DEPDC1 affects autophagy-dependent glycolysis levels in human osteosarcoma cells by modulating RAS/ERK signaling through TTK

Dong Yu[a,*], Lin Chen[b,*], Yingchun Li[c], Bailian Liu[d] and Weiping Xiao[d]

The current treatment for osteosarcoma (OS) is based on surgery combined with systemic chemotherapy, however, gene therapy has been hypothesized to improve patient survival rates. The density-enhanced protein domain 1 protein (DEPDC1) functions as a crucial determinant in the advancement of OS, which is highly expressed in OS cells. The current study was designed to delve into the effect and mechanism of DEPDC1 and phosphotyrosine-picked threonine tyrosine kinase (TTK) in OS. The expression of DEPDC1 and TTK in OS cells was detected by western blotting. Furthermore, the assessment of glycolysis encompassed the quantification of extracellular acidification rate, glucose uptake rate, lactate concentration, and the expression of glucose transporter 1, hexokinase 2, and pyruvate kinase M2. Finally, the functions of DEPDC1 and TTK in autophagy and ras-extracellular signal-regulated kinase signaling were determined by western blotting after interfering with DEPDC1 in SaOS-2 cells. The results revealed that DEPDC1 and TTK were upregulated in OS cell lines and interfering with DEPDC1 inhibited glycolysis and autophagy in OS cells. Furthermore, the STRING database suggested that DEPDC1 and TTK perform targeted binding. Notably, the results of the present study revealed that DEPDC1 upregulated RAS expression through TTK and enhanced ERK activity, thereby affecting glycolysis and autophagy in OS cells. Collectively, the present investigation demonstrated that DEPDC1 affected autophagy-dependent glycolysis levels of OS cells by regulating RAS/ERK signaling through TTK. *Anti-Cancer Drugs* 35: 893–901 Copyright © 2024 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Osteosarcoma (OS), a malignant tumor derived from mesenchymal stem cells, is primarily found in children and adolescents [1]. OS primarily manifests in the long metaphyseal region adjacent to the knee joint, with approximately 80% of OS occurring in the long bones of the extremities [2]. With the current advancement of medical treatment, adjuvant chemotherapy can constructively enhance the 5-year survival rate of patients. However, 30–40% of patients still experience tumor recurrence and metastasis, especially lung metastasis, which often causes respiratory failure and the prognosis is poor [3,4]. Hence, there is an urgent need to further study the molecular mechanisms of OS pathogenesis and its therapeutic targets to improve patient prognosis and survival rates.

Research in recent years has focused on elucidating crucial oncogenes that facilitate the onset and progression of OS. The occurrence and progression of malignant tumors is a continuous and complex process, accompanied by changes in the expression of multiple genes. DEP domain 1 protein (DEPDC1), a novel oncogene harboring the DEP domain, has emerged as a tumor-associated gene, initially identified in bladder cancer [5]. Prior investigations have established that DEPDC1 plays a pivotal role in various cellular processes, such as promoting cell proliferation and inhibiting apoptosis [6–8]. Moreover, DEPDC1 is significantly overexpressed in some cancers, and high expression of DEPDC1 is closely associated with the progression of cancers, including gastric cancer, lung adenocarcinoma, bladder cancer, and hepatocellular carcinoma [9–11]. A recent study has indicated that DEPDC1, a hub gene, is highly expressed in OS, and its high expression is related to poor prognosis [12]. However, the role and underlying mechanisms of DEPDC1 in OS have not been studied. New research demonstrated that DEPDC1 inhibits autophagy in lung adenocarcinoma cells through RAS-ERK1/2 signaling [13]. In addition, numerous studies have reported the impact of RAS/ERK signaling through regulating autophagy and glycolysis levels [14–16]. However, whether

DEPDC1 can affect the level of autophagy-dependent glycolysis in human OS cells by regulating RAS/ERK signaling remains to be investigated.

Of note, the STRING database (https://cn.string-db.org/) revealed that DEPDC1 could bind to phosphotyrosine-picked threonine tyrosine kinase (TTK) in the present study. Furthermore, Wang *et al*. [17] demonstrated that TTK plays a key role in the progression of OS and is also considered a clinical therapeutic target. An existing study also confirmed that dysregulated expression of TTK is a hallmark of various cancers, which is highly expressed in human OS tissues. Moreover, interference with the expression of TTK inhibits the malignant progression of OS [18]. It is worth noting that TTK could affect autophagy and ERK signaling [19,20]. Thus, the present study aimed to explore whether DEPDC1 regulates RAS/ERK signaling through TTK to affect the level of autophagy-dependent glycolysis in human OS cells.

Materials and methods

Cell culture

Human osteoblast cell line (hFOb1.19), SaOS-2, and U2OS cell lines were obtained from EK-Bioscience, and 143B and human osteosarcoma cells (HOS) were purchased from the Chinese Academy of Sciences. All the cells were cultured with Dulbecco's Modified Eagle's Medium, including 10% fetal bovine serum plus penicillin/streptomycin (Invitrogen, Carlsbad, California, USA; Thermo Fisher Scientific, Inc., Cleveland, Ohio, USA) at 37 °C with 5% CO_2.

Cell transfection

The siRNA against DEPDC1, Beclin1, ATG5, and negative control siRNA were synthesized by Sangon Biotech Co. Ltd. A total of 2 μg of TTK overexpression vector and the corresponding control vector were purchased from Origene Technologies, Inc. SaOS-2 cells were transfected with 100 nM of the aforementioned recombinants using lipofectamine 2000 (Thermo Fisher Scientific, Inc.) at 37 °C for 48 h. After transfection, cells were harvested for subsequent experiments.

Western blotting

Cellular proteins were extracted using radio immunoprecipitation assay buffer (supplemented with protease inhibitors) on ice for 30 min, followed by centrifugation. Subsequently, the concentration of the extracted proteins was quantified utilizing a bicinchoninic acid protein assay kit obtained from the Beyotime Institute of Biotechnology. The extracted protein (20 μg) was loaded into SDS-PAGE under reducing conditions for separation, transferred to a polyvinylidene fluoride membrane, and blocked with Tween detergent in phosphate-buffered saline (PBST) containing 5% evaporated skimmed milk for 1 h at room temperature. Following three washes with PBST, the membranes were blocked in 5% nonfat milk for a duration of 2 h and incubated with primary antibodies against DEPDC1, glucose transporter 1 (GLUT1), hexokinase 2 (HK2), pyruvate kinase M2 (PKM2), cytoplasmic light chain 3 (LC3) (LC3-I), lipidated LC3 (LC3-II), p62, TTK, RAS, ERK, phosphorylated (p-) ERK and glyceraldehyde-3-phosphate dehydrogenase at 4 °C overnight. Subsequently, the membranes were washed three times and incubated with horseradish peroxidase-labeled anti-IgG antibody (supplied by Wuhan Boster Biological Technology, Ltd.) at ambient temperature for a period of 1 h. Finally, the signal was measured with enhanced chemiluminescence-Plus reagent (MilliporeSigma) and exposed to film. The gray value of protein bands was quantitatively analyzed utilizing Image Pro Plus 6.0 software (Media Cybernetics, Inc.).

Measurement of extracellular acidification rate

The extracellular acidification rate (ECAR) was evaluated using the Seahorse XFe 96 Extracellular Flux Analyzer (Agilent Technologies, Inc. Santa Clara, California; Seahorse Bioscience) in accordance with the manufacturer's instructions, employing the Seahorse XF Glycolysis Stress Test Kit. ECAR data were analyzed utilizing the Seahorse XF-96 Wave software, then the results were presented in mpH/min.

Glucose uptake and lactic acid concentration

The glucose uptake levels in SaOS-2 cells were quantified utilizing the Glucose Uptake Assay Kit (Colorimetric; catalog number ab136955; Abcam, Cambridge, Massachusetts, USA). Additionally, to assess the lactic acid concentration within the culture medium of SaOS-2 cells, the lactic acid assay kit from Nanjing Jiancheng Bioengineering Institute was employed according to the manufacturer's guidelines. The concentrations of lactate and pyruvate were normalized relative to the cell count.

Immunofluorescent cell staining

SaOS-2 cells were seeded on eight well chamber slides at a density of 6×10^3 cells/well. After incubation, cells were fixed with 4% paraformaldehyde, blocked with 1% fetal bovine serum, and incubated with mouse anti-DEPDC1 antibody. The cells were then incubated with Alexa-Fluor anti-mouse IgG. Finally, the cells were mounted with Vectashield with 4',6-diamidino-2-phenylindole (Vector Laboratories, Inc.). After which, the cells were incubated with Alexa-Fluor anti-rabbit IgG and mounted. Cell nuclei were stained with Hoechst 33342, and fluorescent images were obtained using fluorescence microscopy (BX51; Olympus Corporation) and a camera system (VB7000; Keyence Corporation).

Co-immunoprecipitation

Following protein extraction in Immunoprecipitation lysis buffer containing 20 mM Tris-HCl, 150 mM NaCl, and 1% Triton X-100 at a pH of 7.5, cell lysates were incubated overnight at 4 °C with either DEPDC1 or TTK antibodies. Subsequently, the lysates were transferred to Protein A/G PLUS-agarose beads. The precipitated proteins were resuspended and boiled before elution from

the PBS-rinsed beads. Subsequently, the eluted proteins were collected using magnetic separation, and the immune complexes were analyzed via western blotting.

Statistical analysis

The results were analyzed utilizing GraphPad Prism 8.0 software (Dotmatics), and all data were presented as the mean ± SD. To compare the differences between two groups, an unpaired Student's *t*-test was conducted. For comparisons among multiple groups, a one-way analysis of variance followed by Tukey's post hoc test was employed. Statistical significance was set at a *P* value less than 0.05.

Results

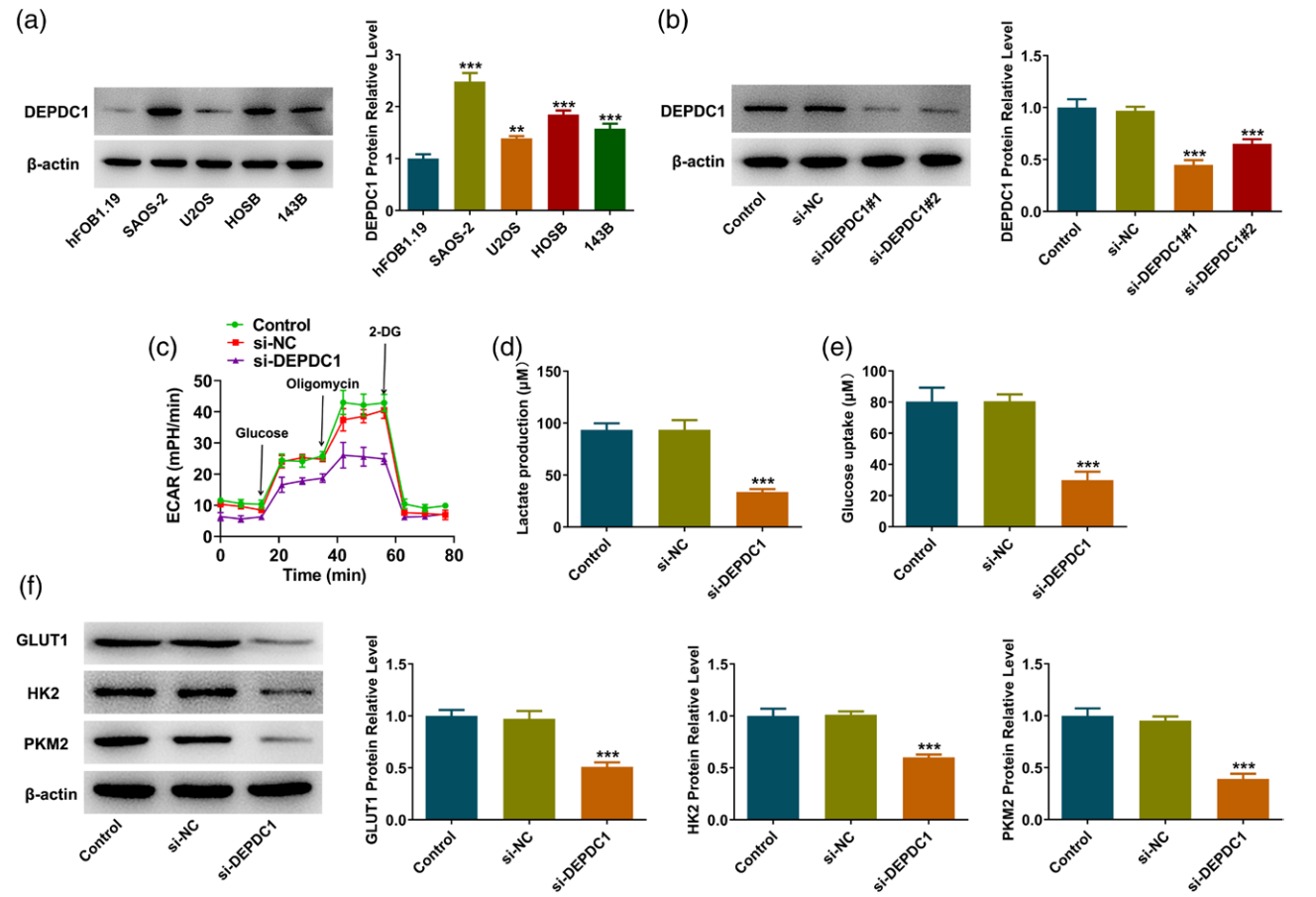
DEPDC1 is highly expressed in osteosarcoma cells, and interfering with DEPDC1 inhibits glycolysis in osteosarcoma cells

The expression of DEPDC1 in OS cell lines SaOS-2, U2OS, 143B, and HOS was measured by western blotting. Results showed that the protein expression of DEPDC1 was significantly higher in OS cell lines compared with human osteoblasts hFOB1.19, with the most pronounced decrease in the SaOS-2 cell line (Fig. 1a). Therefore, the SaOS-2 cell line was selected for subsequent experiments. In addition, to further study the mechanisms of DEPDC1, transfection was used to interfere with the expression of DEPDC1 (Fig. 1b). Subsequently, the effect of interfering with DEPDC1 on glycolysis in OS cells was studied. Compared with the si-NC group, the levels of ECAR, lactate and glucose in OS cell culture medium were significantly reduced (Fig. 1c–e). Moreover, western blotting showed that the expression of GLUT1, HK2, and PKM2 were decreased in the si-DEPDC1 group (Fig. 1f).

Interfering with DEPDC1 affects autophagy-dependent glycolysis levels in osteosarcoma cells

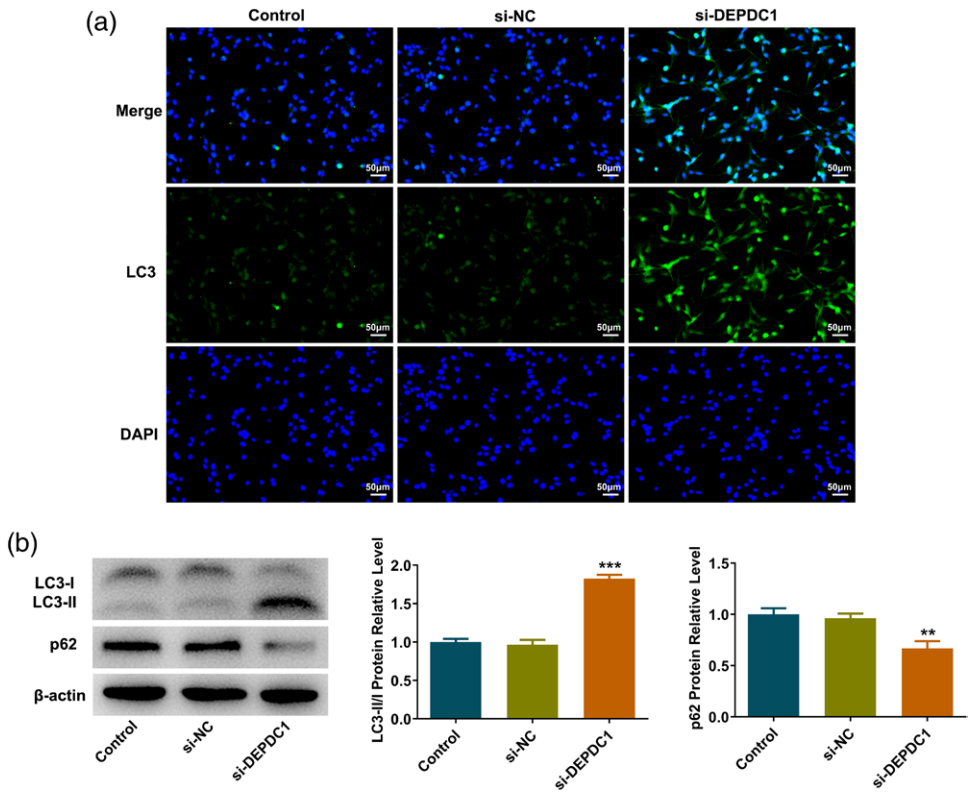
To investigate whether autophagy is related to interference with DEPDC1, the expression levels of green fluorescent protein (GFP)-LC3 in OS cells with interference

Fig. 1



Expression of DEPDC1 in OS cells, and effects of interfering with DEPDC1 on glycolysis in OS cells. (a) Relative DEPDC1 protein expression in OS cell lines; (b) relative DEPDC1 protein expression after interference; (c) ECAR assessment; (d) levels of lactic acid; (e) levels of glucose; (f) protein levels of GLUT1, PKM2, and HK2 were estimated by western blotting. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. si-NC. DEP, density-enhanced protein; DEPDC1, DEP domain 1 protein; ECAR, extracellular acidification rate; GLUT1, glucose transporter 1; HK2, hexokinase 2; NC, negative control; OS, osteosarcoma; PKM2, pyruvate kinase M2.

Fig. 2



Effects of interfering with DEPDC1 on autophagy-dependent glycolysis levels in OS cells. (a) Cell percentage of GFP-LC3+ autophagosomes; (b) The expression levels of autophagy-related proteins LC3-II/I and p62. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. si-NC. DEP, density-enhanced protein; DEPDC1, DEP domain 1 protein; ECAR, extracellular acidification rate; GFP, green fluorescent protein; GFP-LC3; LC3-II/I; NC, negative control; OS, osteosarcoma.

with DEPDC1 were examined. It was found that the percentage of GFP-LC3+ autophagosomes was reduced in OS cells after interfering with DEPDC1 (Fig. 2a). After which, the expression of autophagy-related proteins LC3-II/I and p62, selective substrates of autophagy, was examined. Western blot analysis showed that interfering with DEPDC1 increased the LC3-II/I ratio and downregulated the expression of p62 (Fig. 2b).

Since autophagy is a dynamic process, siBeclin1 and siATG5 were used to further explore the effect of interfering with DEPDC1 on glycolysis in OS cells after inhibiting autophagy. The results showed that siBeclin1 and siATG5 significantly increased the levels of ECAR, glucose consumption, and lactate production rate of si-DEPDC1 OS cells (Fig. 3a–e). Next, it was found that the expression of glycolysis enzymes GLUT1, HK2, and PKM2 were upregulated in si-DEPDC1 OS cells. Therefore, inhibition of autophagy promoted glycolysis in si-DEPDC1 OS cells (Fig. 3f).

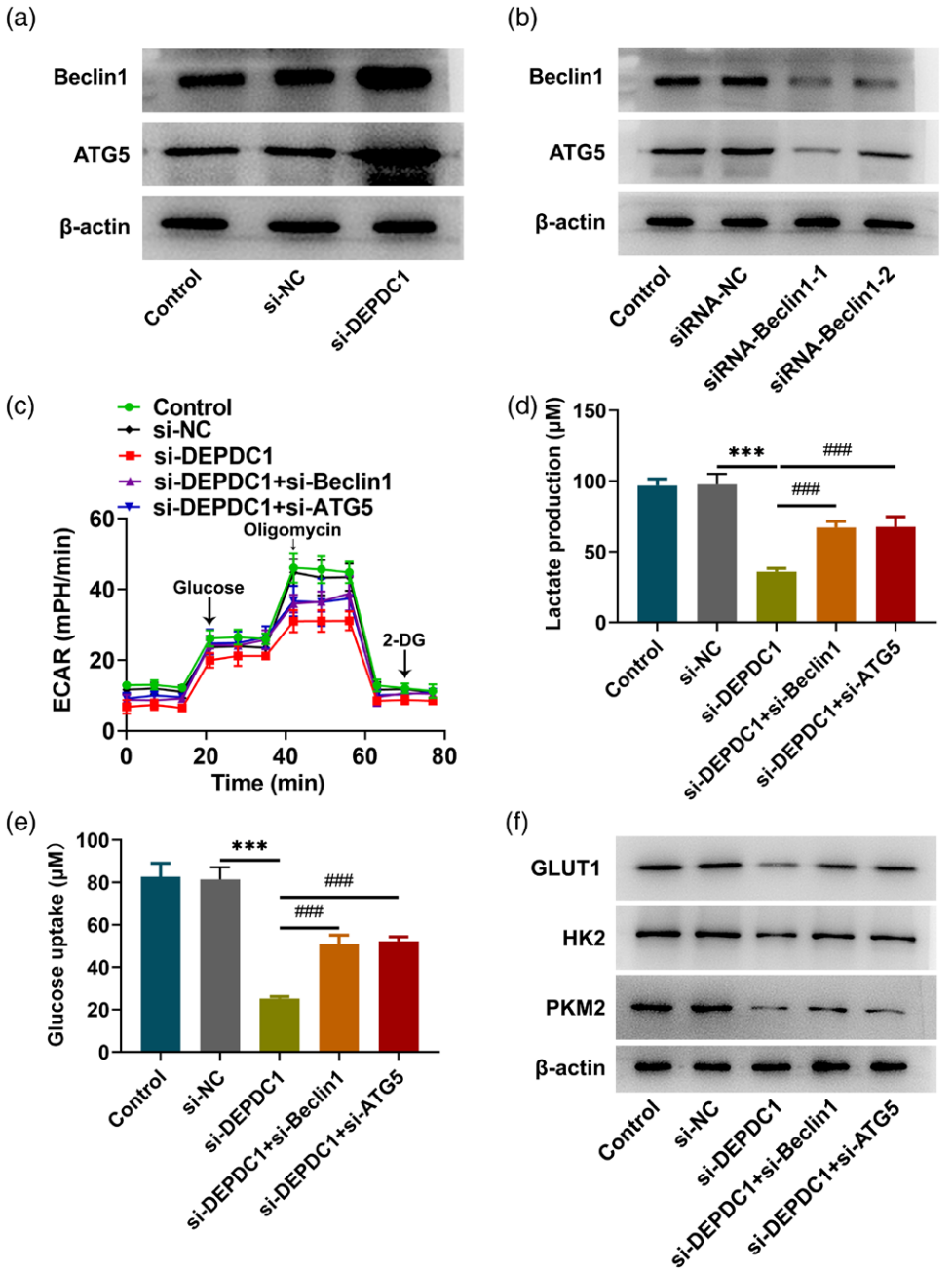
DEPDC1 and threonine tyrosine kinase have interactions in osteosarcoma cells

To further verify the potential mechanism of DEPDC1 affecting autophagy and glycolysis in OS cells, western blotting was used to detect the expression of TTK in SaOS-2 cells (Fig. 4a). Furthermore, the targeted combination of DEPDC1 and TTK was also assessed by the STRING database (Fig. 4b). Subsequently, co-immunoprecipitation was used to verify the target binding relationship between DEPDC1 and TTK in OS cells (Fig. 4c). The data from the present study verified that DEPDC1 and TTK have interactions in OS cells.

DEPDC1 affects the autophagy of osteosarcoma cells through threonine tyrosine kinase

To further explore the effect of DEPDC1 on human OS cell autophagy through TTK, an overexpression plasmid of TTK was constructed, and western blotting was used to detect the expression level of TTK (Fig. 5a). Additionally, immunofluorescence was used to detect the expression level of GFP-LC3 in cells, and it was found that after overexpression of TTK, the expression level of GFP-LC3 was significantly higher than that in the ov-NC group. Moreover, TTK overexpression decreased the LC3-II/I ratio and upregulated the expression of p62 (Fig. 5b,c). These data indicated that DEPDC1 affects the autophagy of OS cells through TTK.

Fig. 3

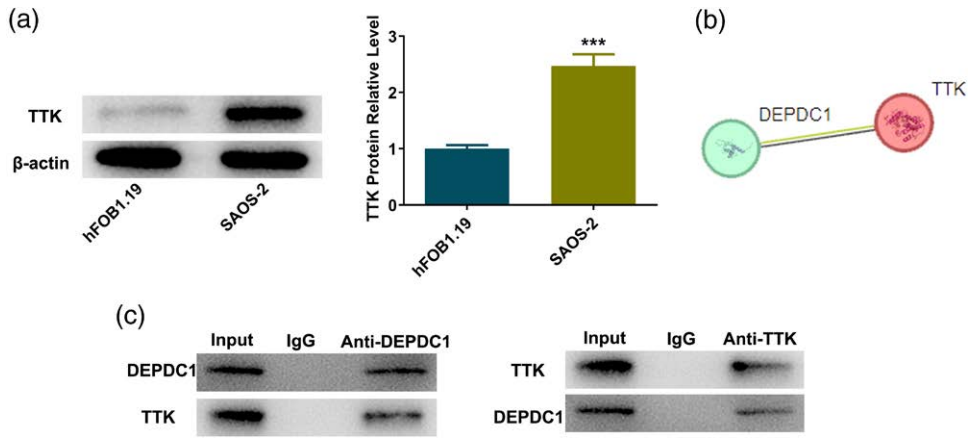


(a) The expression of Beclin1 and ATG5; (b) Relative Beclin1 and ATG5 protein expression after interference (c) ECAR assessment; (d) levels of lactic acid; (E) levels of glucose; (f) protein levels of GLUT1, PKM2, and HK2 were estimated by western blotting. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. si-NC, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. si-DEPDC1. DEP, density-enhanced protein; DEPDC1, DEP domain 1 protein; ECAR, extracellular acidification rate; GLUT1, glucose transporter 1; HK2, hexokinase 2; NC, negative control; OS, osteosarcoma; PKM2, pyruvate kinase M2.

DEPDC1 affects the glycolysis and RAS/ERK pathway of osteosarcoma cells through threonine tyrosine kinase

Subsequently, the effect of TTK overexpression on glycolysis in human OS cells was studied. An overexpression plasmid of TTK was constructed, and western blotting detected that the expression level of TTK was significantly higher than that in ov-NC group. Compared with the si-DEPDC1 + ov-NC group, the levels of ECAR, lactate, and glucose, as well as the expression of GLUT1, HK2, and PKM2 in the OS cell culture medium were significantly increased in the si-DEPDC1 + ov-TTK group (Fig. 6a–d). Moreover, compared with the ov-NC group, the expression of RAS, ERK, and p-ERK were

Fig. 4



Interaction between DEPDC1 and TTK in OS cells. (a) Expression of TTK in SaOS-2 cells; (b) targeted combination of DEPDC1 and TTK; (c) co-immunoprecipitation was used to verify the target binding relationship between DEPDC1 and TTK. ***$P < 0.001$ vs. si-NC. DEP, density-enhanced protein; DEPDC1, DEP domain 1 protein; NC, negative control; OS, osteosarcoma; TTK, threonine tyrosine kinase.

also upregulated after overexpression of TTK (Fig. 6e). These data suggested that DEPDC1 regulates RAS/ERK signaling through TTK to affect the level of autophagy-dependent glycolysis in OS cells.

Discussion

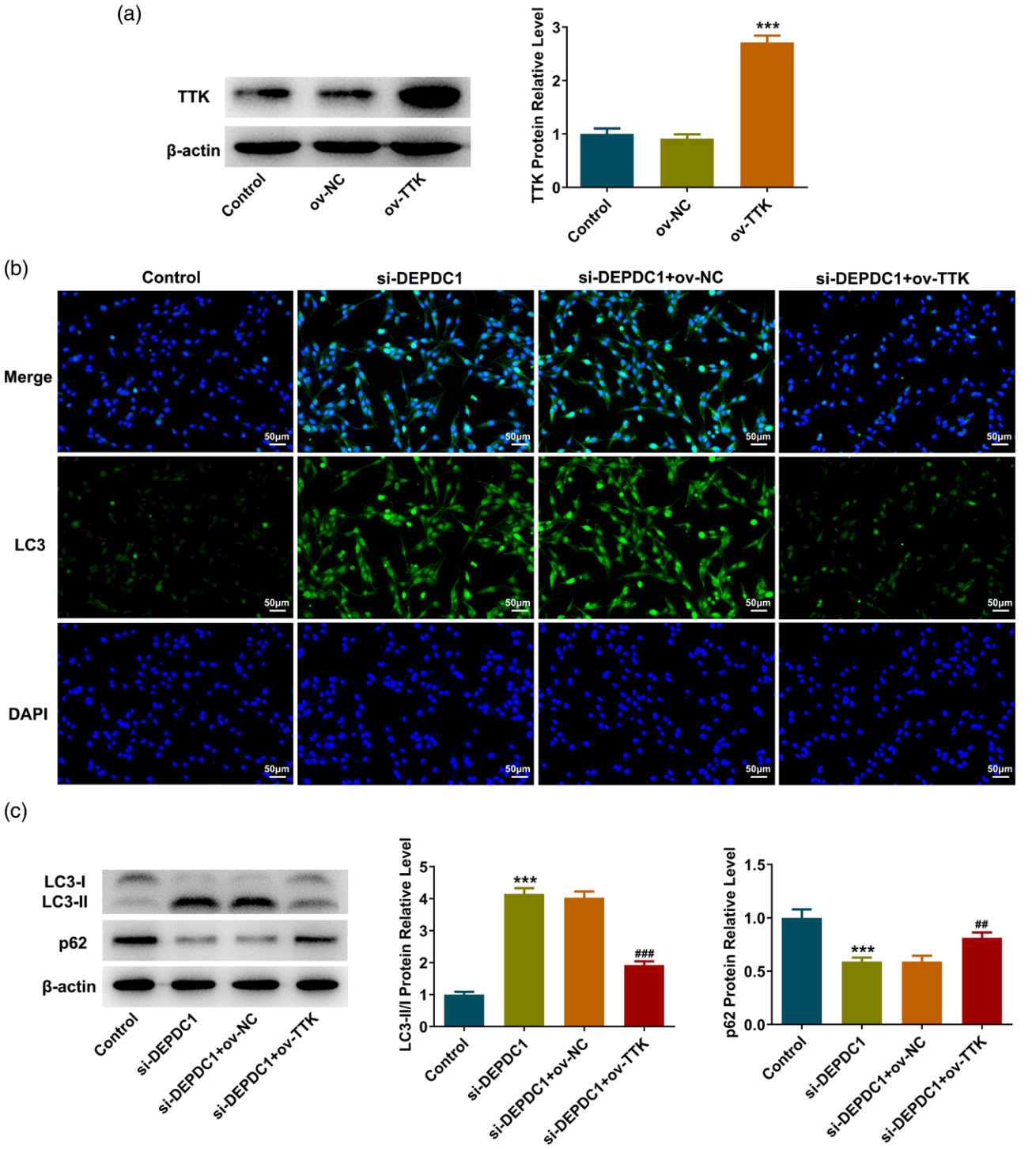
OS is the most common primary bone cancer and the leading malignancy in adolescents [21]. The 5-year survival rate of OS patients has improved over the past 30 years, while the prognosis of drug-resistant or metastatic OS remains unsatisfactory [22]. To gain insights into the pathogenesis of OS, the role and mechanism of DEPDC1 in regulating autophagy-dependent glycolysis of OS was investigated. In the present study, the results showed that DEPDC1 expression is significantly upregulated in human OS cells, which is consistent with previous findings relating to the role of DEPDC1 in other types of tumors. Furthermore, interfering with DEPDC1 affected autophagy-dependent glycolysis levels in OS cells. The present study also indicated that DEPDC1 interacted with TTK and that upregulation of TTK could weaken the effect of DEPDC1 interference to inhibit autophagy and improve glycolysis of OS cells.

DEPDC1 is a novel tumor-related gene with a highly conserved domain [12]. A number of studies have found that proteins with DEP domains can regulate numerous cellular functions, such as signal transduction, cell membrane anchoring, and the regulation of small molecule guanosine triphosphate enzyme activity [23]. Recent studies demonstrated that the expression of DEPDC1 is significantly upregulated in cancers such as bladder cancer, lung adenocarcinoma, OS, and other malignant tumor types [5,24,25]. In addition, DEPDC1 could be used in the diagnosis and treatment of a variety of tumors. However, the role and underlying mechanism of DEPDC1 expression in autophagy and glycolysis of OS remains to be explored. The present study revealed that DEPDC1 plays an essential role in the autophagy and glycolysis of OS cells.

Rapid tumor proliferation and metastasis always require a large amount of energy [26]. Hence, cancer cells reprogram metabolic pathways, including aerobic glycolysis, glutamine metabolism, and fatty acid metabolism [27]. Among them, aerobic glycolysis, also known as the Warburg effect, is important in metabolic reprogramming [28]. This process results in increased glucose uptake, lactate accumulation, and ATP production, which play an essential role in tumor initiation and progression [29]. Furthermore, glycolysis helps cancer cells resist cellular stress, and accelerated aerobic glycolysis is associated with treatment resistance in cancer [30]. Therefore, further exploration of these mechanisms in OS may have significance in improving the prognosis of OS. The present study constructed a DEPDC1 interference plasmid and confirmed that the levels of ECAR, lactate, glucose, and the expression of GLUT1, HK2, and PKM2 were reduced in the si-DEPDC1 group, indicating that interference with DEPDC1 inhibits glycolysis in OS cells.

Autophagy is required to promote the metabolic shift toward glycolysis, which is necessary for cell differentiation [31]. The basic mechanism of this process is autophagy, which provides essential components for energy requirements, controls mitochondrial mass, and regulates the activity of metabolic enzymes [32]. Moreover, the two pivotal proteins for autophagy detection are LC3 and p62, a microtubule-associated protein. Upon activation of autophagy, LC3-I conjugates with phosphatidylethanolamine to yield LC3-II, serving as a marker of autophagosomes [33]. Furthermore,

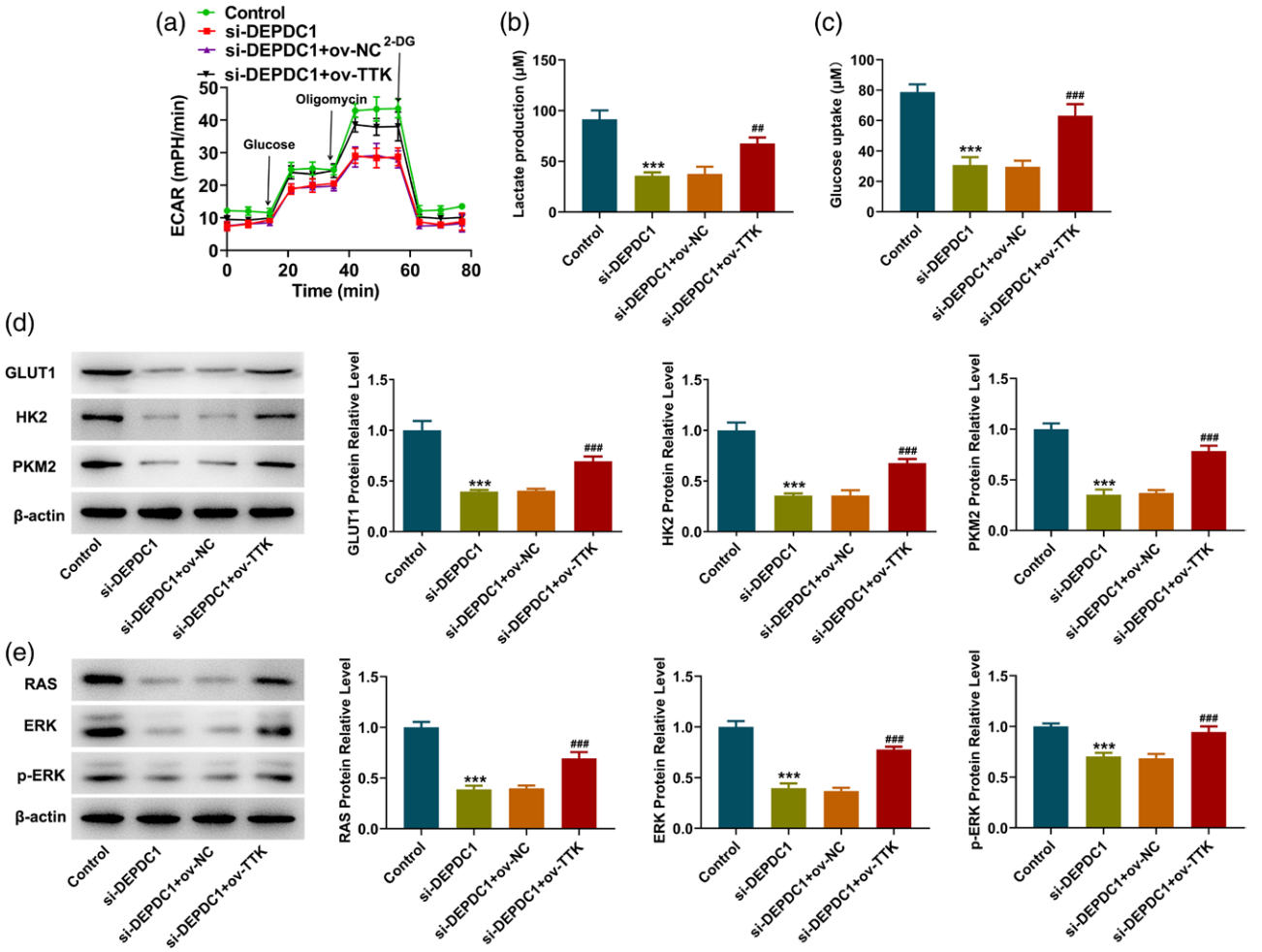
Fig. 5



Effects of DEPDC1 on autophagy of OS cells through TTK. (a) Expression level of TTK, ***$P < 0.001$ vs. ov-NC; (b) expression level of GFP-LC3; (c) expression levels of autophagy-related proteins LC3-II/I and p62. ***$P < 0.001$ vs. control, ##$P < 0.01$, ###$P < 0.001$ vs. si-DEPDC1 + ov-NC. DEPDC1, DEP domain 1 protein; GFP, green fluorescent protein; GFP-LC3; LC3-II/I; NC, negative control; OS, osteosarcoma; TTK, threonine tyrosine kinase.

inhibiting autophagic degradation also increases the level of LC3-II; therefore, the measurement of autophagic flux must clarify the lysosome-dependent degradation of LC3-II within a certain period of time. p62 binds directly to LC3 and is selectively degraded by autophagy. Thus, changes in autophagic flux can

Fig. 6



Effects of DEPDC1 on glycolysis and the RAS/ERK pathway in OS cells through TTK. (a) ECAR assessment; (b) levels of lactic acid; (c) levels of glucose; (d) protein levels of GLUT1, PKM2, and HK2 were estimated by western blotting; (e) protein levels of RAS, ERK, and p-ERK were estimated by western blotting. $^{***}P < 0.001$ vs. control, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. si-DEPDC1 + ov-NC. DEPDC1, DEP domain 1 protein; ECAR, extracellular acidification rate; GLUT1, glucose transporter 1; HK2, hexokinase 2; NC, negative control; OS, osteosarcoma; PKM2, pyruvate kinase M2; p-, phosphorylated; TTK, threonine tyrosine kinase.

be elucidated by detecting the degradation of p62 [34]. The present study found that interfering with DEPDC1 increased the LC3-II/I ratio and downregulated the expression of p62, with a significant difference observed in the si-DEPDC1 group compared with the si-NC group. Moreover, siBeclin1 and siATG5 significantly increased the levels of ECAR, glucose consumption, lactate production rate, and the expression of GLUT1, HK2, and PKM2 in OS cells upon interference with DEPDC1, suggesting that interfering with DEPDC1 could affect autophagy-dependent glycolysis levels in OS cells. The STRING database demonstrated that DEPDC1 can bind to TTK, which plays an important role in OS progression [17]. Therefore, the interaction between DEPDC1 and TTK and whether DEPDC1 can affect OS cells through TTK were further explored. The results of the present study showed that TTK overexpression decreased the LC3-II/I ratio, upregulated the expression of p62, and increased the levels of ECAR, glucose consumption, lactate production rate, and the expression of GLUT1, HK2, and PKM2, thereby inhibiting autophagy and prompting glycolysis in OS cells with DEPDC1 interference.

Numerous studies verified that RAS/ERK signaling plays a key role in autophagy and glycolysis [15,16,35]. Activation of RAS may gradually activate downstream proteins through phosphorylation, resulting in a complex cascade reaction [36,37]. Activation of ERK may be related to autophagic programmed cell death [38]. The present study revealed that interfering with DEPDC1 affects autophagy-dependent glycolysis levels in OS cells. Moreover, it was verified that DEPDC1 and TTK interact in OS cells. DEPDC1 affects the autophagy, glycolysis, and RAS/ERK pathway of OS cells through TTK.

To summarize, the current study identified that DEPDC1 regulated RAS/ERK signaling through TTK, affecting the level of autophagy-dependent glycolysis in human OS cells.

Acknowledgements

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

D.Y. and W.X. conceived and designed the study. L.C. acquired and interpreted the data. D.Y. was assisted in the writing of the manuscript. Y.L. and B.L. collected and analyzed data. All authors confirmed the authenticity of all the raw data and read and approved the final version of the manuscript.

Conflicts of interest

There are no conflicts of interest.

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