



TRIP13 knockdown inhibits the proliferation, migration, invasion, and promotes apoptosis by suppressing PI3K/AKT signaling pathway in U2OS cells

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Abstract

Background Although osteosarcoma (OS) is the most common malignant bone tumor, the biological mechanism underlying its incidence and improvement remains unclear. This study investigated early diagnosis and treatment objectives using bioinformatics strategies and performed experimental verification.

Methods and results The top 10 OS hub genes—*CCNA2*, *CCNB1*, *AURKA*, *TRIP13*, *RFC4*, *DLGAP5*, *NDC80*, *CDC20*, *CDK1*, and *KIF20A*—were screened using bioinformatics methods. *TRIP13* was chosen for validation after reviewing literature. *TRIP13* was shown to be substantially expressed in OS tissues and cells, according to Western blotting (WB) and quantitative real-time polymerase chain reaction data. Subsequently, *TRIP13* knockdown enhanced apoptosis and decreased proliferation, migration, and invasion in U2OS cells, as validated by the cell counting kit-8 test, Hoechst 33,258 staining, wound healing assay, and WB. In addition, the levels of p-PI3K/PI3K and p-AKT/AKT in U2OS cells markedly decreased after *TRIP13* knockdown. Culturing U2OS cells, in which *TRIP13* expression was downregulated, in a medium supplemented with a PI3K/AKT inhibitor further reduced their proliferation, migration, and invasion and increased their apoptosis.

Conclusions *TRIP13* knockdown reduced U2OS cell proliferation, migration, and invasion via a possible mechanism involving the PI3K/AKT signaling pathway.

Keywords Osteosarcoma · Thyroid hormone receptor interacting protein 13 · PI3K/AKT signaling pathway · Bioinformatics · U2OS

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Introduction

Osteosarcoma (OS) is an essential malignant tumor originating from mesenchymal stem cells [1]. OS most commonly occurs in adolescents under 18 years and elderly over 60 years of age [2]. The five-year survival rate of OS has increased to more than 60% in the past decade due to the rapid development of treatment methods [3]. However, metastasis occurs in most patients diagnosed with OS due to the lack of early diagnostic markers. Patients with metastatic OS have a poor survival rate (25–30%) and prognosis [4]. Therefore, it is essential to deeply understand the biological characteristics of OS, which can obtain the targets of diagnosis and treatment.

Thyroid hormone receptor-interacting protein 13 (TRIP13) is an anti-actin antibody-ATP family member. It is found on chromosome 5 and codes for a 432-amino-acid-residue protein. TRIP13 plays a vital role in meiosis and

mitosis, especially in spindle assembly checkpoints, chromosome synapses, and DNA break/repair [5]. TRIP13 is notably expressed in a variety of malignancies (breast, bladder, colon, and lung squamous cell cancers) and has been linked to a poor prognosis in numerous studies [6]. For example, the overexpression of TRIP13 enhances cell proliferation and invasion in lung cancer cells by stimulating the Wnt signaling pathway and epithelial-mesenchymal transformation (EMT) [7]. In bladder cancer, high TRIP13 expression can promote cancer cell growth and colony-forming ability and enhance the resistance of bladder cancer cells to cisplatin and doxorubicin [8]. Alternatively, TRIP13's role and probable mechanism in OS are unknown.

In this study, we first used bioinformatics to screen the differentially expressed genes (DEGs) of OS, and to identify the hub genes. We then verified a gene, *TRIP13*, selected from the hub genes by referring to literature. TRIP13's effects on U2OS cell proliferation, migration, invasion, and apoptosis, as well as its potential mechanism, were also investigated. These results provide a theoretical basis for targeted OS therapy.

Materials and methods

DEG screening in OS

GSE16088 and GSE14359, two OS-related GEO datasets, were retrieved from the GEO database. The RMA algorithm was first utilized to contextualize raw data. The limma package and ggplot2 package in R language were used to screen for dysregulated genes, and to draw a volcano plot showing the dysregulated gene expression levels. Screening thresholds were set for \log_2 fold change (FC) > 1 and adjusted P-value 0.05. The fold change refers to OS relative to the normal tissue. The dysregulated genes from GSE16088 and GSE14359 were introduced into the Venn diagram to obtain duplicate genes. These duplicate genes were used as the DEGs.

Functional enrichment of DEGs

Metascape (<http://metascape.org>) is a user-friendly integrated portal based on 40 independent databases for gene annotation, interactome analysis, and functional enrichment [9]. DEGs were imported into the Metascape database for GO enrichment (biological process [BP], cell components [CC], and cellular components [MF]) and KEGG pathway enrichment. The following were the filter conditions: min overlap=3, min enrichment=1.5, and P-value =0.05.

Protein-protein interaction (PPI) network construction and hub gene screening.

STRING is an online database that displays the interactive relationships between proteins. CytoHubba is a plug-in utility in Cytoscape that can identify the Hub gene according to different algorithms. The PPI network of DEGs with the minimum interaction score of 0.4 was built by using the STRING database. Then, we imported the TSV files of DEGs into Cytoscape and used CytoHubba to identify the top 10 hub genes, based on the MCC algorithm.

Cancer tissues and cell lines

We collected OS (n=20) and adjacent normal tissues (n=20) from the orthopedic department of Lanzhou University Second Hospital. These patients did not receive radiotherapy or chemotherapy before surgery. The removed tissue was immediately immersed in liquid nitrogen before being transferred to -80 °C refrigerator. The ethical committee of Lanzhou University Second Hospital approved this study (project number: 2021 A-407), and the patient or guardian provided written informed permission. The OS cell line, U2OS, and the osteoblast cell line, hFOB1.19, were purchased from Fu Heng Biology (Shanghai, China). MEM and DMEM were used to cultivate U2OS and hFOB1.19 cells, respectively. The culture medium has a 10% fetal bovine serum (purchased from American Gibco) concentration. All cells were grown at 37 °C with 5% CO₂.

Cell transfection

Lentiviruses expressing short hairpin RNA targeting *TRIP13* (shTRIP13) and scrambled shRNA (shCtrl) were obtained from the Ji Kai Gene (Shanghai, China). The sequence of shTRIP13 was 5'-TACTCAACAGACATAATA-3', and that of shCtrl was 5'-TTCTCCGAACGTGTACACGT-3'. First, the U2OS cells were treated with or without 50 μM LY294002, a PI3K / AKT inhibitor (catalog number: S1737) purchased from Beyotime Biotechnology Company (Shanghai) [10]. U2OS cells at 40% confluence were infected with lentiviral particles of shTRIP13 and shCtrl. After 72 h of infection, puromycin was added to the culture medium at a concentration of 2 μg/ml. The cells were cultured following the above method for one week to obtain stably transfected cells.

Western blotting (WB)

To obtain total protein, the OS cells and tissues were lysed in RIPA lysis buffer. A BCA protein analysis kit was used to quantify the protein content. The proteins were separated using a 12% SDS-PAGE gel and then transferred to a polyvinylidene fluoride (PVDF) membrane. After 2 h of blocking in a Tris-buffered saline (TBS) solution containing 5% skim milk, the

membranes were incubated with primary antibodies, namely TRIP13 (1:1000, Abcam, USA), Cleaved caspase-9 (1:1000, Abcam, USA), CDK4 (1:1000, Abcam, USA), CyclinD1 (1:1000, Abcam, USA), Ki-67 (1:1000, Abcam, USA), MMP2 (1:1000, Abcam, USA), MMP9 (1:1000, Abcam, USA), E-cadherin (1:1000, Abcam, USA), N-cadherin (1:1000, Abcam, USA), Bax (1:1000, Abcam, USA), Bcl-2 (1:1000, Abcam, USA), Cleaved caspase-3 (1:1000, Abcam, USA), PCNA (1:1000, Abcam, USA), and β -actin (1:1000, Abcam, USA), in 4 °C overnight. It was then incubated for 2 h at room temperature with secondary antibodies. The membranes were cleaned three times with TBST for ten minutes each time. Finally, the blots were visualized using the VersaDoc Imaging System (BioRad Laboratories Co., San Francisco, CA, USA). Image J software was used for the quantitative analysis.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Using TRIzol reagent, total RNA was extracted from frozen tissues and cells, followed by cDNA synthesis. qRT-PCR was carried out with the SYBR Green Pro Taq HS Premix (Accurate Biology, China) using LC96 (Roche, Switzerland). The relative gene expression was computed using the $2^{-\Delta\text{ct}}$ method with GAPDH as a reference. The following primers were used for qRT-PCR: TRIP13 forward (5'-3'): TCGGAAAGTGGCAAGCTGGTAAC; TRIP13 reverse (5'-3'): CATTTCGGGCGGCTGTGAGAC; GAPDH forward (5'-3'): CATGAGAAGTATGACAACAGCCT; GAPDH reverse (5'-3'): AGTCCTTCCACGATACCAAAGT.

Cell counting kit-8 (CCK-8)

At a density of 1000 cells/well, stable transfected U2OS cells were seeded into 96-well plates. 10 μ l CCK-8 (Yeasen, China) solution was added to each well after the cells had been grown for 0, 24, 48, and 72 h. The absorbance of the cells was measured at 450 nm using an ELx800UV reader (Bio Tek Instruments, Winooski, VT, USA) after 2 h of incubation at 37 °C.

Wound healing assay

In 6-well plates, stable transfected U2OS cells were injected. When the cell fusion rate reached 100%, the cell layer was scratched using a 1-mL pipette tip. After adding medium containing 2% FBS, the cells were placed in an incubator at 37 °C and 5% CO₂ for 72 h. Cell migration was observed under a microscope at 0, 24, 48, and 72 h after scratching. The migration rate at each time point (24, 48, and 72 h) relative to 0 h was then calculated. The calculation formula for the 24 h relative migration rate is 1–blank area (24 h)/blank area (0 h). The relative migration rates of the other two time points were calculated using the same method.

Hoechst 33,258 staining

Hoechst 33,258 staining (Solarbio, China) was used to detect apoptosis in stably transfected U2OS cells. The cells were first treated for 20 min with a paraformaldehyde glutaraldehyde mixed fixative. The cells were then rinsed in PBS and stained for 20 min with Hoechst 33,258 reagent. Finally, the cells were put under a fluorescent microscope after being rinsed with PBS to examine and photograph their state. We identified cells as apoptotic cells when the nuclei were dense and dense staining, or fragmented dense and dense staining.

Statistical analysis

GraphPad Prism version 8 was used for all statistical analyses. The mean and standard deviation are used to show all data. The discrepancies in the data were analyzed using the student's *t*-test and one-way ANOVA. The threshold for statistical significance was established at $p < 0.05$.

Results

Identification of DEGs in OS

The expression profiles of OS tissues were obtained from GSE16088 and GSE14359. According to the screening threshold, 1453 and 765 DEGs from extracted from GSE16088 and GSE14359 respectively, met the criteria. The distribution of DEGs is shown as a volcano plot (Fig. 1a, b). The Venn diagram showed a total of 211 shared genes from the two datasets (Fig. 1c).

GO and KEGG pathway enrichment of DEGs in OS

Using the Metascape database, we performed GO (BP, CC, and MF) and KEGG pathway enrichment analyses to better understand the biological roles of DEGs. BP enrichment revealed that DEGs were mainly enriched in regulation of proteolysis, extracellular matrix organization, mitotic cell cycle phase transition, response to oxidative stress, blood vessel development, regulation of chromosome separation, response to growth factors, leukocyte activation involved in immune response, skeletal system development, and regulation of cell adhesion (Fig. 2a).

In terms of CC, DEGs were significantly enriched in focal adhesions, collagen-containing extracellular matrix, tertiary granules, the cytoplasmic vesicle membrane, chromosomal regions, the lysosomal lumen, melanosomes, actin filaments, the MHC class II protein complex, and leading-edge membranes. (Fig. 2b).

The enriched MF items of DEGs mainly included integrin binding, protein heterodimerization activity, hyaluronic acid

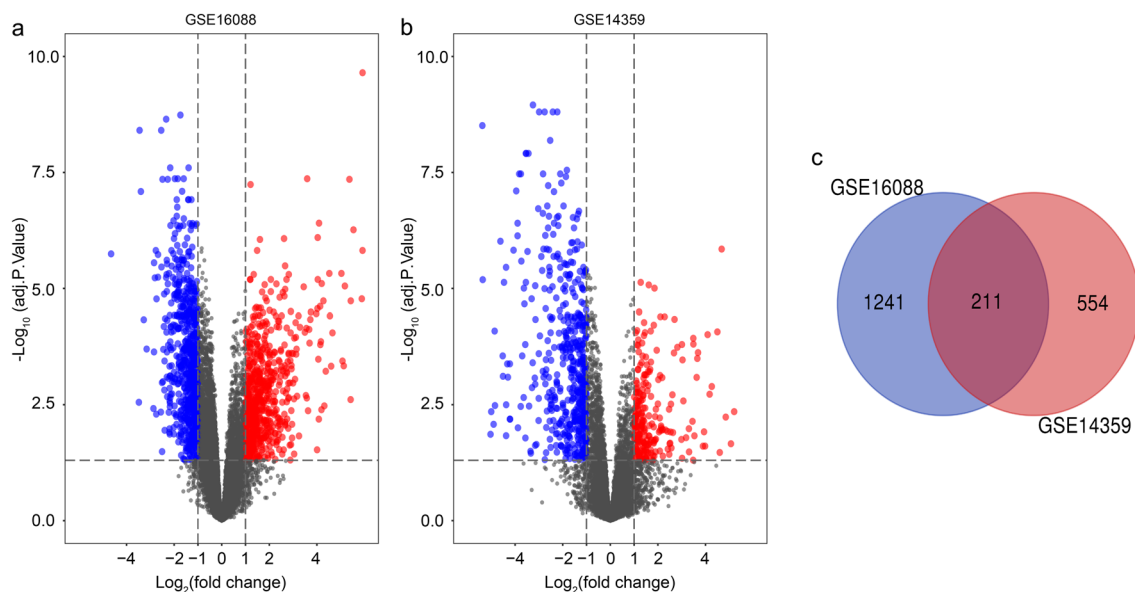


Fig. 1 Volcano plots and Venn diagrams of differentially expressed genes (DEGs) in OS samples and normal ones. **a** Volcano plot of GSE16088. **b** Volcano plot of GSE14359. **c** Venn diagrams of overlapping DEGs from GSE16088 and GSE14359

binding, exopeptidase activity, chemokine binding, peptide antigen binding, kinase binding, collagen binding, histone deacetylase binding, and histone kinase activity (Fig. 2c).

According to KEGG pathway enrichment analysis, the DEGs were substantially related with the following entries: cell cycle, Epstein Barr virus infection, p53 signaling pathway, focal adhesion, proteoglycans in cancer, complement and regulation cascades, DNA replication, PI3K Akt signaling pathway, lysosome, and plate activation (Fig. 2d).

PPI network construction and hub gene identification

The constructed PPI network included 210 nodes and 880 edges (Fig. 3a). MCC was used to pick out the hub genes in the PPI network. As shown in Fig. 3b, the top 10 hub genes were: cyclin-A2 (*CCNA2*), kinetochore protein NDC80 homolog (*NDC80*), G2/mitotic-specific cyclin-B1 (*CCNB1*), thyroid hormone receptor interactor 13 (*TRIP13*), aurora kinase A (*AURKA*), replication factor C subunit 4 (*RFC4*), kinesin-like protein KIF20A (*KIF20A*), cyclin-dependent kinase 1 (*CDK1*), disks large-associated protein 5 (*DLGAP5*), and cell division cycle protein 20 homolog (*CDC20*).

Expression of *TRIP13* in human OS tissues and cell lines

Based on the literature, we selected *TRIP13* to conduct the following research. First, we used WB to evaluate *TRIP13* expression in OS and adjacent normal tissues. *TRIP13* expression in OS tissues was dramatically higher than in

adjacent normal tissues (Fig. 4a, b). Using qRT-PCR, the same results were achieved (Fig. 4c). Second, we investigated *TRIP13* expression in U2OS cells using WB and qRT-PCR. *TRIP13* expression in U2OS cells was much higher than in hFOB1.19 cells (Fig. 4d–f). These results demonstrate that *TRIP13* may function as an oncogene in OS.

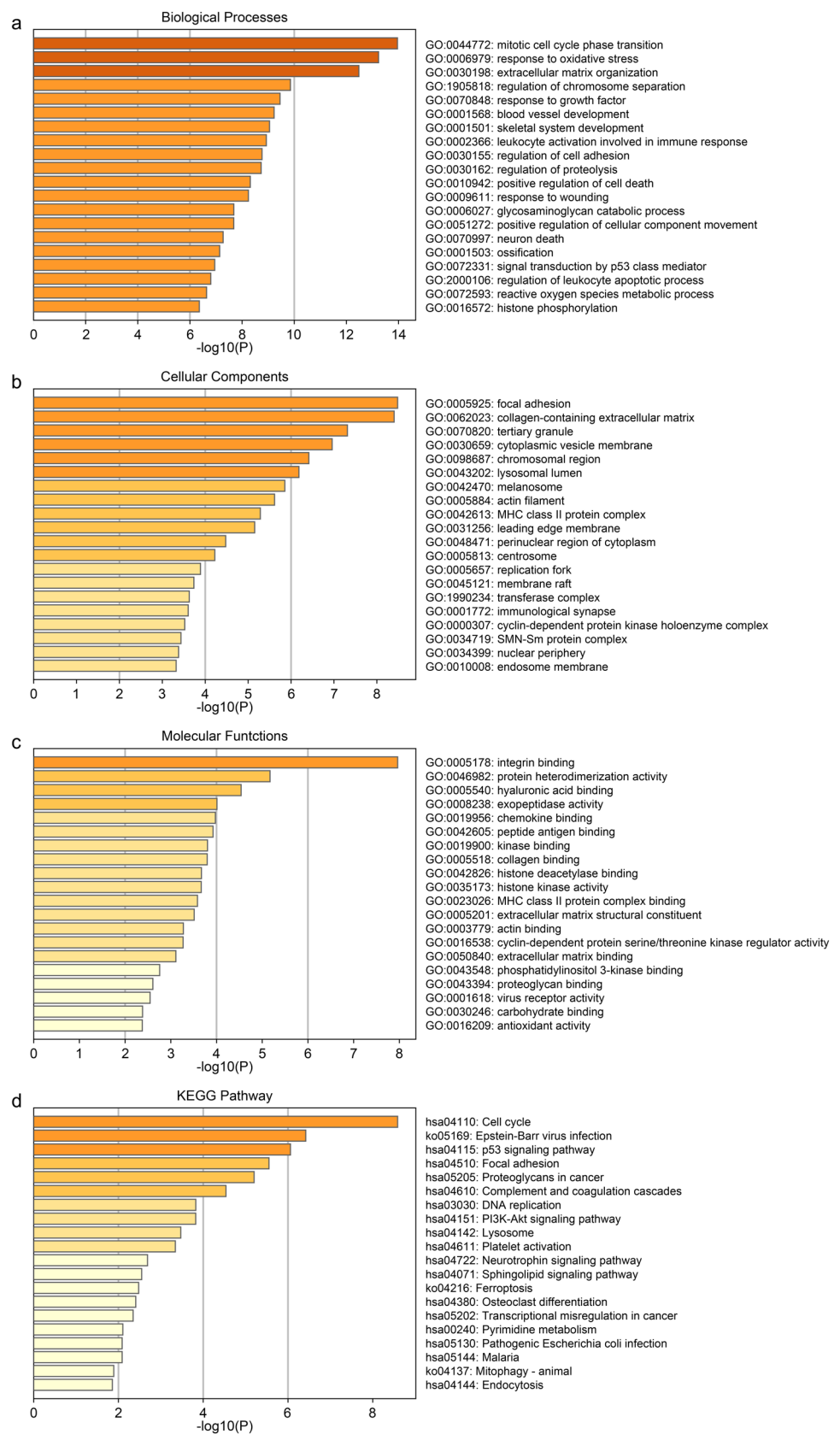
TRIP13 knockdown inhibits U2OS cell proliferation

We used lentiviruses carrying specific sequences to knock down *TRIP13* expression in U2OS cells to analyze the potential function of *TRIP13* in OS. WB and qRT-PCR results are shown in Fig. 4h–j. The blank control and shCtrl groups had substantially higher *TRIP13* expression than the sh*TRIP13* group. Next, we used CCK8 to explore the proliferation ability of these three groups of U2OS cells. *TRIP13* knockdown repressed U2OS cell proliferation substantially (Fig. 4g). Following that, WB revealed that in the sh*TRIP13* group, the expression levels of proliferation-related proteins (PCNA, CDK4, Ki67, and CyclinD1) were low (Fig. 4k, l). These results indicated that *TRIP13* knockdown inhibited U2OS cell proliferation.

TRIP13 knockdown prevents U2OS cells from migrating and invading

OS is a cancer that is very malignant and aggressive. WB and wound healing assays were used to investigate the effect of *TRIP13* on U2OS cell migration and invasion. WB was used to examine the expression of migration-related proteins, and the findings revealed that MMP2, MMP9, and

Fig. 2 Go enrichment and KEGG pathway enrichment of DEGs. **a** Biological processes of DEGs. **b** Cellular components of DEGs. **c** Molecular functions of DEGs. **d** KEGG pathway of DEGs



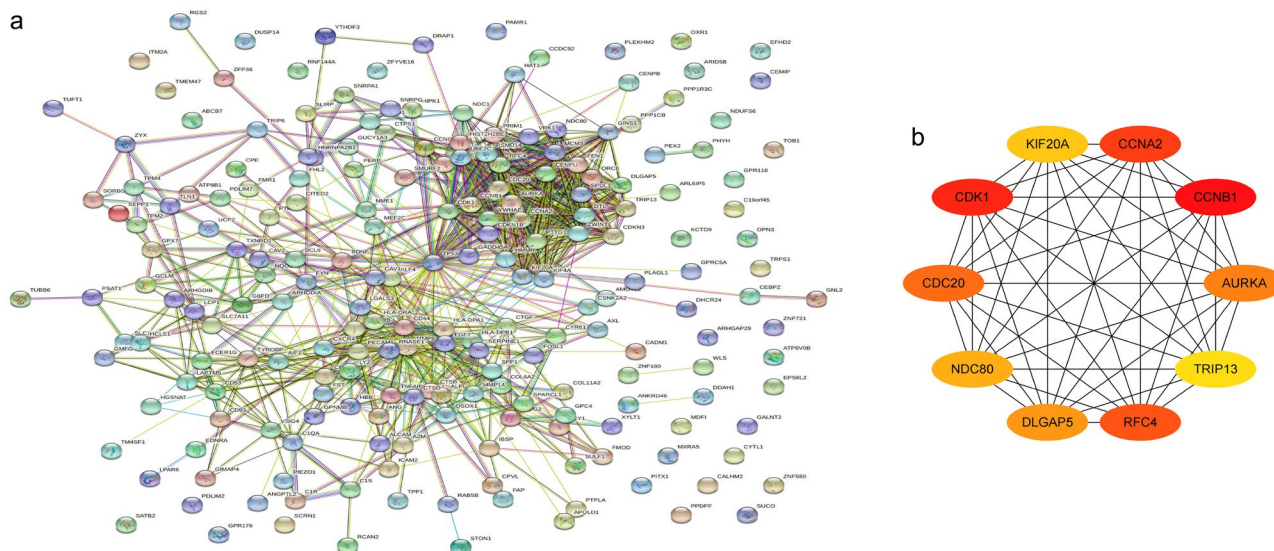


Fig. 3 Protein and protein interaction network (PPI) network of DEGs and screening of Hub gene. **a** PPI network of DEGs. **b** Top ten hub genes

N-cadherin levels in the *TRIP13* knockdown group were distinctly lower than those in the blank control and shCtrl groups. In contrast, E-cadherin expression levels were higher in *TRIP13* knockdown group (Fig. 4m, n). The wound healing assay also revealed that the negative and blank control groups' cell migration rates were considerably greater than the sh*TRIP13* group's (Fig. 4o, p). *TRIP13* knockdown decreased U2OS cell migration and invasion, according to these findings.

***TRIP13* knockdown induces U2OS cell apoptosis**

TRIP13's role in promoting apoptosis in OS was explored using Hoechst 33,258 staining. As shown in Fig. 4q, r, the apoptosis rate of U2OS cells in the sh*TRIP13* group was the highest. WB was used to analysis the expression levels of apoptosis-related proteins. Among them, the expression of cleaved caspase-3, Bax, and cleaved caspase-9 were significantly higher in the sh*TRIP13* group than those in the shCtrl and blank control groups. In contrast, Bcl-2 expression was lower in sh*TRIP13* group (Fig. 4s, t). These results suggest that *TRIP13* knockdown can promote apoptosis of U2OS cells.

***TRIP13* knockdown affects cell function in U2OS cells via suppressing the PI3K/AKT signaling pathway**

The enrichment of DEGs in KEGG pathways suggested that the PI3K/Akt pathway may be important in the development and progression of OS. As a result, we used WB to determine the expression levels of critical proteins in the PI3K/AKT pathway. The sh*TRIP13* group had substantially lower levels of p-AKT/AKT and p-PI3K/PI3K than the shCtrl and blank control groups (Fig. 5a, b). This indicated that *TRIP13* might act as an oncogene in OS via the PI3K/AKT pathway. Furthermore, we used the PI3K/Akt pathway inhibitor, LY294002, to specifically block the PI3K/Akt pathway in cells from the *TRIP13* knockdown group (Fig. 5c, d). Then, the functional changes in U2OS cells were evaluated following pathway inhibition.

First, CCK8 was utilized to observe the changes in U2OS cells proliferation ability after inhibition. The proliferation of U2OS cells treated with inhibitors was significantly stronger than that of cells without inhibitor treatment (Fig. 5g). Second, using a wound healing assay, we evaluated the migratory ability of U2OS cells. The migration ability of *TRIP13* knockdown U2OS cells was drastically decrease

in the inhibitor-treated group than in the untreated group (Fig. 5e, f). Subsequent WB results also showed that E-cadherin expression was drastically lower in the untreated inhibitor group than in the inhibitor-treated group. The untreated group, on the other hand, had greater levels of N-cadherin expression (Fig. 5c, d). Finally, Hoechst 33,258 staining and WB were used to investigate the effect of inhibitors on apoptosis in *TRIP13* knockdown U2OS cells. Hoechst 33,258 staining showed that the apoptotic rate of sh*TRIP13* U2OS cells in the inhibitor-treated group was significantly higher than that of the cells in the untreated group (Fig. 5j, k). Subsequent WB results also demonstrated that the expression levels of pro-apoptotic proteins (cleaved caspase-3, Bax, and cleaved caspase-9) in inhibitor-treated sh*TRIP13* knockdown U2OS cells were significantly higher than in untreated cells, but the expression of anti-apoptotic protein (Bcl-2) was lower (Fig. 5h, i). These results demonstrated that *TRIP13* knockdown affected the function of U2OS cells through the PI3K/Akt pathway.

Discussion

In children and adolescents, OS is the most common primary malignant bone tumor [11]. The advancement of treatment methods has resulted in a considerable increase in the survival rate of individuals with OS. However, because OS is prone to metastasis, recurrence, and drug resistance, patient prognosis remains poor [12, 13]. It is therefore crucial to improve our understanding regarding the occurrence and developmental mechanisms of OS and seek effective biological targets for diagnosis and treatment.

In this investigation, we first screened for 211 DEGs by analyzing OS-related chip data in the GEO database. After that, we ran GO and KEGG enrichment studies on DEGs. These genes were primarily correlated with chromosome separation, adhesion, and cell cycle, such as mitotic cell cycle phase transition, focal adhesion, regulation of chromosome separation, and cell cycle. Studies have shown that the knockdown of upregulated gene 4 (*URG4*) in OS cells can increase the proportion of G1 phase cells and reduce the proportion of S phase cells, affecting the OS cell cycle [14]. Abnormal chromosome segregation leads to chromosomal instability and often occurs in tumors [15]. Previous studies have shown that 3',4'-dihydroxyflavonol (DiOHF) can regulate the cell cycle and nuclear division, thereby inhibiting OS cell proliferation [16]. Additionally, fucoidan plays an

anticancer role in OS by reducing focal adhesion and inhibiting cell proliferation [17]. Subsequently, the PPI network of differentially expressed genes was constructed, and the top 10 hub genes (*CCNA2*, *CCNB1*, *AURKA*, *TRIP13*, *RFC4*, *DLGAP5*, *NDC80*, *CDC20*, *CDK1*, and *KIF20A*) were determined using Cytoscape. We selected the *TRIP13* gene for further study based on relevant literature.

TRIP13 was reported to be significantly expressed in OS cells and tissues. We then explored whether *TRIP13* affects the biological functions of OS cells. *TRIP13* knockdown decreased proliferation, migration, and invasion in U2OS cells while promoting apoptosis. This suggests that *TRIP13* is an oncogene. Consistent with our results, previous investigations found that *TRIP13* knockout can enhance glioma cell proliferation, migration, and invasion [18]. *TRIP13* knockout can also lower ovarian cancer cell proliferation, migration, and invasion while increasing apoptosis via the Notch signaling pathway [19]. The epithelial-mesenchymal transition (EMT) is important in tumor occurrence, migration, and invasion because it involves the loss of epithelial traits and the acquisition of mesenchymal features, which causes a loss of E-cadherin and an increase in vimentin expression [20]. EMT involves drastic cellular changes, namely that epithelial cells lose polarity, lose connections with other cells, and obtain stronger migration and invasion abilities [21]. Studies have shown that *TRIP13* regulates bladder cancer cell proliferation, migration, and invasion by controlling EMT [22]. Our study discovered that E-cadherin expression elevated in *TRIP13* knockdown U2OS cells, while the opposite was true for N-cadherin expression. This indicates that *TRIP13* knockdown inhibits U2OS cell migration and invasion by inhibiting EMT.

This study confirmed that *TRIP13* plays a biological role in OS. However, the mechanism by which *TRIP13* affects U2OS cells remains unclear. We explored the role of the PI3K/AKT signaling pathway in OS based on the results of KEGG pathway enrichment. The PI3K/AKT signaling pathway is involved in cell regulation, including epigenetics, autophagy, proliferation, migration, invasion, and EMT [23–26]. A growing body of evidence suggests that the PI3K/AKT signaling pathway is important in OS. Treatment of OS is largely based on the administration of cisplatin and other DNA-damaging drugs. Growth hormone-releasing hormone (GHRH) antagonists plus cisplatin have the additive anti-proliferative effect in several human tumors, including OS. The additive effect might be due to the antagonist's suppression of PI3K/AKT activation. Chemotherapeutic

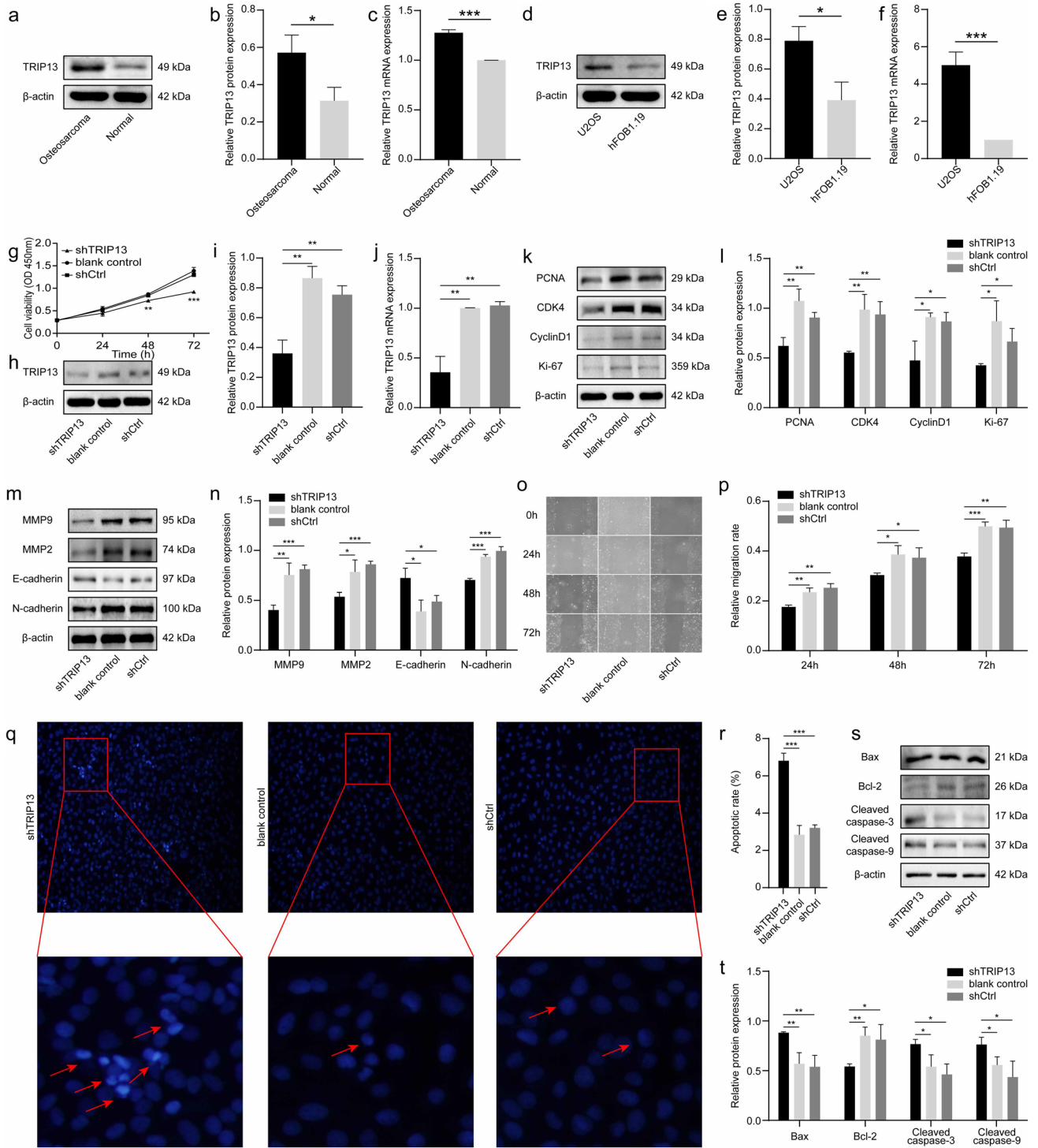


Fig. 4 TRIP13 knockdown inhibited the proliferation, migration, and invasion, and promoted apoptosis in U2OS cells. **a, b, d, and e** The protein expression levels of TRIP13 in osteosarcoma samples and U2OS cells were detected by western blot analysis. **c and f** The mRNA expression levels of TRIP13 in osteosarcoma samples and U2OS cells were detected by qRT-PCR. **g** U2OS cells proliferation after TRIP13 knockdown. **h and i** The protein expression levels of TRIP13 in U2OS cells following the infection of TRIP13 shRNA were detected by western blot analysis. **j** The mRNA expression levels of TRIP13 in U2OS cells following the infection of TRIP13 shRNA were detected by qRT-PCR. **k and l** After TRIP13 knockdown, the expressions of PCNA, CDK4, CyclinD1, and Ki-67 in U2OS cells were measured by western blot analysis. **m and n** After TRIP13 knockdown, the expressions of MMP9, MMP2, E-cadherin, and N-cadherin in U2OS cells were measured by western blot analysis. **o and p** After TRIP13 knockdown, the migration distance of U2OS cells was measured at the indicated time points by wound healing experiments. **q and r** After TRIP13 knockdown, the apoptotic rate of U2OS cells was measured by Hoechst 33,258 assay. **s and t** After TRIP13 knockdown, the expressions of Bax, Bcl-2, Cleaved caspase-3, and Cleaved caspase-9 in U2OS cells were measured by western blot analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

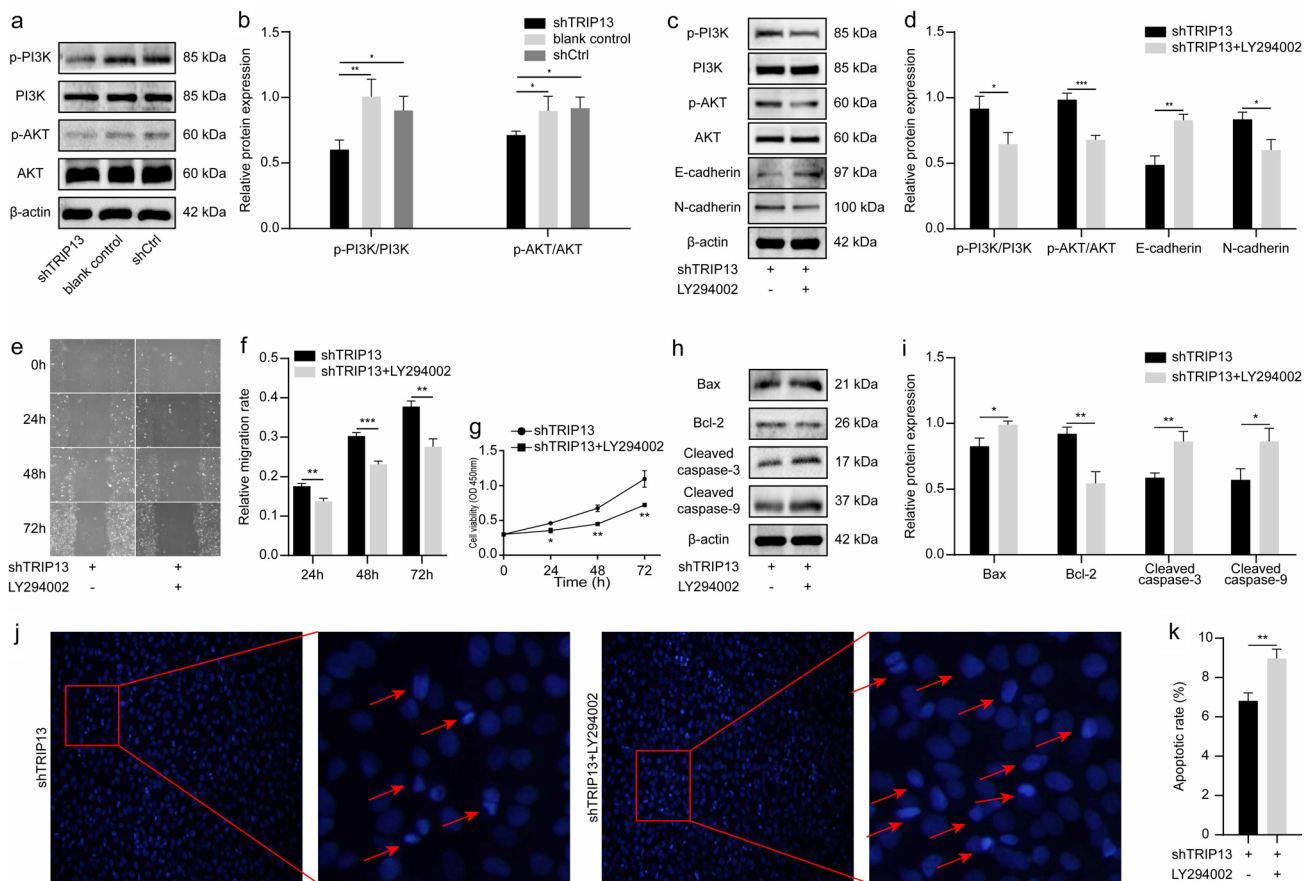


Fig. 5 TRIP13 knockdown inhibited the PI3K/Akt signaling pathway in U2OS cells. **a and b** After TRIP13 knockdown, the expressions of p-PI3K, PI3K, p-AKT, and AKT in U2OS cells were measured by western blot analysis. **c, d, h and i** After being treated with PI3K / Akt pathway inhibitor (LY294002), the expression of p-PI3K, PI3K, p-AKT, AKT, E-cadherin, N-cadherin, Bax, Bcl-2, Cleaved caspase-3, and Cleaved caspase-9 were measured in shTRIP13 transfected U2OS cells by western blot analysis. **e and f** After being

resistance is known to be conferred via Akt induction. As a result, treating OS patients with GHRH antagonists concurrently may improve chemosensitivity and hence the effectiveness of cytotoxic drugs such as cisplatin [27, 28]. Wu et al. demonstrated that PB2 reduced OS cell proliferation and increased U2OS cell apoptosis by inhibiting PI3K/AKT activity in vivo and in vitro [29]. In addition, Aziz et al. found that DK1 plays an anti-migration and anti-angiogenesis role in OS by influencing PI3K/AKT signaling [30]. *TRIP13* can increase the development of HCC via stimulating the AKT/mTOR pathway [31].

In conclusion, we demonstrated that *TRIP13* is highly expressed in OS and acts as an oncogene. *TRIP13* knockdown can reduce U2OS cell proliferation, migration, and invasion via a possible mechanism involving the PI3K/AKT signaling pathway. As a results, *TRIP13* is expected to be a focus for OS diagnosis and treatment.

treated with PI3K / Akt pathway inhibitor (LY294002), the migration distance of shTRIP13 transfected U2OS cells were measured at the indicated time points by wound healing experiments. **g** The shTRIP13 transfected U2OS cells proliferation after being treated with PI3K/ Akt pathway inhibitor (LY294002). **j and k** After being treated with PI3K/Akt pathway inhibitor (LY294002), the apoptotic rate of shTRIP13 transfected U2OS cells were measured by Hoechst 33,258 assay. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

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Author contributions Y-DC, C-XY and Z-HY conceived and designed the idea to this paper; L-MQ, G-XD, G-TW, ZK, Z-XB, Z-RH and H-YC participated in its design and coordination and supervised the study. Y-DC, Y-DQ and Y-XL analyzed the data and revised the final paper. All authors read and approved the final version of the manuscript. Y-DC was the first author of this article, Y-DC and C-XY contributed equally to this work.

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Data Availability The data used to support the findings of this study are available from the corresponding author upon request.

Code Availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Our study was approved by the ethics committee of the Second Hospital of Lanzhou University (Project Number: 2021 A-407).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication The authors affirm that human research participants provided informed consent for publication of the images in Fig. 4a and c.

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