

# Berberine Inhibits Growth of Liver Cancer Cells by Suppressing Glutamine Uptake

This article was published in the following Dove Press journal:  
*OncoTargets and Therapy*

Pengcheng Zhang  
Quancheng Wang   
Zhibin Lin  
Peijun Yang   
Kefeng Dou  
Ruohan Zhang

Department of Hepato-Biliary and  
Pancreto-Splenic Surgery, Xijing Hospital,  
The Fourth Military Medical University,  
Xi'an, People's Republic of China

**Introduction:** Glutamine metabolism is essential for the proliferation of cancer cells. Transported by SLC1A5, a Na<sup>+</sup> dependent transporter, glutamine is absorbed for further use. Recent studies have revealed the anti-tumor effect of berberine. The present study aimed to evaluate the effect of berberine on cancer cell glutamine metabolism.

**Materials and methods:** The inhibitory effect of berberine on liver cancer cells was analyzed by CCK-8 and EdU assay. The glutamine concentrations were detected by ELISA and UHPLC-MRM-MS analysis. Glutamine metabolism-related proteins were determined by Western blot, immunofluorescent analysis and immunohistochemistry.

**Results:** Berberine inhibited the proliferation of Hep3B and BEL-7404 cell in vitro. Berberine suppressed the glutamine uptake by inhibiting SLC1A5. The upregulation of SLC1A5 led to an increased glutamine uptake and improved tolerance to berberine. Berberine suppresses SLC1A5 expression by inhibiting c-Myc. Furthermore, berberine suppresses the growth of tumor xenografts, and the expression of SLC1A5 and c-Myc in vivo. The high expression of SLC1A5 in hepatocellular carcinoma (HCC) tissues is associated with poor prognosis.

**Conclusion:** Berberine can suppress the proliferation of liver cancer cells by reducing SLC1A5 expression.

**Keywords:** berberine, hepatocellular carcinoma, SLC1A5, glutamine metabolism

## Introduction

Hepatocellular carcinoma is the sixth most common cancer and the second most common cause of cancer mortality worldwide.<sup>1</sup> Several treatments can benefit patients, including surgical resection, ablation, transplantation, transarterial chemoembolisation and tyrosine-kinase inhibitors.<sup>2</sup> However, the curative treatments for HCC, such as liver resection, transplantation and ablation, are merely indicated for patients in the early stage.<sup>3</sup> For advanced-stage patients, these curative strategies are not suitable.<sup>4</sup> The therapeutic goal is to inhibit the proliferation of cancer cells and improve the survival of patients. Unfortunately, these reprogrammed metabolic pathways permit cancer cells to survive,<sup>5</sup> which impair the efficacy of present therapeutic regimens. Therefore, there is an urgent need to identify new drugs that could influence the metabolism of cancer cells and enhance the present therapy.

Glutamine metabolism is essential for cancer cells. Proliferating tumor cells require large amounts of biosynthesis. Glutamine acts as a nitrogen donor and a carbon donor, in addition to protein synthesis.<sup>6</sup> The oxidative stress encountered during cancer progression, metastasis and exposure to anti-tumor therapeutics raises the need of cancer cells for anti-oxidative defenses.<sup>7</sup> The product of glutamine metabolism and glutathione plays an important role in promoting anti-oxidative defenses. Therefore, cancer cells exhibit

Correspondence: Ruohan Zhang; Kefeng Dou  
Department of Hepato-Biliary and  
Pancreto-Splenic Surgery, Xijing Hospital,  
The Fourth Military Medical University,  
No. 169 West Changle Road, Xi'an  
710000, People's Republic of China  
Tel +86 150 2909 8264;  
+86 135 7189 0758  
Email zhangrh1990@outlook.com;  
doukef@fmmu.edu.cn

a great demand for glutamine. Consistent with the increased demand for glutamine, several transporters have been upregulated in many types of cancers.<sup>8</sup> One of the most studied proteins is plasma membrane transporter SLC1A5, which is also known as ASCT2. This transports glutamine in a Na<sup>+</sup>-dependent manner.<sup>9</sup> The gene of SLC1A5 is located at 19q13.3 with eight exons, and SLC1A5 forms a homotrimeric complex.<sup>10</sup> Overexpressed SLC1A5 can potentially be a drug target for cancer therapy, and blockade of this transporter might cause metabolic disorders and growth arrest in tumor cells.<sup>11</sup>

Berberine is the main ingredient for many Chinese herbal medicines.<sup>12</sup> Its multiple pharmacological properties make berberine have antioxidant, anti-inflammatory and antimicrobial effects.<sup>12</sup> Recently, several studies have shown that berberine could inhibit proliferation and induce apoptosis in a variety of cancer cells.<sup>13–15</sup> However, the mechanism by which berberine suppresses tumor growth remains elusive. In the present study, it was determined whether berberine could interfere with glutamine metabolism via the downregulation of SLC1A5.

## Materials and Methods

### Cell Lines and Culture Conditions

HCC cell lines Hep3B and BEL-7404 (obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences) were cultured in DMEM, supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA), in a humidified 5% CO<sub>2</sub> atmosphere at 37°C.

### Cell Proliferation and Colony Formation Assays

Cell proliferation was detected by Cell Counting Kit-8 (CCK-8) assay. Cells were seeded in 96-well plates at a density of 1×10<sup>3</sup> cells/well (Hep3B) and 8×10<sup>2</sup> cells/well (BEL-7404), and treated with berberine. At the indicated time points (12, 24, 36 and 48 hrs), 90 μL of culture medium containing 10% serum and 10 μL of CCK-8 solution were added to each well. Then, these cells were incubated for one hour at 37°C, and the absorbance was measured at 450 nm using a spectrophotometer.

For the colony formation assay, cells were seeded at a density of 1×10<sup>3</sup> cells/well in a 6-well plate, and cultured with 2 mL of DMEM supplemented with 10% FBS for five days. Then, the colonies were treated with berberine for nine days. After two weeks, the colonies were fixed in methanol and stained with a 0.25% crystal violet solution for counting.

### EdU Assay

EdU was obtained from RiboBio (Guangzhou, China). Cells were cultured in 24-well plates at a density of 1×10<sup>5</sup> cells/well. After treatment with berberine for 48 hrs, cells were incubated with DMEM with 10 mM of EdU for two hours, and washed with phosphate buffered saline (PBS). Then, these cells were fixed with 2% formaldehyde for 15 mins, and permeabilized with 0.5% triton X-100 for 30 mins. After extensive washing, cells were incubated with Apollo 567 dye for 30 mins, and stained with Hoechst or DAPI for 15 mins. The images of these cells were captured using a fluorescent microscope.

### Western Blot Analysis

Cells were harvested in RIPA lysis buffer (Beyotime, Shanghai, China) containing protease inhibitors (Beyotime, Shanghai, China) for 10 mins, and the lysates were centrifuged at 12,000 rpm for 10 mins at 4°C. Then, the supernatants were collected to determine the protein concentrations using a BCA Protein Assay Kit (Beyotime, Shanghai, China). Afterwards, these proteins were separated using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride (PVDF) membrane that was activated in advance by methanol. After blocking with 5% non-fat dry milk in Tris-buffered saline with Tween (TBST) at room temperature for one hour, these membranes were incubated with the primary antibodies overnight against SLC1A5 (Abcam, MA, USA), GLS (Proteintech, Wuhan, China), c-Myc (Proteintech, Wuhan, China), β-actin (CWBiotech, Beijing, China), PHGDH (Proteintech, Wuhan, China), and PSPH (Proteintech, Wuhan, China). Then, these membranes were incubated with secondary antibodies (CWBiotech, Beijing, China) the following day. After incubation, the membrane was scanned.

### Immunofluorescent Analysis

Cells were cultured in 24-well plates at a density of 1×10<sup>5</sup> cells/well, and treated with berberine for 48 hrs. Then, these cells were fixed with PBS containing 2% formaldehyde for 30 mins, and incubated with blocking buffer (Beyotime, Shanghai, China) for 10 mins. After extensive washing, these cells were incubated with primary antibodies overnight against SLC1A5 (Cell Signaling Technology, Danvers, MA, USA) and c-Myc (Proteintech, Wuhan, China). On the following day, these cells were incubated with the secondary antibody, Dylight 488 (Abbkine, Wuhan, China), for two hours, and DAPI

for 15 mins. The images of these cells were captured using a fluorescent microscope.

### UHPLC-MRM-MS Analysis

After the addition of 900  $\mu$ L of extraction solution (acetonitrile-methanol-water, 2:2:1), these samples were vortexed for 30 seconds, homogenized at 45 Hz for four minutes, and sonicated for five minutes in an ice-water bath. Then, the homogenate and sonicate cycle was repeated for three times, followed by incubation at  $-40^{\circ}\text{C}$  for one hour and centrifugation at 12,000 rpm for 15 mins at  $4^{\circ}\text{C}$ . A 100- $\mu$ L aliquot of the clear supernatant was transferred to an auto-sampler vial for the UHPLC-MS/MS analysis. The UHPLC separation was carried out using the Agilent 1290 Infinity II series UHPLC System (Agilent Technologies), which was equipped with a Waters ACQUITY UPLC BEH Amide column (100 $\times$ 2.1 mm, 1.7  $\mu$ m). The mobile phase A was 1% formic acid in water, and the mobile phase B was acetonitrile. An Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies), equipped with an AJS electrospray ionization (AJS-ESI) interface, was applied for assay development. The MRM parameters for each of the targeted analytes were optimized using flow injection analysis, and were performed by injecting the standard solutions of the individual analytes into the API source of the mass spectrometer. The Agilent MassHunter Work Station Software (B.08.00, Agilent Technologies) was employed for MRM data acquisition and processing.

### Enzyme-Linked Immunosorbent Assay (ELISA)

The glutamine uptake in the medium was determined by ELISA. Cells were treated with berberine at gradient concentrations for 48 hrs. The cell culture media were collected and centrifuged at 12,000 rpm for 10 mins. The concentration of glutamine in the media was assessed using an ELISA kit (Aviva Systems Biology, San Diego, USA). Briefly, the media was added into an anti-glutamine microplate and incubated with a glutamine-biotin complex for 60 mins. Then, the liquid was discarded, and an avidin-HRP conjugate was added to each well of the plate. After 45 mins, a TMB substrate was added to each well, and the plate was placed in the dark at  $37^{\circ}\text{C}$  for 20 mins. Finally, a stop solution was added to each well, and the OD absorbance was read at 450 nm using a standard microplate reader.

### Plasmids and Cell Transfection

The lentiviral plasmids encoding SLC1A5 or a negative control were designed and produced by GenePharma (Shanghai, China). The lentiviral plasmids encoding c-Myc or a negative control were designed by Genechem (Shanghai, China). Hep3B and BEL-7404 cells were grown in 6-well plates to 20–30% confluence, and the culture medium was replaced with serum-free medium containing HitransG A (Genechem, Shanghai, China) and the lentivirus at a multiplicity of infection (MOI) of 20. After 24 hrs, the medium was replaced with complete medium, and the cells were cultured for 72 hrs. Then, these cells were selected with 1  $\mu$ g/mL of puromycin for three days, and harvested for further studies.

### Xenograft Tumor Models in Nude Mice

BALB/C nude mice (six weeks old) were purchased from the Central Laboratory of Animal Science, the Fourth Military Medical University, China. Then, 200  $\mu$ L of Hep3B cell suspension with approximately  $3\times 10^6$  cells was subcutaneously injected. The drug treatments were initiated at four days after the cell injections. These animals were administered with saline or berberine (20 mg/kg, intraperitoneally) daily. The tumor size and body weight were measured every four days, and tumor volume was calculated using the equation: volume =  $\frac{1}{2}$  (length  $\times$  width<sup>2</sup>). Mice were sacrificed at 20 days after implantation. All experimental procedures that involved mice were performed in accordance to the Guide for the Care and Use of Laboratory Animals, and were approved by The Research Animal Care and Use Committee of the Fourth Military Medical University.

### Immunohistochemistry

The slide was deparaffinized, hydrated, and boiled in 10 mM of citrate buffer (pH 6.0) for antigen retrieval. Then, the slide was incubated in 3% hydrogen peroxide for 10 mins to inactivate the endogenous peroxidases. Afterwards, the slide was blocked by 5% normal goat serum at room temperature for one hour. Next, the tissue sections were incubated with a primary antibody at  $4^{\circ}\text{C}$  overnight. After washing with PBS, the slide was incubated with a biotinylated secondary antibody at room temperature for one hour, and stained with diaminobenzidine for 10 mins. The, hematoxylin was used to counter-stain the sections.

## Evaluation of the Immunohistochemical (IHC) Staining

A total of 32 paraffin-embedded HCC samples and their corresponding adjacent liver tissues were used. These tissues were stained by immunohistochemistry. Multiple tissue arrays were independently scored by a pathologist, who was blinded to the clinical information, using an immunoreactivity score system based on the proportion and intensity of the stained cancer cells. The standard used: (1) the number of cells with positive staining (<5%: 0; 6–25%: 1; 26–50%: 2; 51–75%: 3; >75%: 4) and (2) the staining intensity (colorless: 0; pallide-flavens: 1; yellow: 2; brown: 3). The score in (1) and (2) was multiplied, and the mean score of five fields was calculated.

## Statistical Analysis

Data were presented as the mean  $\pm$  standard deviation (SD) of at least three independent experiments or multiple independent mice, as indicated. The Kaplan-Meier survival data were analyzed using the log rank test. GraphPad Prism 7.0 (GraphPad software, La Jolla, CA, USA) was used to evaluate the statistical significance through Student's *t*-test. These results were considered statistically significant when  $P < 0.05$ .

## Results

### Berberine Inhibits the Proliferation of Hep3B and BEL-7404 Cells in vitro

Berberine at 50, 75, 100 and 125  $\mu$ M significantly inhibited the viability of Hep3B and BEL-7404 cells after 48 hrs (Figure 1A). In order to confirm the time effect, cancer cells were treated with berberine for 12, 24 and 48 hrs. The CCK-8 assay revealed that the inhibitory effect of berberine was enhanced after prolonging the treatment time (Figure 1B). Furthermore, colony formation assay was performed to strengthen this conclusion. The formation of Hep3B and BEL-7404 cells was significantly reduced by the berberine treatment (Figure 1C). The EdU assay also revealed that berberine suppressed the growth of liver cancer cell lines (Figure 1D). All these above results indicate that berberine inhibits Hep3B and BEL-7404 cell proliferation in a dose- and time-dependent manner.

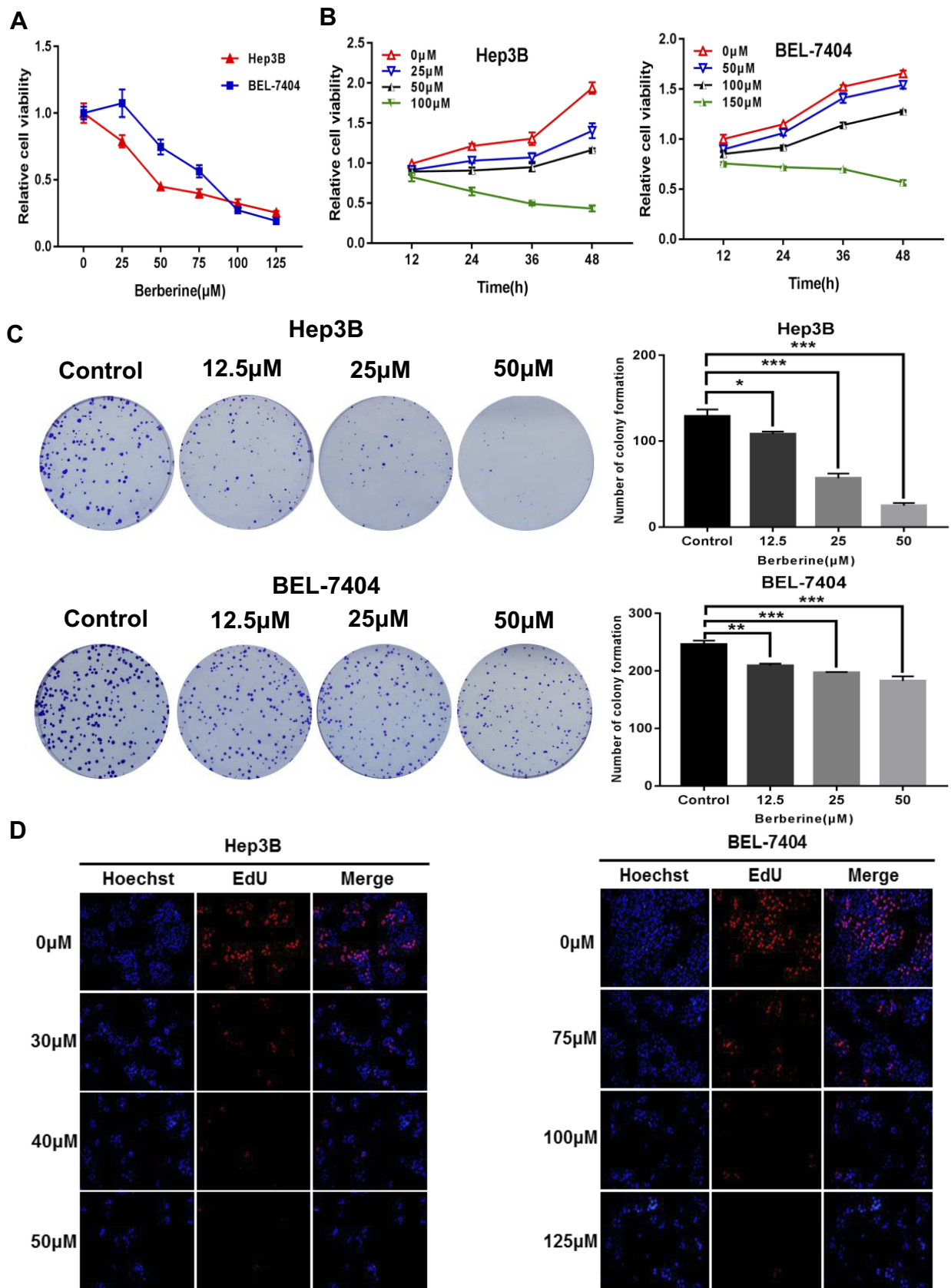
### Berberine Suppresses Glutamine Uptake by Inhibiting SLC1A5

Cancer cells undergo specific metabolic reprogramming to sustain cell proliferation. In order to observe the effects of

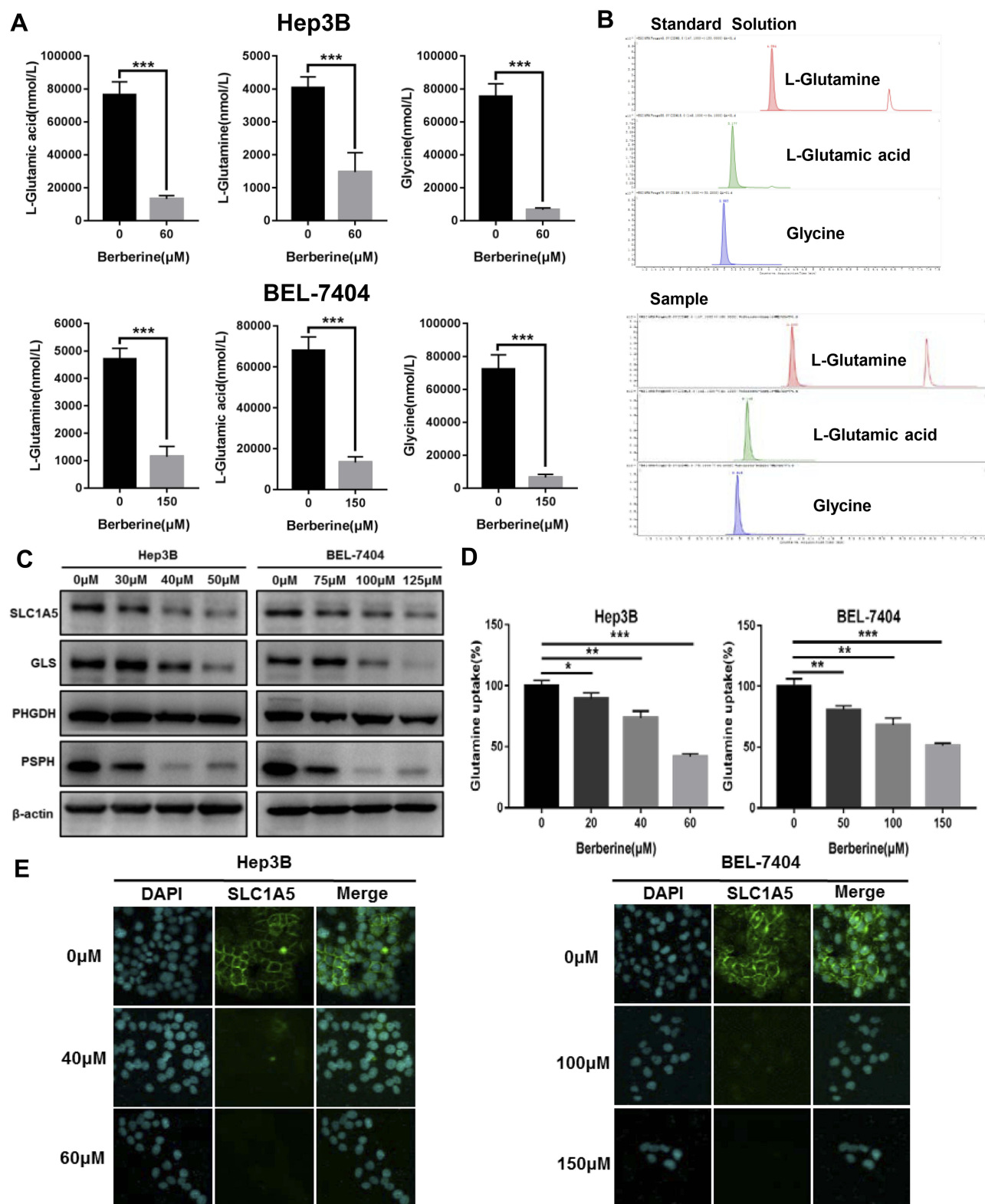
berberine on tumor amino acid metabolism, UHPLC-MS/MS analysis was performed to determine the concentration of glutamine, glutamate and glycine in berberine-treated Hep3B and BEL-7404 cells (Figure 2A and B). It was found that the concentration of glutamine, glutamate and glycine significantly decreased in berberine-treated cancer cells, when compared with vehicle-treated cells ( $P < 0.001$ , Figure 2A). In order to explore the mechanisms whereby berberine influences the contents of glutamine, glutamate and glycine, the expression of amino acid metabolism-related proteins (SLC1A5, GLS, PHGDH and PSPH) were measured by Western blot. The expression of SLC1A5, GLS and PSPH decreased, and such decrease was enhanced with the increase in berberine dose (Figure 2C). Since transport is the former procedure, and SLC1A5 is the transporter of glutamine, focus was given on the effect of berberine on SLC1A5 and glutamine uptake. The glutamine concentration in the cell culture medium was detected by ELISA, and glutamine uptake rates were calculated. The results indicated that berberine decreased the rates of glutamine uptake in a dose-dependent manner (Figure 2D). In accordance with the Western blot results, the immunofluorescent images revealed that the SLC1A5 expression was downregulated by berberine (Figure 2E).

### The Upregulation of SLC1A5 Leads to Increased Glutamine Uptake and Improves Tolerance to Berberine

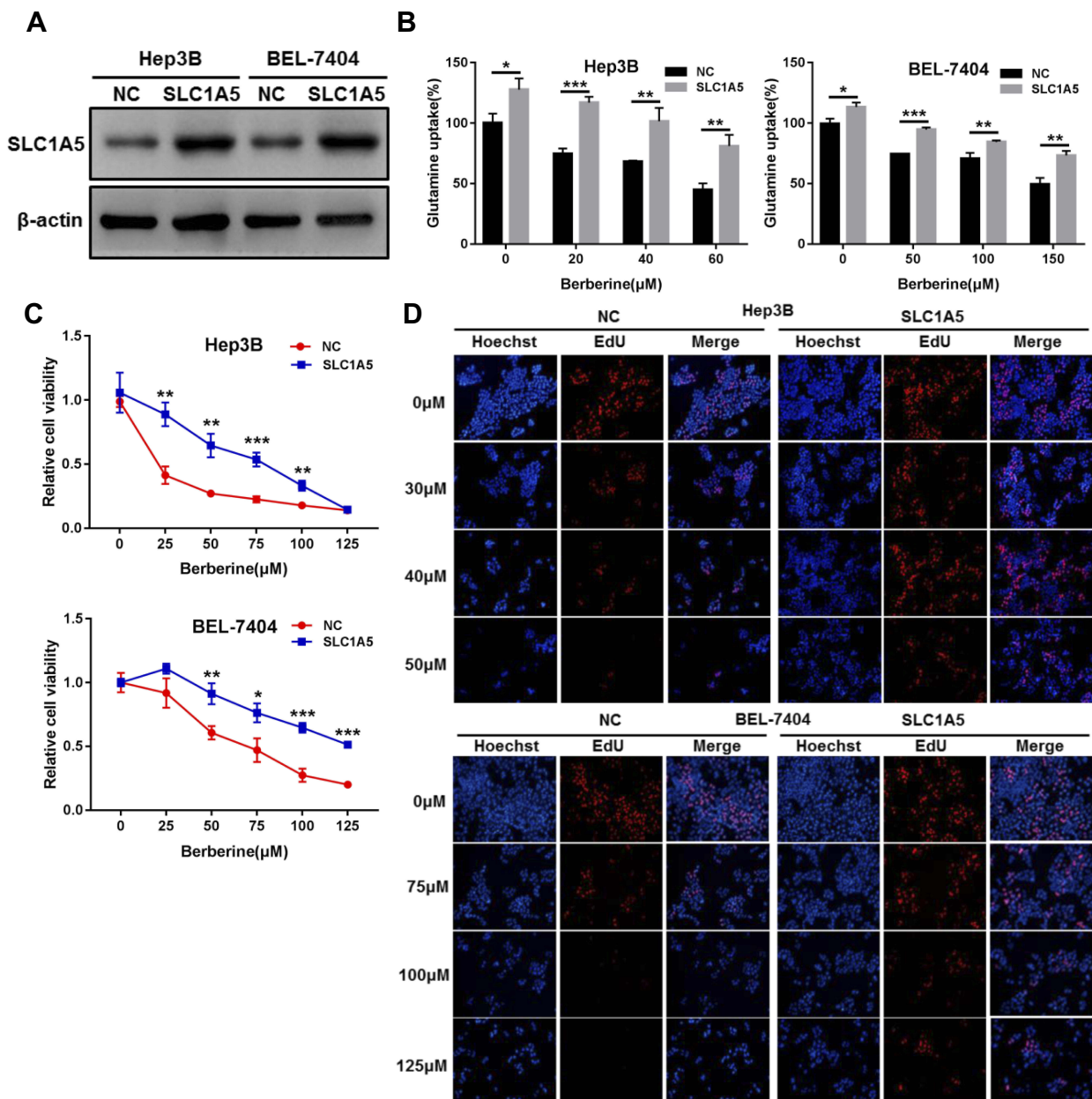
Since these above observations indicated berberine as an inhibitor of SLC1A5, it was determined whether the overexpression of SLC1A5 could improve the proliferation in the condition of berberine. Hep3B and BEL-7404 cells were transfected with lentiviral plasmids encoding SLC1A5 or a negative control. The Western blot revealed that SLC1A5 was successfully upregulated (Figure 3A). Next, it was determined whether the upregulation of SLC1A5 could increase the glutamine uptake rates. Compared with the NC group, glutamine uptake improved with the upregulation of SLC1A5 (Figure 3B). The CCK-8 assay suggested that SLC1A5 upregulation also ameliorated the inhibitory effect on cell viability induced by berberine treatment (Figure 3C). In order to confirm this observation, EdU assay was performed, and it was revealed that the overexpression of SLC1A5 could reverse the berberine-induced inhibition of proliferation to some extent (Figure 3D). Taken together, SLC1A5 upregulation increases glutamine uptake and improves the tolerance to berberine.



**Figure 1** Berberine inhibited the viability of hepatocellular carcinoma cells. **(A)** The effect of different concentrations of berberine on the viability of Hep3B and BEL-7404 cells after 48 hrs. **(B)** The effect of different treatment times of berberine on the viability of Hep3B and BEL-7404. **(C)** The effect of different doses of berberine on the colony formation of Hep3B and BEL-7404 cells. **(D)** The EdU staining of Hep3B and BEL-7404 after berberine treatment for 48 hrs. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Figure 2** Berberine suppressed the glutamine uptake and inhibited the SLC1A5 expression. **(A)** The concentration of glutamine, glutamate and glycine in Hep3B and BEL-7404 cells after treatment with berberine for 48 hrs. **(B)** The extracted ion chromatographs (EICs) from a standard solution, and a sample of the targeted analytes under optimal conditions. **(C)** The SLC1A5, GLS, PHGDH and PSPH expression in berberine-treated Hep3B and BEL-7404 cells. **(D)** The glutamine uptake rates in Hep3B and BEL-7404 cells after treatment with berberine for 48 hrs. **(E)** The immunofluorescent images depicting the SLC1A5 expression in berberine-treated Hep3B and BEL-7404 cells. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

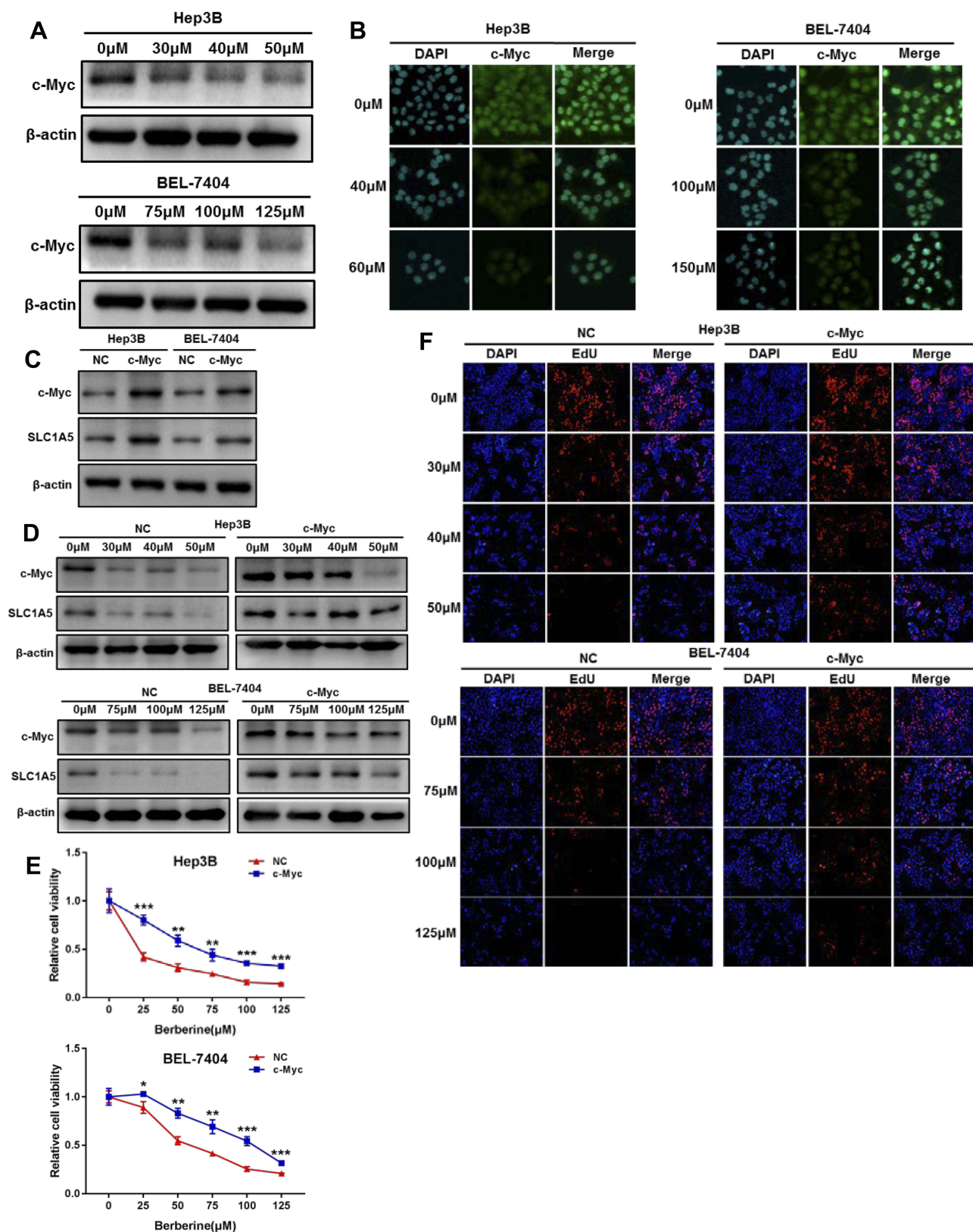


**Figure 3** The SLC1A5 upregulation increased the glutamine uptake and improved berberine tolerance. **(A)** The SLC1A5 expression in Hep3B and BEL-7404 cells transduced with the SLC1A5-lentivirus or a negative control was analyzed by Western blot. **(B)** The glutamine uptake rates in the SLC1A5 group and NC group. **(C)** The effect of the transfection with SLC1A5 on cell viability in the condition of berberine treatment. **(D)** The EdU staining in the SLC1A5 group and NC group after berberine treatment for 48 hrs. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

## Berberine Suppresses SLC1A5 Expression by Inhibiting c-Myc

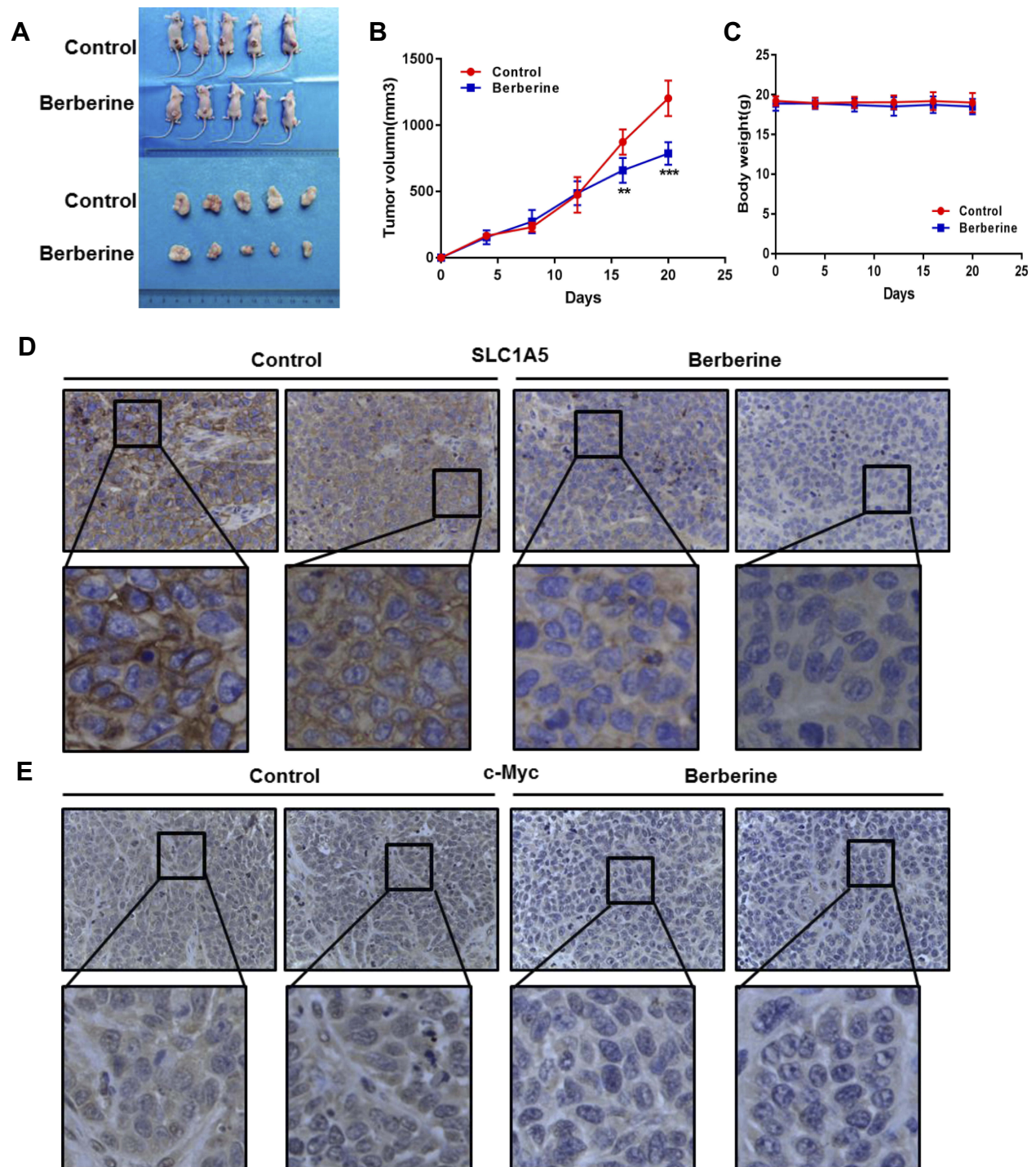
A recent study reported that SLC1A5 is regulated by c-Myc, and the ChIP-seq data revealed that Myc binds with SLC1A5 in the promoter region.<sup>16</sup> In order to better understand the molecular mechanism of the berberine inhibitory effect on SLC1A5, it was determined

whether berberine could influence the expression of c-Myc. The Western blot results revealed that c-Myc expression was downregulated by berberine in a dose-dependent manner (Figure 4A), and this was supported by the immunofluorescent analysis (Figure 4B). The Hep3B and BEL-7404 cells transfected with the c-Myc-lentivirus exhibited a relatively higher expression of



**Figure 4** Berberine suppressed c-Myc, and the upregulation of c-Myc reversed the SLC1A5 inhibition of berberine. (A) The c-Myc expression in berberine-treated Hep3B and BEL-7404 cells. (B) The immunofluorescent images depicting the c-Myc expression in berberine-treated Hep3B and BEL-7404 cells. (C) The c-Myc and SLC1A5 expression in Hep3B and BEL-7404 cells transfected with the c-Myc-lentivirus or a negative control for 48 hrs was analyzed by Western blot. (D) The Hep3B and BEL-7404 cells transfected with c-Myc-lentivirus or a negative control after gradient berberine treatment for 48 hrs was analyzed by Western blot. (E) The upregulation of c-Myc enhancing berberine tolerance (F) The EdU staining in the c-Myc group and NC group after berberine treatment for 48 hrs. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

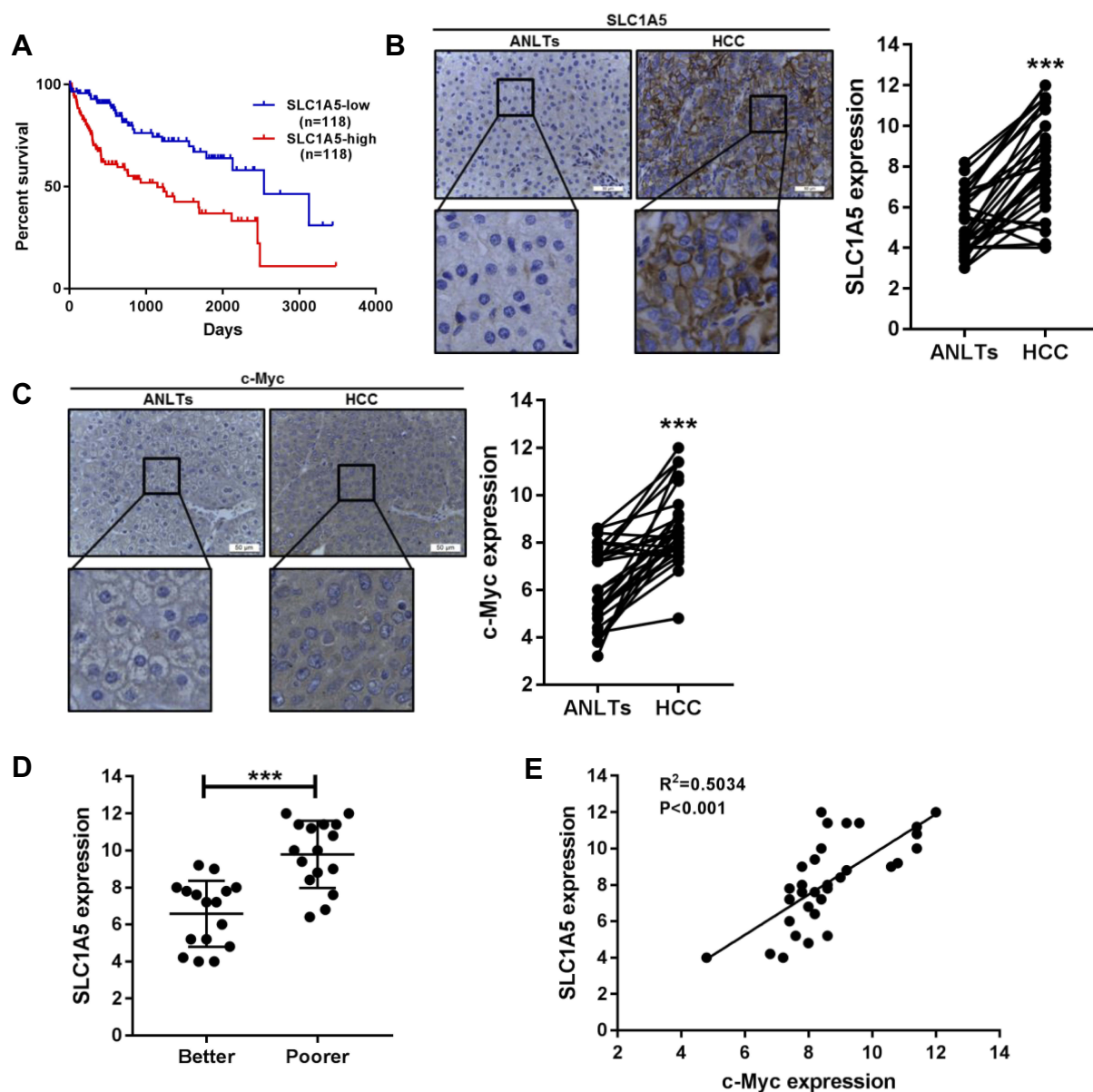




**Figure 5** Berberine inhibited the growth of tumor xenografts in vivo. (A) An image of nude mice that were sacrificed to form the xenograft tumor model by subcutaneously injecting Hep3B cells. (B) The growth curve of tumor volumes in the berberine-treated group and control group. (C) The analysis of body weight alterations. (D) The representative immunohistochemical staining of SLC1A5. (E) The representative immunohistochemical staining of c-Myc. \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

c-Myc and SLC1A5 (Figure 4C). The upregulation of c-Myc reversed the inhibitory effect of berberine on SLC1A5 expression (Figure 4D) and cancer cell prolif-

eration (Figure 4E and F). Overall, the present data indicates that berberine suppresses SLC1A5 expression by inhibiting c-Myc.



**Figure 6** The overexpression of SLC1A5 was associated with worse clinical outcome in HCC. **(A)** The comparison of the survival of patients with high SLC1A5 expression (SLC1A5-High, n=118) and low SLC1A5 expression (SLC1A5-Low, n=118) using data obtained from The Cancer Genome Atlas (TCGA). **(B)** The SLC1A5 expression in HCC tissues and adjacent non-tumor liver tissues (ANLTs). **(C)** The c-Myc expression in HCC tissues and ANLTs. **(D)** The SLC1A5 expression levels assessed in human HCC tissues characterized by better or poorer prognosis. **(E)** The Pearson's correlation coefficient between c-Myc and SLC1A5. \*\*\* $P < 0.001$ .

## Berberine Suppresses the in vivo Growth of Tumor Xenografts and Expression of SLC1A5 and c-Myc

In order to further determine the effect of berberine on HCC cell growth in vivo, Hep3B cells were subcutaneously injected into the nude mice to form xenograft tumors (Figure 5A). The administration of 20 mg/kg of berberine revealed significant anti-tumor effect in the Hep3B cell xenograft model (Figure 5A and B). Furthermore, the tumor volume of mice in the berberine-treated group was markedly

lower than that of mice in the control group. Drug-associated cytotoxicity was monitored by analyzing the changes in body weight. Compared with control group mice, the berberine treatment did not significantly alter the body weight, suggesting that the drug cytotoxicity was tolerable (Figure 5C). In order to determine whether berberine could downregulate the expression of SLC1A5 and c-Myc in vivo, immunohistochemistry staining was performed. The protein expression of SLC1A5 and c-Myc decreased in tumor tissues after berberine treatment (Figure 5D and E). These results show

that berberine could impair the growth of HCC cells, and suppress SLC1A5 and c-Myc expression *in vivo*.

## The Relatively High Expression of SLC1A5 Is Associated with Poor Prognosis

In order to explore the clinical significance of the expression of SLC1A5, the RNA-Seq data obtained from The Cancer Genome Atlas (TCGA) Project was analyzed. The Kaplan-Meier survival analysis revealed that HCC patients with low SLC1A5 expression exhibited prolonged survival (Figure 6A). Furthermore, the SLC1A5 protein expression in tissues obtained from HCC patients was further detected by immunohistochemistry. The expression of SLC1A5 and c-Myc were higher in HCC tissues, when compared to that in adjacent non-tumor liver tissues (Figure 6B and C). HCC tissues obtained from patients with poorer prognosis exhibited a relatively higher SLC1A5 expression (Figure 6D). Furthermore, a correlation study determined that SLC1A5 expression was positively correlated with c-Myc expression in these cancer tissue samples (Figure 6E). The SLC1A5 overexpression in HCC tissues and its association with poor prognosis suggests that berberine, as an inhibitor of SLC1A5, may have anti-tumor potential in clinic.

## Discussion

Glutamine is the most abundant amino acid in the circulation,<sup>17</sup> which makes it easy to take in for rapidly proliferating cells, especially for cancer cells. Glutamine plays an important role in cancer cell energy generation. After entering the cell, glutamine is converted by mitochondrial glutaminases (GLS) to glutamate, and glutamate can be converted to  $\alpha$ -ketoglutarate, which enters the TCA cycle to generate ATP.<sup>18</sup> Accompanied by ATP generation, the mitochondrial electron transport chain generates superoxide. The hypermetabolism in tumor cells induces an elevated reactive oxygen species (ROS) level, which could seriously damage the biomacromolecule.<sup>19</sup> Although increased glutamine oxidation correlates with increased ROS production,<sup>20</sup> glutamine metabolic pathways have an essential effect in controlling the ROS levels. The most well-known pathway is through the synthesis of glutathione. The tri-peptide glutathione eliminates peroxide free radicals, and glutamine uptake is the speed limit step for the synthesis of glutathione.<sup>21</sup> Glutamine can be converted to other amino acids through transamination, supporting the levels of many amino acid pools in cells.<sup>22</sup>

Tracer experiments have revealed that half of non-essential amino acids in the protein synthesis by cancer cells can be derived from glutamine.<sup>23</sup> This glutamine addiction has made it an attractive anti-cancer therapeutic target. Many compounds that target glutamine metabolism have been studied. However, glutamine mimic and glutamine depletion drugs have been limited by their toxicity,<sup>24,25</sup> and the inhibitors of glutamine transporter SLC1A5 and GLS have shown promise in preclinical models of cancer.<sup>26,27</sup> Recent studies have shown that decreased SLC1A5 expression led to lower glutamine uptake.<sup>28</sup> The anti-SLC1A5 monoclonal antibody could induce oxidative stress and suppress cancer growth.<sup>29</sup> The present study revealed that berberine could suppress cancer cell proliferation, as a SLC1A5 inhibitor *in vitro* and *in vivo*. However, different from other SLC1A5 inhibitors, berberine could also reduce the GLS expression, which suggests that berberine can act as a multiple-target drug that interferes in both the transport and conversion of glutamine. To our surprise, it was found berberine could inhibit the expression of PSPH, which is essential for serine synthesis.<sup>30</sup> Since serine can be converted to glycine, this might explain the decreased level of glycine in berberine-treated cells, to some extent.

In the present study, an attempt was made to illuminate the mechanism by which berberine inhibits the SLC1A5 expression. MYC proteins have been proven to be implicated in tumorigenesis.<sup>31</sup> Their expression is deregulated and enhanced via multiple mechanisms in tumor cells.<sup>32</sup> In recent years, MYC was found to upregulate glutamine transporters<sup>33</sup> and c-Myc can bind to SLC1A5 in the promoter-region.<sup>16</sup> Thus, it was assumed that the inhibition of SLC1A5 is dependent of c-Myc. Consistent with several studies in other tumors,<sup>13,34</sup> the present study demonstrated that berberine could suppress c-Myc expression in liver cancer cells. When c-Myc was upregulated, the expression of SLC1A5 increased, and cancer cells exhibited a significant tolerance to berberine treatment. These results suggest that berberine decrease SLC1A5 expression by suppressing c-Myc.

It was reported that berberine could inhibit liver cancer cell invasion without cytotoxicity in healthy hepatocytes.<sup>35</sup> The present study revealed the high expression of SLC1A5 in cancer tissues and its relative low expression in adjacent non-tumor liver tissues. This difference in expression could provide berberine with a specific anti-tumor property, and may explain the low cytotoxicity in healthy hepatocytes. It was also found

that the high expression of SLC1A5 was associated with poor prognosis, which suggests that berberine might benefit patients with advanced liver cancer. However, pharmacokinetic studies have shown that berberine is poorly absorbed, and it is difficult to maintain the plasma concentration after oral administration.<sup>36</sup> Recent studies in designing carriers may help to solve this problem, in which silver nanoparticles, zinc oxide nanoparticles and nanostructured lipid were used to carry berberine.<sup>37</sup> Nevertheless, more studies are definitely needed to translate these preclinical data into clinical practice.

## Conclusion

Berberine suppresses the proliferation of liver cancer cells by reducing SLC1A5 expression and glutamine uptake. Berberine might have clinical potential as an anti-tumor drug.

## Data Sharing Statement

The datasets analyzed in the study are available at [http://www.oncolnc.org/kaplan/?lower=33&upper=33&cancer=LIHC&gene\\_id=6510&raw=SLC1A5&species=mRNA](http://www.oncolnc.org/kaplan/?lower=33&upper=33&cancer=LIHC&gene_id=6510&raw=SLC1A5&species=mRNA).

## Acknowledgment

This study was supported by the National Natural Science Foundation of China (81702329 and 2016YFC0905902).

## Disclosure

The authors report no conflicts of interest in this work

## References

- Grandhi MS, Kim AK, Ronnekleiv-Kelly SM, et al. Hepatocellular carcinoma: from diagnosis to treatment. *Surg Oncol*. 2016;25:74–85. doi:10.1016/j.suronc.2016.03.002
- Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet*. 2018;391:1301–1314. doi:10.1016/S0140-6736(18)30010-2
- Couri T, Pillai A. Goals and targets for personalized therapy for HCC. *Hepatol Int*. 2019;13:125–137. doi:10.1007/s12072-018-9919-1
- Eggert T, Greten TF. Current standard and future perspectives in non-surgical therapy for hepatocellular carcinoma. *Digestion*. 2017;96:1–4. doi:10.1159/000464282
- Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. *Nat Cell Biol*. 2015;17:351–359. doi:10.1038/ncb3124
- Zhang J, Pavlova NN, Thompson CB. Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. *EMBO J*. 2017;36:1302–1315. doi:10.15252/embj.201696151
- Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. *Nat Rev Drug Discov*. 2013;12:931–947. doi:10.1038/nrd4002
- Bhutia YD, Ganapathy V. Glutamine transporters in mammalian cells and their functions in physiology and cancer. *Biochim Biophys Acta*. 2016;1863:2531–2539. doi:10.1016/j.bbamcr.2015.12.017
- Scalise M, Pochini L, Console L, et al. The human SLC1A5 (ASCT2) amino acid transporter: from function to structure and role in cell biology. *Front Cell Dev Biol*. 2018;6:96. doi:10.3389/fcell.2018.00096
- Garaeva AA, Oostergetel GT, Gati C, et al. Cryo-EM structure of the human neutral amino acid transporter ASCT2. *Nat Struct Mol Biol*. 2018;25:515–521. doi:10.1038/s41594-018-0076-y
- McCracken AN, Edinger AL. Nutrient transporters: the Achilles' heel of anabolism. *Trends Endocrinol Metab*. 2013;24:200–208. doi:10.1016/j.tem.2013.01.002
- Wang K, Feng X, Chai L, et al. The metabolism of berberine and its contribution to the pharmacological effects. *Drug Metab Rev*. 2017;49:139–157. doi:10.1080/03602532.2017.1306544
- Wu YY, Li TM, Zang LQ, et al. Effects of berberine on tumor growth and intestinal permeability in HCT116 tumor-bearing mice using polyamines as targets. *Biomed Pharmacother*. 2018;107:1447–1453. doi:10.1016/j.biopha.2018.08.130
- Wang Y, Zhang S. Berberine suppresses growth and metastasis of endometrial cancer cells via miR-101/COX-2. *Biomed Pharmacother*. 2018;103:1287–1293. doi:10.1016/j.biopha.2018.04.161
- Lin YS, Chiu YC, Tsai YH, et al. Different mechanisms involved in the berberine-induced antiproliferation effects in triple-negative breast cancer cell lines. *J Cell Biochem*. 2019;120:13531–13544. doi:10.1002/jcb.28628
- Liu P, Ge M, Hu J, et al. A functional mammalian target of rapamycin complex 1 signaling is indispensable for c-Myc-driven hepatocarcinogenesis. *Hepatology*. 2017;66:167–181. doi:10.1002/hep.v66.1
- Mayers JR, Vander Heiden MG. Famine versus feast: understanding the metabolism of tumors in vivo. *Trends Biochem Sci*. 2015;40:130–140. doi:10.1016/j.tibs.2015.01.004
- Moreadith RW, Lehninger AL. The pathways of glutamate and glutamine oxidation by tumor cell mitochondria. Role of mitochondrial NAD(P)<sup>+</sup>-dependent malic enzyme. *J Biol Chem*. 1984;259:6215–6221.
- Hamanaka RB, Chandel NS. Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. *Trends Biochem Sci*. 2010;35:505–513. doi:10.1016/j.tibs.2010.04.002
- Weinberg F, Hamanaka R, Wheaton WW, et al. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. *Proc Natl Acad Sci U S A*. 2010;107:8788–8793. doi:10.1073/pnas.1003428107
- Welbourne TC. Ammonia production and glutamine incorporation into glutathione in the functioning rat kidney. *Can J Biochem*. 1979;57:233–237. doi:10.1139/o79-029
- Hosios AM, Hecht VC, Danai LV, et al. Amino acids rather than glucose account for the majority of cell mass in proliferating mammalian cells. *Dev Cell*. 2016;36:540–549. doi:10.1016/j.devcel.2016.02.012
- Alberghina L, Gaglio D. Redox control of glutamine utilization in cancer. *Cell Death Dis*. 2014;5:e1561. doi:10.1038/cddis.2014.513
- Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. *Trends Biochem Sci*. 2010;35:427–433. doi:10.1016/j.tibs.2010.05.003
- Earhart RH, Koeller JM, Davis HL. Phase I trial of 6-diazo-5-oxo-L-norleucine (DON) administered by 5-day courses. *Cancer Treat Rep*. 1982;66:1215–1217.
- Wang Q, Beaumont KA, Otte NJ, et al. Targeting glutamine transport to suppress melanoma cell growth. *Int J Cancer*. 2014;135:1060–1071. doi:10.1002/ijc.v135.5
- Jacque N, Ronchetti AM, Larrue C, et al. Targeting glutaminolysis has antileukemic activity in acute myeloid leukemia and synergizes with BCL-2 inhibition. *Blood*. 2015;126:1346–1356. doi:10.1182/blood-2015-01-621870
- Zhao X, Petrashen AP, Sanders JA, et al. 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*. 2019;18:e12947. doi:10.1111/acel.12947

29. Osanai-Sasakawa A, Hosomi K, Sumitomo Y, 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.* 2018;8:1499–1513.
30. Amelio I, Cutruzzola F, Antonov A, et al. Serine and glycine metabolism in cancer. *Trends Biochem Sci.* 2014;39:191–198. doi:10.1016/j.tibs.2014.02.004
31. Kress TR, Sabo A, Amati B. MYC: connecting selective transcriptional control to global RNA production. *Nat Rev Cancer.* 2015;15:593–607. doi:10.1038/nrc3984
32. Dejure FR, Eilers M. MYC and tumor metabolism: chicken and egg. *EMBO J.* 2017;36:3409–3420. doi:10.15252/embj.201796438
33. Wise DR, DeBerardinis RJ, 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;105:18782–18787. doi:10.1073/pnas.0810199105
34. Zhang R, Qiao H, Chen S, et al. Berberine reverses lapatinib resistance of HER2-positive breast cancer cells by increasing the level of ROS. *Cancer Biol Ther.* 2016;17:925–934. doi:10.1080/15384047.2016.1210728
35. Liu B, Wang G, Yang J, et al. Berberine inhibits human hepatoma cell invasion without cytotoxicity in healthy hepatocytes. *PLoS One.* 2011;6:e21416. doi:10.1371/journal.pone.0021416
36. Ye M, Fu S, Pi R, et al. Neuropharmacological and pharmacokinetic properties of berberine: a review of recent research. *J Pharm Pharmacol.* 2009;61:831–837. doi:10.1211/jpp.61.07.0001
37. Farooqi AA, Qureshi MZ, Khalid S, et al. Regulation of cell signaling pathways by berberine in different cancers: searching for missing pieces of an incomplete jig-saw puzzle for an effective cancer therapy. *Cancers (Basel).* 2019;11:478. doi:10.3390/cancers11040478

## OncoTargets and Therapy

Dovepress

### Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic

agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/oncotargets-and-therapy-journal>