mitochondrial glutamine transporter for metabolic reprogramming in cancer cells. Cell Metab., 2020, 31(2): 267-283.

[10] Wise D R, Thompson C B. Glutamine addiction: A new therapeutic target in cancer. Trends Biochem. Sci., 2010, 35(8): 427-433.

[11] Cassago A, Ferreira A P, Ferreira I M, et al. Mitochondrial localization and structure-based phosphate activation mechanism of Glutaminase C with implications for cancer metabolism. Proc. Natl. Acad. Sci. U. S. A., 2012, 109(4): 1092-1097.

[12] Udagawa M, Horie Y, Hirayama C. Aberrant porphyrin metabolism in hepatocellular carcinoma. Biochem. Med., 1984, 31(2): 131-139.

[13] Gao P, Tchernyshyov I, Chang T C, et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature, 2009, 458(7239): 762-765.

[14] Son J, Lyssiotis C A, Ying H, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature, 2013, 496(7443): 101-105.

[15] Wise D R, Deberardinis R J, Mancuso A, et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc. Natl. Acad. Sci. U. S. A., 2008, 105(48): 18782-18787.

[16] Arriza J L, Kavanaugh M P, Fairman W A, et al. Cloning and expression of a human neutral amino acid transporter with structural similarity to the glutamate transporter gene family. J. Biol. Chem., 1993, 268(21): 15329-15332.

[17] Nakaya M, Xiao Y, Zhou X, et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. Immunity, 2014, 40(5): 692-705.

[18] Scalise M, Pochini L, Galluccio M, et al. Glutamine transport and mitochondrial metabolism in cancer cell growth. Front. Oncol., 2017, 7: 306.

[19] Scalise M, Pochini L, Panni S, et al. Transport mechanism and regulatory properties of the human amino acid transporter ASCT2 (SLC1A5). Amino Acids, 2014, 46

(11): 2463-2475.
[20] Ren P, Yue M, Xiao D, et al. ATF4 and N-Myc coordinate glutamine metabolism in MYCN-amplified neuroblastoma cells through ASCT2 activation. J. Pathol., 2015, 235(1): 90-100.
[21] van Geldermalsen M, Wang Q, Nagarajah R, et al. ASCT2/SLC1A5 controls glutamine uptake and tumour growth in triple-negative basal-like breast cancer. Oncogene, 2016, 35(24): 3201-3208.
[22] Ye J, Huang Q, Xu J, et al. Targeting of glutamine transporter ASCT2 and glutamine synthetase suppresses gastric cancer cell growth. J. Cancer Res. Clin. Oncol., 2018, 144(5): 821-833.
[23] Sun H W, Yu X J, Wu W C, et al. GLUT1 and ASCT2 as predictors for prognosis of hepatocellular carcinoma. PLoS One, 2016, 11(12): e168907.
[24] Hu W, Feng Z. The role of p53 in reproduction, an unexpected function for a tumor suppressor. J. Mol. Cell Biol., 2019, 11(7): 624-627.
[25] Icard P, Fournel L, Wu Z, et al. Interconnection between metabolism and cell cycle in cancer. Trends Biochem. Sci., 2019, 44(6): 490-501.
[26] Han T S, Ban H S, Hur K, et al. The epigenetic regulation of HCC metastasis. Int. J. Mol. Sci., 2018, 19(12): 3978.
[27] Sim H W, Knox J. Hepatocellular carcinoma in the era of immunotherapy. Curr. Probl. Cancer, 2018, 42(1): 40-48.
[28] Brown K K, Spinelli J B, Asara J M, et al. Adaptive reprogramming of de novo pyrimidine synthesis is a metabolic vulnerability in triple-negative breast cancer. Cancer Discov., 2017, 7(4): 391-399.
[29] Alberghina L, Gaglio D. Redox control of glutamine utilization in cancer. Cell Death Dis., 2014, 5: e1561.
[30] Geck R C, Toker A. Nonessential amino acid metabolism in breast cancer. Adv. Biol. Regul., 2016, 62: 11-17.
[31] Yoo H C, Park S J, Nam M, et al. A variant of SLC1A5 is a mitochondrial glutamine transporter for metabolic reprogramming in cancer cells. Cell Metab., 2020, 31(2): 267-283.
[32] Broer A, Gauthier-Coles G, Rahimi F, et al. Ablation of the ASCT2 (SLC1A5) gene encoding a neutral amino acid transporter reveals transporter plasticity and redundancy in cancer cells. J. Biol. Chem., 2019, 294(11): 4012-4026.
[33] Bothwell P J, Kron C D, Wittke E F, et al. Targeted suppression and knockout of ASCT2 or LAT1 in epithelial and mesenchymal human liver cancer cells fail to inhibit growth. Int. J. Mol. Sci., 2018, 19(7): 2093.
[34] Lee P, Malik D, Perkons N, et al. Targeting glutamine metabolism slows soft tissue sarcoma growth. Nat. Commun., 2020, 11(1): 498.
[35] Wang V M, Ferreira R, Almagro J, et al. CD9 identifies pancreatic cancer stem cells and modulates glutamine metabolism to fuel tumour growth. Nat. Cell Biol., 2019, 21(11): 1425-1435.
[36] Wang Y, Bai C, Ruan Y, et al. Coordinative metabolism of glutamine carbon and nitrogen in proliferating cancer cells under hypoxia. Nat. Commun., 2019, 10(1): 201.
[37] Biancur D E, Paulo J A, Malachowska B, et al. Compensatory metabolic networks in pancreatic cancers upon perturbation of glutamine metabolism. Nat. Commun., 2017, 8: 15965.
[38] Pochini L, Scalise M, Galluccio M, et al. Membrane transporters for the special amino acid glutamine: Structure/function relationships and relevance to human health. Front. Chem., 2014, 2: 61.
[39] Hoxhaj G, Manning B D. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. Nat. Rev. Cancer, 2020, 20(2): 74-88.
[40] Pereira O, Teixeira A, Sampaio-Marques B, et al. Signalling mechanisms that regulate metabolic profile and autophagy of acute myeloid leukaemia cells. J. Cell Mol. Med., 2018, 22(10): 4807-4817.
[41] Stiles B L. PI-3-K and AKT: Onto the mitochondria. Adv. Drug Deliv. Rev., 2009, 61(14): 1276-1282.
[42] Robey R B, Hay N. Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt. Oncogene, 2006, 25(34): 4683-4696.
[43] Pelicano H, Xu R H, Du M, et al. Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. J. Cell Biol., 2006, 175(6): 913-923.
[44] Li T, Han J, Jia L, et al. PKM2 coordinates glycolysis with mitochondrial fusion and oxidative phosphorylation. Protein Cell, 2019, 10(8): 583-594.

丙氨酸-丝氨酸-半胱氨酸转运体2对肝细胞肝癌增殖和侵袭的影响

于淑琦[1],张诗童[1],王德政[1],孙漪[1],程美玲[1],阚晨[1*],倪芳[1,2,3*]

1. 安徽医科大学基础医学院病理生理学教研室,安徽合肥 230032;
2. 中国科学技术大学附属第一医院(安徽省立医院)血液科,安徽合肥 230001;
3. 中国科学技术大学生命科学与医学部基础医学院,安徽合肥 230027

* 通讯作者. E-mail:ckanahmu@163.com; fangni@ustc.edu.cn

摘要: 代谢重编程是肿瘤的主要特征,肿瘤细胞通过上调丙氨酸-丝氨酸-半胱氨酸转运体2(ASCT2)来适应其对谷氨酰胺的需求. 本研究发现肝细胞肝癌(HCC)组织中ASCT2的表达明显高于正常肝组织,并且,高表达ASCT2的患者长期生存率较低. 在体外实验中,敲低ASCT2能显著抑制HCC细胞的增殖、克隆形成、迁移和

侵袭. 细胞周期分析表明,敲低 ASCT2 抑制了 HCC 细胞 S 期的比例. 裸鼠成瘤实验证实敲低 HCC 细胞中的 ASCT2 能显著抑制肿瘤的生长. 进一步的研究显示,敲低 ASCT2 可显著抑制 HCC 细胞线粒体氧化磷酸化(OXPHOS)、ATP 生成以及 AKT/S6 通路的磷酸化水平. 本研究结果表明,敲低 ASCT2 可抑制 HCC 细胞的恶性行为. 此外,ASCT2 下调后的 HCC 细胞线粒体代谢以及 AKT/S6 通路的磷酸化水平也受到抑制,提示线粒体代谢失调以及 AKT/S6 通路的异常活化与 HCC 的进展密切相关.

关键词: ASCT2;肝细胞肝癌;增殖;侵袭;代谢