

SLC25A10 performs an oncogenic role in human osteosarcoma

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Abstract. Osteosarcoma is one of the most common primary malignant bone tumors in adolescents. It is associated with high risk of relapse and the outcomes of patients with high-grade osteosarcoma remain poor. Therefore, additional studies investigating the molecular mechanisms involved in tumor initiation, growth, migration and invasion of osteosarcoma are necessary. In the present study, the protein levels of solute carrier family 25 member 10 (SLC25A10) were increased in osteosarcoma tissue, compared with normal bone tissue. In patients with osteosarcoma, high expression levels of SLC25A10 were associated with poor clinicopathological parameters, including metastasis, clinical Enneking stage, relapse-free survival and overall survival rates. Short hairpin RNA knockdown of SLC25A10 significantly suppressed cell proliferation as determined by cell counting, MTT assay and cell colony formation assays. In addition, SLC25A10 knockdown caused an increase in apoptosis and a decrease in mitosis in osteosarcoma cells. Cyclin E1 (CCNE1) was positively regulated by SLC25A10, while P21 and P27 were negatively regulated by SLC25A10. Therefore, SLC25A10 may play an oncogenic role in human osteosarcoma, which could be mediated by CCNE1, P21 and P27.

Introduction

Osteosarcoma mainly arises from human mesenchymal cells and is one of the most common primary malignant bone tumors (1-3). The incidence of osteosarcoma in adolescents is relatively high (4,5). The main treatment option for low-grade osteosarcoma is surgery alone, and the prognosis for patients with low-grade osteosarcoma is relatively good. High-grade osteosarcoma or osteosarcoma with metastasis can be treated with surgery and chemotherapy; however, the mean 5-year survival rate is <25% (1,6,7). Although the treatment for osteosarcoma has improved, few effective methods are available (8,9). Research has focused on the mechanisms involved in the initiation and development of osteosarcoma, yet the intrinsic mechanisms involved in the malignant behaviors of osteosarcoma remain unclear. Further studies are needed to improve the understanding of the molecular mechanisms involved in osteosarcoma initiation, growth, migration and invasion, which could help the development of novel treatments for osteosarcoma.

Solute carrier 25 family member 10 (SLC25A10), also known as dicarboxylate carrier, is an important regulator of human energy metabolism and redox homeostasis (10,11). SLC25A10 contains six transmembrane regions and three cognate repeats, and is localized in the cytoplasm and the inner mitochondrial membrane (12,13). SLC25A10 transports dicarboxylate substrates and supplies substrates for several pathways, including sulfur metabolism and gluconeogenesis (10,14). SLC25A10 is involved in fatty acid synthesis, glucose-stimulated insulin secretion and additional physiological processes (12,15,16). Moreover, Zhou *et al* (10) suggested that knockdown of SLC25A10 in human cancer cells markedly decreased cell growth and increased sensitivity to anticancer drugs, demonstrating its role as an oncogene. However, the role of SLC25A10 in different types of human cancer, including osteosarcoma, remains unclear. Therefore, further studies focusing on osteosarcoma are required.

The present study demonstrated that the expression levels of SLC25A10 were higher in human osteosarcoma tissues, compared with normal bone tissues. In addition, in patients with osteosarcoma, the expression levels of SLC25A10 were positively associated with tumor metastasis, clinical Enneking stage, poor relapse-free survival (RFS) and overall survival

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Abbreviations: SLC25A10, solute carrier 25 family member 10; RFS, relapse-free survival; OS, overall survival; shRNA, short hairpin RNA; PARP, poly ADP-ribose polymerase

Key words: SLC25A10, proliferation, apoptosis, mitosis, osteosarcoma

(OS) rates. Knockdown of SLC25A10 with short hairpin RNA (shRNA) significantly decreased cell proliferation, increased cell apoptosis and suppressed cell mitosis in osteosarcoma cells. Moreover, cyclin E1 (CCNE1) was positively regulated by SLC25A10, while P21/P27 were negatively regulated by SLC25A10. CCNE1 was previously described as an important tumor promoter in many types of human cancer, and P21/P27 were found to be tumor suppressors in many human cancer types (17-21). Collectively, CCNE1, P21 and P27 may mediate the oncogenic role of SLC25A10 in human osteosarcoma cells.

Materials and methods

Clinical osteosarcoma and normal bone samples. In total, 60 osteosarcoma tissues and 60 normal bone tissues were collected in The Department of Orthopedics and The Department of Pathology in The First Affiliated Hospital of Anhui Medical University. These tissues were collected from patients with osteosarcoma or bone diseases who underwent resection in The First Affiliated Hospital of Anhui Medical University between January 2011 and December 2013. These osteosarcoma tissues and normal bone tissues were not from the same patients. The clinicopathological features of the enrolled patients with osteosarcoma were collected from The Department of Pathology, The First Affiliated Hospital of Anhui Medical University. The 60 patients with osteosarcoma were followed-up for >5 years, and the RFS and OS rates were determined. Ethical approval from The Institutional Review Boards of Anhui Medical University was obtained prior to the study. All experiments involving human patients were performed according to The Code of Ethics of The World Medical Association (Declaration of Helsinki). Informed consent was obtained from all patients involved in the present study.

Immunohistochemistry. The protein levels of SLC25A10 in 4- μ m thick paraffin sections of osteosarcoma tissues and normal bone tissues (10% formalin fixed at room temperature for 24 h) were detected by immunohistochemistry, as previously described (22,23). Sections were deparaffinized in xylene, rehydrated in a series of ethanol solutions (100, 100, 95, 85 and 75%) and heated in 0.01 M sodium citrate buffer at 100°C for 10 min for antigen retrieval. Sections were incubated with 3% hydrogen peroxide incubation at room temperature for 10 min, and then incubated with primary antibody [SLC25A10 rabbit polyclonal antibody (1:200; 12086-1-AP; ProteinTech Group, Inc.)] for 3 h at room temperature, followed by incubation for 15 min at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibody (1:1; MaxVision-HRP, KIT-5030; Fuzhou Maixin Biotech Co., Ltd.) 3,3'-diaminobenzidine tetrahydrochloride (Fuzhou Maixin Biotech Co., Ltd.) was used for visualization. Sections with <10% positive stained cells were considered as SLC25A10-negative, and sections with \geq 10% positive stained cells were considered to be SLC25A10-positive using a light microscope (Olympus Corporation) at x20 magnification.

Cells and cell culture. The human MG-63 and U2OS osteosarcoma cell lines (both from American Type Culture Collection) were used in the present study. MG-63 and U2OS cells were cultured using DMEM medium (Gibco; Thermo Fisher

Scientific, Inc.) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) in humidified conditions at 37°C and 5% CO₂.

shRNA transfection. shRNAs targeting SLC25A10 (shSLC25A10-1 and shSLC25A10-2) and control shRNA (shCtrl) were used for cell functional experiments. All of the shRNAs were obtained from Shanghai GenePharma Co., Ltd. Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used for shRNA transfection (75 pmol/transfection), which was performed as previously described (22,23). The shRNA sequences were as follows: shSLC25A10-1, 5'-GTT TAGCTGGAGGCTTCGTGG-3'; shSLC25A10-2, 5'-CAA GCAGCTGGTCCCTAGCAC-3'; and shCtrl, 5'-TCAAGC TGCTAGGCCTATCCG-3'.

Western blotting. Western blotting experiments were performed to detect the protein expression levels of SLC25A10, CCNE1, P21 and P27 in human osteosarcoma cells. Western blot analysis was carried out as previously described (22,23) and β -actin was used as a control. Polyvinylidene difluoride-membranes (EMD Millipore) were used for electrotransfer. Membranes were blocked using incubation with 5% (w/v) non-fat milk powder at room temperature for 2 h. Membranes were incubated with respective antibodies (rabbit polyclonal antibodies anti-SLC25A10 (1:1,000; 12086-1-AP; ProteinTech Group, Inc.), anti-CCNE1 (1:1,000; 11554-1-AP; ProteinTech Group, Inc.), anti-P21 (1:1,000; 10355-1-AP; ProteinTech Group, Inc.), anti-P27 (1:1,000; 25614-1-AP; ProteinTech Group, Inc.), rabbit polyclonal antibody anti-caspase-3 (1:500; #9662; Cell Signaling Technology, Inc.), rabbit polyclonal anti-poly ADP-ribose polymerase (PARP; 1:500; #9542; Cell Signaling Technology, Inc.) and rabbit polyclonal antibody anti- β -actin (1:1,000; 20536-1-AP; ProteinTech Group, Inc.) overnight at 4°C. Then, then membranes were incubated with goat anti-rabbit IgG (H+L) HRP-conjugated secondary antibody (1:50,000; 31460; Invitrogen; Thermo Fisher Scientific, Inc.) for 2 h at room temperature. Femto (34095; Thermo Fisher Scientific, Inc.) and Pico (34077; Thermo Fisher Scientific, Inc.) were used for visualization. Protein bands were identified using Image Quant LAS 4000 (Cytiva) and analyzed using ImageJ version 1.8.0 software (National Institutes of Health).

Cell proliferation assay. Cell counting, MTT and cell colony formation assays were performed to evaluate the role of SLC25A10 in the proliferation of human osteosarcoma cells, as previously described (22,23). MG-63 and U2OS cells were transfected with shRNAs, and collected after 24 h. In the cell counting assay, 10,000 cells/well were seeded into six-well plates, and the total cell number was calculated every day during a period of 5 days. In the MTT assay, 2,000 cells/well were seeded into 96-well plates, and the MTT detection was performed for 5 days (the purple formazan was dissolved in DMSO, and the absorbance was measured at 570 nm). In the cell colony formation assay, 1,000 cells/well were seeded into six-well plates, and cell colony formation was examined after 10 days.

Flow cytometry. After transfection, flow cytometry was carried out to examine apoptosis and mitosis in MG-63 and

U2OS cells. After transfection with shRNA for 72 h, MG-63 or U2OS cells were collected, fixed in 70% ethanol solution at 4°C for 30 min and incubated with Annexin V-FITC (Beyotime Institute of Biotechnology) and propidium iodide (Sigma-Aldrich; Merck KGaA) for 30 min at room temperature. Flow cytometric analysis was performed to examine cell apoptosis. Cells were collected, fixed in 70% ethanol solution at 4°C for 30 min and incubated with propidium iodide and RNase A for 30 min at room temperature. Subsequently, a flow cytometer (LSR II; BD Biosciences) was used to examine cell mitosis. Data were analyzed using FlowJo version 7.6 software (Tree Star, Inc.).

Statistical analysis. The data are presented as the mean, calculated from three independent experiments. SPSS 25.0 (IBM Corp) was used for statistical analysis. For cell functional experiments, the respective results were standardized by percentage. For cell functional experiments and flow cytometry, one-way ANOVA followed by Bonferroni or Tamhane post hoc tests were used. Immunohistochemical results were analyzed using Pearson's χ^2 test. For RFS and OS analysis, Kaplan-Meier curves were calculated and log-rank test was used to analyze the statistical significance. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Clinical analysis of SLC25A10 expression in patients with osteosarcoma. In total, 60 paraffin-embedded osteosarcoma tissue samples and 60 paraffin-embedded normal bone tissue samples were collected. The protein levels of SLC25A10 in these tissues were detected by immunohistochemistry, and a positive signal of SLC25A10 was mainly detected in the cytoplasm. Among these 60 osteosarcoma tissues, 18 (30.0%) were SLC25A10-negative and 42 (70.0%) were SLC25A10-positive ($P < 0.05$; Table I). Among the 60 normal bone tissues, 32 (53.3%) were SLC25A10-negative and 28 (46.7%) were SLC25A10-positive (Table I). The representative SLC25A10 expression levels in osteosarcoma and normal bone tissues are presented in Fig. 1A. Therefore, the protein levels of SLC25A10 were higher in human osteosarcoma tissues, compared with normal bone tissues.

The association between SLC25A10 expression and various clinicopathological features and survival rates in the 60 patients with osteosarcoma was analyzed. Positive expression of SLC25A10 was found to be associated with a higher risk of tumor metastasis ($P = 0.037$) and worse clinical Enneking stage ($P = 0.042$) in the 60 patients with osteosarcoma (Table II). However, there was no significant statistical significance in SLC25A10 expression between patient groups of different age, sex or tumor size (all $P > 0.05$; Table II).

Moreover, these 60 patients with osteosarcoma were all followed-up for > 5 years, and potential associations between SLC25A10 expression and RFS and OS rates were examined. Patients with osteosarcoma who displayed positive expression of SLC25A10 showed decreased OS and RFS rates, compared with patients with negative SLC25A10 expression (both $P < 0.05$; Fig. 1B and C). Therefore, high expression of SLC25A10 was associated with poor prognosis in patients with osteosarcoma.

Table I. Expression of SLC25A10 in osteosarcoma and normal tissues.

Group	n	SLC25A10 expression	
		Negative, n (%)	Positive, n (%)
Osteosarcoma	60	18 (30.0)	42 (70.0) ^a
Normal	60	32 (53.3)	28 (46.7)

^a $P < 0.05$. $\chi^2 = 6.720$. SLC25A10, solute carrier 25 family member 10.

Role of SLC25A10 in osteosarcoma cell proliferation. The human osteosarcoma cell lines MG-63 and U2OS were selected for cell functional experiments. Protein levels of SLC25A10 decreased after transfection with shSLC25A10-1 or shSLC25A10-2 compared with shCtrl in both MG-63 and U2OS cells (Fig. 2A). As determined by the cell counting assay (Fig. 2B and C), both shSLC25A10-1 and shSLC25A10-2 significantly reduced the total cell number, compared with shCtrl in both MG-63 and U2OS cells over 5 days (standardized by percentage: MG-63 shCtrl, 100%; MG-63 shSLC25A10-1, 55.25%; MG-63 shSLC25A10-2, 41.61%; $P < 0.01$; U2OS shCtrl, 100%; U2OS shSLC25A10-1, 61.55%; U2OS shSLC25A10-2, 46.29%; $P < 0.01$). Moreover, as detected by the MTT assay (Fig. 2D and E), cell proliferation decreased significantly during a period of 5 days in both MG-63 and U2OS cells after transfection with shSLC25A10-1 or shSLC25A10-2, compared with shCtrl (MTT assay at day 5, standardized by percentage: MG-63 shCtrl, 100%; MG-63 shSLC25A10-1, 74.79%; MG-63 shSLC25A10-2, 67.23%; $P < 0.01$; U2OS shCtrl, 100%; U2OS shSLC25A10-1, 75.57%; U2OS shSLC25A10-2, 68.70%; $P < 0.01$). In addition, MG-63 shSLC25A10-1 and MG-63 shSLC25A10-2 cells showed decreased cell colony formation compared with MG-63 shCtrl cells (standardized by percentage: MG-63 shCtrl 100%, MG-63 shSLC25A10-1 22.31%, MG-63 shSLC25A10-2 20.77%; $P < 0.01$). Moreover, U2OS shSLC25A10-1 and U2OS shSLC25A10-2 cells also showed decreased colony formation compared with U2OS shCtrl cells (Standardized by percentage: U2OS shCtrl 100%, U2OS shSLC25A10-1 33.56%, U2OS shSLC25A10-2 28.64%; $P < 0.01$; Fig. 2F and G). Collectively, SLC25A10 knockdown suppressed cell proliferation in human osteosarcoma cells.

Role of SLC25A10 in osteosarcoma cell apoptosis and mitosis. The role of SLC25A10 in apoptosis and mitosis of osteosarcoma cells was evaluated by flow cytometry. SLC25A10 shRNA knockdown significantly increased the percentage of apoptotic cells in both MG-63 and U2OS cells (MG-63 shCtrl, 6.97%; MG-63 shSLC25A10-1, 15.82%; MG-63 shSLC25A10-2, 16.84%; $P < 0.01$; U2OS shCtrl, 6.89%; U2OS shSLC25A10-1, 16.23%; U2OS shSLC25A10-2, 16.67%; $P < 0.01$; Fig. 3A and B). Moreover, transfection of shSLC25A10-1 or shSLC25A10-2 significantly increased the percentage of cells in G₁ phase and decreased the percentage of cells in S phase in both MG-63 and U2OS cells (Fig. 3C and D). Therefore, the present data suggested that SLC25A10 promoted cell mitosis and suppressed cell apoptosis in human osteosarcoma cells.

Table II. Association of SLC25A10 expression with clinicopathological parameters from patients with osteosarcoma.

Parameter	n	SLC25A10 expression, n (%)		P-value	χ^2
		Negative	Positive		
Age, years					
≤ 20	27	7 (25.9)	20 (74.1)	0.533	0.388
> 20	33	11 (33.3)	22 (66.7)		
Sex					
Male	37	11 (29.7)	26 (70.3)	0.954	0.003
Female	23	7 (30.4)	16 (69.6)		
Tumor size, cm					
≤ 5	36	9 (25.0)	27 (75.0)	0.301	1.071
> 5	24	9 (37.5)	15 (62.5)		
Metastasis					
No	42	16 (38.1)	26 (61.9)	0.037	4.369
Yes	18	2 (11.1)	16 (88.9)		
Enneking stage					
I-II	28	12 (42.9)	16 (57.1)	0.042	4.133
III	32	6 (18.8)	26 (71.2)		

SLC25A10, solute carrier 25 family member 10.

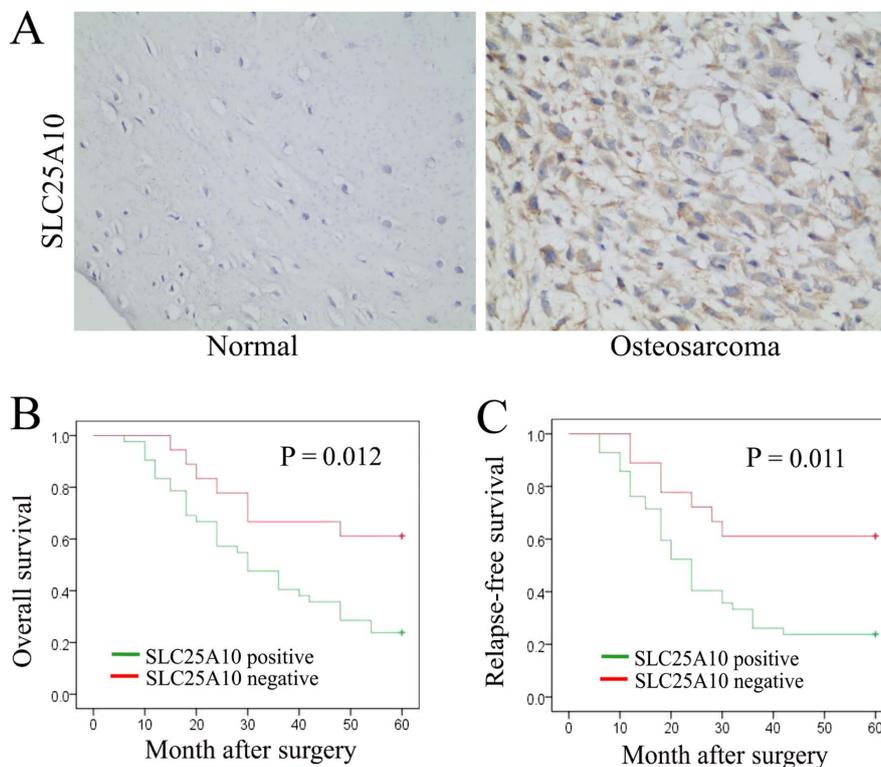


Figure 1. SLC25A10 expression in human osteosarcoma and normal bone tissues, and association between SLC25A10 expression and survival rates in patients with osteosarcoma. (A) Protein levels of SLC25A10 in 60 osteosarcoma tissues and 60 normal bone tissues were examined by immunohistochemistry. Magnification, x200. Kaplan-Meier curves were calculated to analyze (B) overall survival and (C) relapse-free survival rates between the SLC25A10-positive group and the SLC25A10-negative group in 60 patients with osteosarcoma. SLC25A10, solute carrier 25 family member 10.

Regulation analysis of SLC25A10 in osteosarcoma cells. To investigate the downstream mechanisms of SLC25A10, several candidate genes, including MYC, tumor protein 53 (TP53),

cyclin D1 (CCND1), CCNE1, PTEN, STAT3, epidermal growth factor (EGFR), P21 and P27 were examined in MG-63 cells after transfection with shSLC25A10-1, shSLC25A10-2

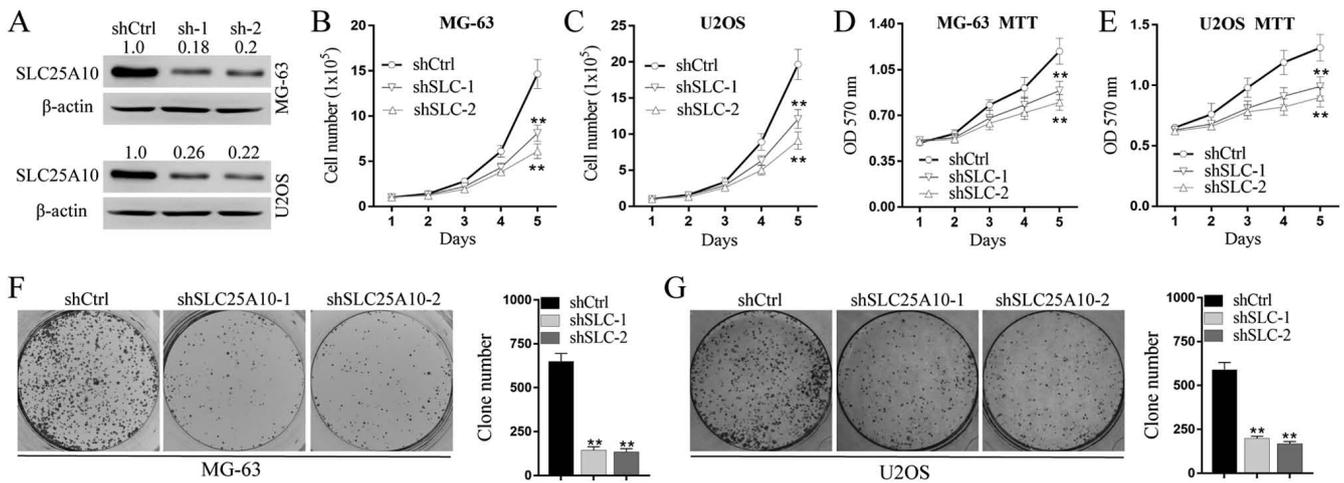


Figure 2. SLC25A10 promotes cell proliferation of human osteosarcoma cells. Osteosarcoma cells MG-63 and U2OS were transfected with shSLC25A10-1, shSLC25A10-2 or shCtrl. (A) Protein levels of SLC25A10 were examined by western blotting. β -actin was used as the loading control. Cell counting assays in (B) MG-63 and (C) U2OS cells were carried out and cell growth curves were calculated during a period of 5 days. MTT assays in (D) MG-63 cells and (E) U2OS cells were carried out and cell growth curves were calculated during a period of 5 days. Cell colony formation assays in (F) MG-63 cells and (G) U2OS cells were carried out and the cell colony number after 10 days were calculated. ** $P < 0.01$ vs. shCtrl. sh, short hairpin; SLC25A10/SLC, solute carrier 25 family member 10; Ctrl, control; OD, optical density.

or shCtrl. These genes were selected according to previous studies which demonstrated their oncogenic role (24-31). Thus, it was hypothesized that these gene candidates were regulated by SLC25A10, and that they might mediate the role of SLC25A10 in osteosarcoma cells.

As determined by the western blotting assay, the protein level of CCNE1 decreased markedly after transfection with shSLC25A10-1 or shSLC25A10-2, whereas the protein levels of P21 and P27 increased after transfection with shSLC25A10-1 or shSLC25A10-2 in both MG-63 and U2OS cells, compared with shCtrl (Fig. 4A). However, there were no marked changes in MYC, TP53, CCND1, PTEN, STAT3 and EGFR protein levels after shSLC25A10 transfection compared with shCtrl (data not shown). Therefore, only CCNE1 was positively regulated by SLC25A10. Moreover, P21 and P27 were negatively regulated by SLC25A10 in human osteosarcoma cells.

In addition, cleaved caspase-3 and its substrate cleaved PARP markedly increased after transfection with shSLC25A10-1 or shSLC25A10-2 in both MG-63 and U2OS cells. Therefore, knockdown of SLC25A10 promoted caspase-3-mediated apoptosis in human osteosarcoma cells.

Discussion

In the present study, several systematical experiments were performed to examine the role of SLC25A10 in human osteosarcoma. As examined by immunohistochemistry, the protein levels of SLC25A10 were much higher in osteosarcoma tissues compared with normal bone tissues. A high expression level of SLC25A10 was associated with worse clinicopathological parameters, including tumor metastasis and clinical Enneking stage in patients with osteosarcoma. Patients with osteosarcoma with high expression levels of SLC25A10 showed lower RFS and OS rates, compared with patients with low expression levels of SLC25A10. In MG-63 and U2OS human osteosarcoma cells, shRNA-mediated SLC25A10 knockdown significantly suppressed cell proliferation as determined by

cell counting, MTT and colony formation assays. In addition, knockdown of SLC25A10 promoted apoptosis and suppressed mitosis in human osteosarcoma cells, as determined by flow cytometry. In addition, knockdown of SLC25A10 promoted caspase-3-mediated apoptosis. Zhou *et al* (10) demonstrated that SLC25A10-knockdown in A549 cells decreased cell proliferation, leading to a less malignant phenotype, as well as increased glutamine dependency and sensitivity to oxidative stress. The data from the present study are in line with these previous findings. However, few previous studies have documented the role of SLC25A10 in other human cancer types. Therefore, to the best of the authors' knowledge, the present study is the first to show that SLC25A10 may play a tumor-promoting role in human osteosarcoma.

In addition, the downstream pathway underlying the oncogenic role of SLC25A10 was examined, and CCNE1 was found to be positively regulated by SLC25A10. CCNE1 is a member of the cyclin family, which contributes to the activity of the cell cycle. As reported in previous study, CCNE1 is upregulated, and plays an oncogenic role in human osteosarcoma (17). MicroRNA (miR)-874 and miR-132 directly targeted CCNE1, and both acted as tumor suppressors, inhibiting the malignant features of human osteosarcoma cells. As a tumor promoter, CCNE1 mediated the tumor-suppressing effect of miR-874 and miR-132 (26,32). Moreover, CCNE1 was found to act as an oncogene in various other types of human cancer, including breast cancer (18,33), lung cancer (34), gastric cancer (35) and hepatocellular carcinoma (36). Therefore, the tumor-promoting role of SLC25A10 in human osteosarcoma might be partly mediated by CCNE1.

Furthermore, in the present study, P21 and P27 were found to be negatively regulated by SLC25A10 in osteosarcoma cells. P21 is an important cyclin-dependent kinase inhibitor, which suppresses both cell proliferation and metastasis in human osteosarcoma cells. Moreover, overexpression of P21 increases sensitivity of osteosarcoma cells to anti-cancer drugs (37-39). Furthermore, P21 acts as tumor suppressor in

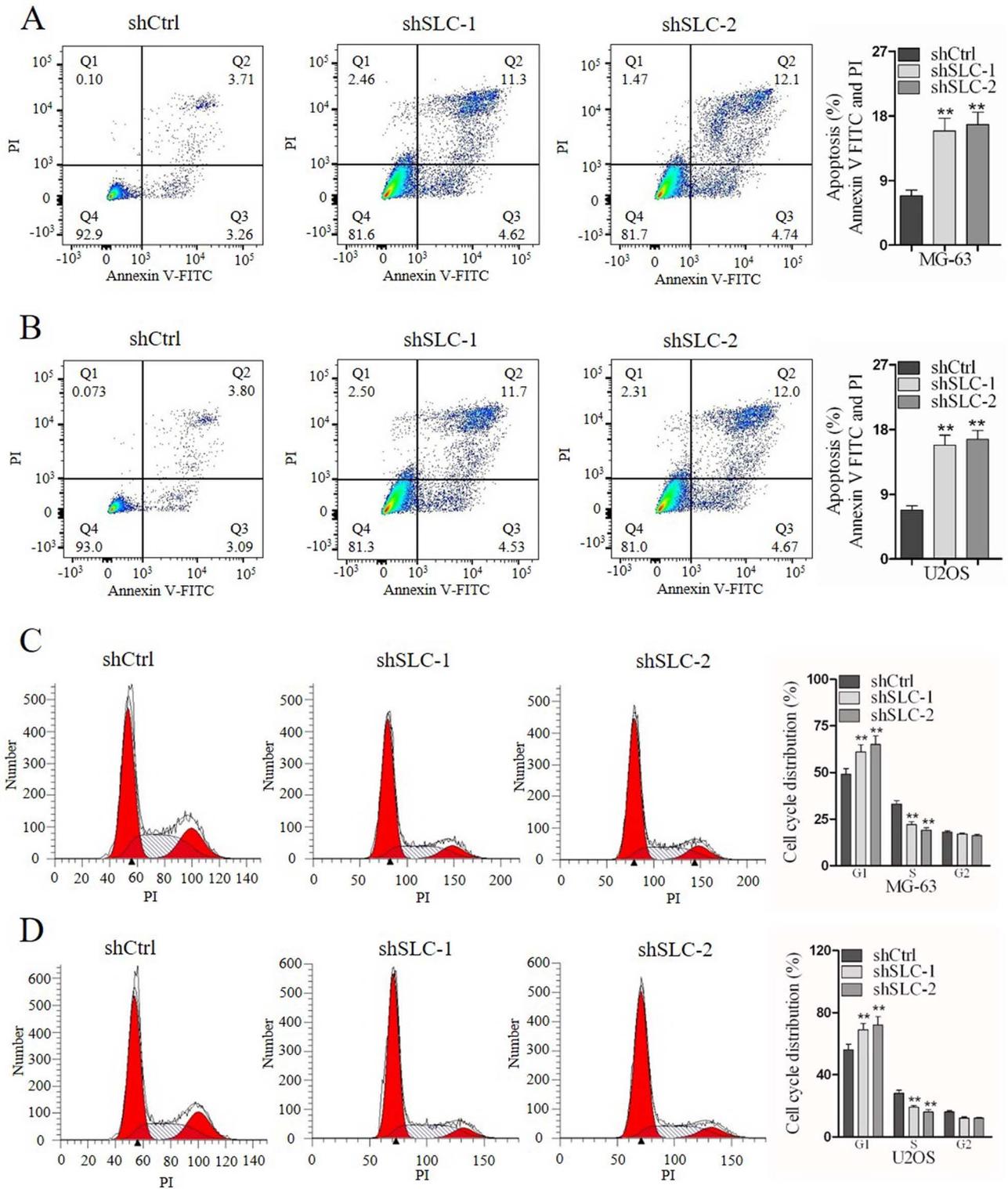


Figure 3. SLC25A10 knockdown promotes cell apoptosis and suppresses cell mitosis in human osteosarcoma cells. Osteosarcoma cells MG-63 and U2OS were transfected with shSLC25A10-1, shSLC25A10-2 or shCtrl. (A) Flow cytometry with Annexin V-FITC and PI staining in (A) MG-63 cells and (B) U2OS cells were performed to evaluate cell apoptosis. Flow cytometry with PI staining in (C) MG-63 cells and (D) U2OS cells were performed to evaluate the proportions of cells in the G₁, S and G₂ phases. **P<0.01 vs. shCtrl. sh, short hairpin; SLC25A10/SLC, solute carrier 25 family member 10; PI, propidium iodide; Ctrl, control.

human nasopharyngeal carcinoma (19), cervical cancer (20), breast cancer (40), non-small cell lung cancer (41). P27 is a tumor suppressor gene in many types of human cancer. In osteosarcoma, Liao *et al* (42) identified that P27 is negatively

correlated with S-phase kinase associated protein 2 and serves a tumor-suppressing role. Hu *et al* (43) demonstrated that inhibition of P27 mediated the tumor-promoting role of miR-227 in both cell proliferation and metastasis of

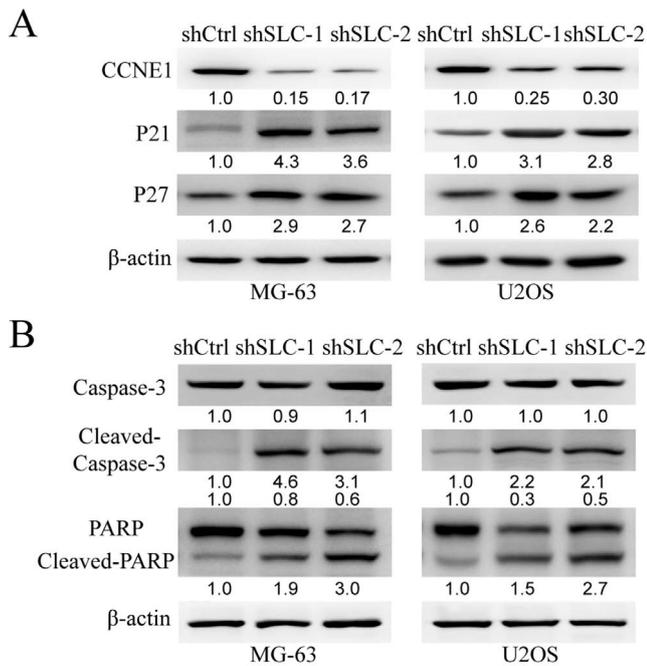


Figure 4. CCNE1, P21 and P27 are regulated by SLC25A10 in human osteosarcoma cells. (A) Protein levels of CCNE1, P21 and P27 in MG-63 and U2OS cells after transfection with shSLC25A10-1, shSLC25A10-2 or shCtrl were examined by western blot analysis. β -actin was used as loading control. The numbers below were fold change compared with reference protein. (B) Protein levels of caspase-3, cleaved caspase-3, PARP and cleaved PARP in MG-63 and U2OS cells following transfection with shSLC25A10-1, shSLC25A10-2 or shCtrl were examined by western blotting. The numbers below were fold change compared with reference protein respectively. sh, short hairpin; SLC25A10/SLC, solute carrier 25 family member 10; CCNE1, cyclin E1; PARP, poly ADP-ribose polymerase; Ctrl, control.

osteosarcoma cells. Accumulating evidence has demonstrated the tumor-suppressing role of P27 in human cervical cancer (20), pancreatic cancer (21), papillary thyroid cancer (44) and other types of cancer. Therefore, the downregulation of P21 and P27 increased by high expression levels of SLC25A10 in osteosarcoma cells may also mediate the tumor-promoting role of SLC25A10. The SLC25A10/CCNE1/P21/P27 signaling pathway may play an important role in human osteosarcoma.

Previous studies suggested that SLC25A10 was involved in sustaining metabolic shift and redox balance (10,13). Abnormal redox condition was found to be associated with abnormal expression of P21 and P27, thus influencing cell proliferation and mitosis (45,46). CCNE1 is an important gene involved in cell cycle (47). Therefore, SLC25A10 may regulate the expression of CCNE1, P21 and P27 by influencing the redox state in osteosarcoma cells. Moreover, P21 may be regulated by SLC25A10. However, a previous study demonstrated that the upstream factor TP53 in the TP53/P21 pathway showed no significant changes after knockdown of SLC25A10 (48). Therefore, SLC25A10 may regulate P21 through TP53-independent mechanisms in osteosarcoma cells. In addition, knockdown of SLC25A10 may induce G₁ cell cycle arrest by undetermined mechanisms, thus increasing the expression levels of P21, P27 and CCNE1. The changes in the levels of CCNE1, P21 and P27 could be an indirect consequence of cell cycle arrest. The exact molecular mechanisms underlying the regulation of CCNE1, P21 and P27

by SLC25A10 in osteosarcoma cells require further examination. The present study did not clarify the energy metabolism, redox homeostasis and the location of SLC25A10 in osteosarcoma cells, which was a limitation to the present study and should be investigated in future experiments.

In summary, the present study identified an oncogenic role for SLC25A10 in human osteosarcoma. *In vitro* functional experiments and analysis of tumor tissues collected from patients with osteosarcoma were performed. Decreased expression of SLC25A10 was associated with less malignant features in osteosarcoma cells and improved clinicopathological parameters and prognosis in patients with osteosarcoma. In addition, CCNE1 was positively regulated and P21/P27 were negatively regulated by SLC25A10. The present results suggested that the SLC25A10/CCNE1/P21/P27 pathway may be fundamental in human osteosarcoma.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

GW, XC and BX designed experiments. GW, JX, CC and JQ performed experiments. GW, JX, PS and ZP analyzed data. XC and BX wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by Institutional Review Boards of Anhui Medical University. All participants signed informed consent before admission.

Patient consent for publication

All patients signed informed consent for the possible publication of the present study.

Competing interests

The authors declare that they have no competing interests.

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