Long Non-Coding RNA-DUXAP8 Regulates TOP2A in the Growth and Metastasis of Osteosarcoma via MicroRNA-635

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Research

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Abstract

Purpose: Osteosarcoma (OS) is a malignant tumor disease with high morbidity and mortality in children and adolescents. Evidence indicates that long non-coding RNAs (lncRNAs) may be important players in human cancer progression, including OS. In this study, we identified the role of lnc-DUXAP8 in the development of OS.

Materials and Methods: Expression of lncRNA DUXAP8 was determined by real-time quantitative PCR and Western blotting in OS tissues. Cell proliferation was evaluated using CCK8 and colony formation assay; Transwell assay was conducted to measure cell invasion. Cell migration was evaluated using Wound Healing assay. The binding site between the lnc-DUXAP8 and miR-635 RNAs was evaluated using a luciferase reporter assay.

Results: The expression of the lnc-DUXAP8 was significantly upregulated in OS samples and OS cell lines compared to normal tissue. High expression of lncRNA DUXAP8 was associated with shorter overall survival. Knockdown of lncRNA DUXAP8 inhibited proliferation and migration, and invasion in OS cells. More importantly, mechanism investigation revealed that lncRNA DUXAP8 was predominantly acted as a competing endogenous RNA (ceRNA) in OS by regulating miR-635/ TOP2A axis.

Conclusion: LncRNA DUXAP8 is upregulated in OS, and LncRNA DUXAP8 knockdown plays a vital anti-tumor role in OS cell progression through a miR-635/ TOP2A axis. Our study suggests that LncRNA DUXAP8 may be a novel, promising biomarker for diagnosis and prognosis of OS.

1. Introduction

Osteosarcoma (OS) is the most common primary sarcoma of the bone and mainly affects adolescents and children[1]. Due to its high degree of malignancy, early metastasis, less chance of surgery, easy recurrence and high mortality, OS causes an unacceptable mortality rate. Although significant improvements have been made in the treatment of OS during the past decade, the prognosis of osteosarcoma remains poor[2]. Reports suggested that the 5-year survival rate of OS patients with non-distant metastasis was approximately 60–70%[3]. However, 5-year survival rate of osteosarcoma patients with distant metastasis is only 20–30%[4]. Therefore, it is of paramount importance to investigate the molecular mechanisms underlying and pathogenesis of the development of OS.

Long non-coding RNAs (LncRNAs) are a type of non-coding nucleic acid with a length of > 200 nucleotides with diverse and largely uncharacterized biological functions[5]. Recently, increasing evidence demonstrated that lnc RNAs participate in fundamental cellular processes, including proliferation, migration and apoptotic processes, which are important in the development of cancer[6–7]. Previous studies have reported that lncRNAs function as oncogenes or tumor suppressors, are linked to cancer initiation and development, and lncRNAs may be dysregulated in various types of human cancer, including for OS[8]. For example, lncRNA SUMO1P3 promoted gastric cancer progression and invasion by

regulating EMT signaling pathway[9] and LncRNA AFAP1-AS1 accelerates nasopharyngeal carcinoma metastasis via sponging miR-423-5p to regulate the Rho/Rac pathway[10]. These findings indicate that lncRNAs could be vital regulators during tumorigenesis and tumor progression.

In recent years, pseudogene-derived lncRNA double homeobox A pseudogene 8 (DUXAP8) has been shown to be up-regulated in various malignant tumors. As a recently identified that DUXAP8 works as an oncogene in renal cell carcinoma, gastric cancer, and other tumors[11–12]. A recent study reports that in HCC, DUXAP8 repressed tumor suppressor KLF2 transcription through interacting with EZH2[13]. However, the expression status and prognostic value of DUXAP8 in OS is still unknown.

MicroRNAs (miRNAs) are approximately 22-nucleotide-long noncoding RNA molecules that could regulate the target gene expression levels via binding to the 3'untranslated regions (3'UTR) of target gene at post-transcriptional level and promote degradation or inhibit translation[14]. The miR-635 is located in 17q and has been recently identified in colorectal cancer[15]. Weber et al. found that miR-635 can significantly accelerate the invasion of A375 melanoma cells[16]. While the mechanism of miR-635 regulation in OS needs further investigation. Topoisomerase alpha 2 (TOP2A) is a marker of proliferation and chemotherapy resistance in different cancers, such as adrenocortical carcinoma and breast carcinoma[17–18]. Moreover, it has been reported that several miRNAs play a regulatory role by directly inhibiting target TOP2A in cancers[19].

In the current study, we showed for the first time that DUXAP8 were enhanced in OS cell lines and tissues. Downregulation of DUXAP8 remarkably suppressed OS cell viability and invasion. Additionally, we also confirmed that DUXAP8 could promote the development of OS cells by modulating miR-635/ topoisomerase 2 alpha (TOP2A). Our study may offer a novel diagnostic and therapeutic candidate for OS treatment.

2. Materials And Methods

2.1 Patient samples

A total of 35 OS patients' cancer tissues, that did not receive chemotherapy or radiotherapy before surgery, together with their adjacent normal tissues were collected from the Affiliated Hospital of Bei hua University (Ji lin, China). The tissues were subsequently stored at in liquid nitrogen prior to further experiments. All research protocols in this study was approved by the Ethics Committee of the the Affiliated Hospital of Bei hua University. Signed informed consent was got from every patient.

2.2 Cell lines and cell culture.

Human osteosarcoma cell lines, including KHOS-240S, SaOS2, MG-63, SOSP-9607 and U2OS and one normal osteoblastic cell line (hFOB1.19) were obtained from American Type Culture Collection (ATCC, Rockville, Maryland, USA) and the Cell Bank of the Chinese Academy of Sciences (Shanghai, China),

respectively. All cell lines were cultured according to the supplier's protocols. Cells were cultured in the Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 10% fetal bovine serum (FBS) and all incubations were performed at 37 °C in a 5% CO^2-containing atmosphere.

2.3 Cell transfection.

The short interference RNAs that targeted lncRNA DUXAP8 (si lncRNA DUXAP8), corresponding siRNA negative controls (siNC), miR-635 mimics and negative control (NC) miRNA were purchased from Gene Pharma (Shanghai, China). Transfections were performed using the Lipofectamine 3000 kit (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. The knockdown efficiency was assessed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) 48 h after transfection.

2.4 RNA extraction and RT-qPCR.

Total RNA from tissues and cells was isolated using Trizol (Invitrogen; Thermo Fisher Scientific, Inc.) per the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using Prime Script® RT reagent Kit (Takara, Japan). qPCR was performed using SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd., Dalian, China), detection method on an ABI 7500 RT- qPCR system. The primer sequences were as follows: DUXAP8 F:5'-AGGATGGAGTCTCGCTGTATTGC-3' and R: 5'- GGA GGTTTGTTTTCTTCTTTTTT-3'; miR-635 F: 50-TATAGCATATGCAGGGTG-30;

R: 5-CGCATTCGGAGTGCGAGTT-30; TOP2A F: 5’- GATTGATTATGACAAAGT

ATA-3’R: 5’-TACTTTGTCATAATCAATCAG-3’; GAPDH F:5'-CGCTCTCTGCTC

CTCCTGTTC-3' and R: 5'-ATCCGTTGACTCCGACCTTCAC-3'. The thermocycling conditions were as follows: Initial denaturation at 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec and extension at 60˚C for 1 min. The relative expressions of

DUXAP8, miR-635, and TOP2A mRNA were calculated using the 2-ΔΔCt method. The median value was the cutoff between low and high DUXAP8 expression in patients with osteosarcoma. The median value was included in the low group.

2.5 Cell proliferation assay

Cell proliferation was quantified using the Cell Counting Kit-8 (CCK-8; Beyo time Institute of Biotechnology, Shanghai, China) following the manual. Briefly, 1×10^5/well cells were seeded and transfected in a 96-well plate (Corning Incorporation). After indicated time, 10 μl of CCK-8 solution was added into each well, and the cells were incubated for 4 h at 37 ˚C. The absorbance was measured using a microplate reader (Bio-Tek Instruments,VT, USA) at 450 nm.

2.6 Wound healing assay

To measure the migratory ability of osteosarcoma cells, a wound-healing assay was performed. Cells were seeded and cultured to a confluent monolayer in the rectangular cell culture plate. The medium was removed, and then draw the teeth of the cell comb across the cell monolayer with sufficient force. Cells were then washed, replaced with fresh medium and incubated for additional 24 h. The wound closure was monitored with a light microscope (BX51, Olympus,Tokyo, Japan). Gap distance was quantified using NIH image J software.

2.7 Transwell assay

Transwell membranes with 8μm pore size (Corning Costar, MA, USA) coated with Matrigel (BD Biosciences, NJ, USA) were used for cancer cell invasion assay as previously described. After indicated transfection, cells were resuspended into fresh serum-free medium, and re-plated into the upper chamber. Fresh medium was directly added to the lower chamber. After additional 24 h incubation, the invasive cells penetrated to the lower surface, and were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) and stained with crystal violet (Sigma-Aldrich, MO, USA). The number of invasive cells was counted with a microscope (BX51, Olympus).

2.8 Colony formation assay

A colony formation assay was also performed. Cells were added to 6-well plates ($2x10^3$ cells/well) after transfection for 2 weeks. Colonies were fixed with 100% methanol at room temperature for 20 min and stained with 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) at 25˚C for 30 min. The total number of visible colonies were imaged and counted using a light microscope. All experiments were repeated three times.

2.9 Luciferase activity assay

All luciferase reporter vectors (DUXAP8-WT, DUXAP8-MUT) were constructed by Promega. DUXAP8-WT vector or DUXAP8-MUT vector, miR-635 mimics, or negative control miRNAs were co-transfected into OS cells, and after 48 hours of transfection, the relative luciferase activity was determined using Dual-luciferase Reporter Assay System (Promega).)

2.10 Western blotting

Western blotting was performed following the protocol as previously described. In brief, cells were lyzed with RIPA lysis buffer (Thermo Fisher Scientific), and incubated on ice for 30 min, and centrifuged at 11,000×g for 30 min at 4◦C. The supernatant was then collected and protein concentration was determined using BCA protein quantitation kit (Beyotime Institute of Biotechnology, Shanghai, China). 20 μg cell lysates were subjected to 10% SDS-PAGE per lane and transferred to polyvinylidene fluoride membranes (GE Healthcare, PA, USA). Then membranes were blocked with 5% bovine serum albumin in PBS for 1 h, and incubated with primary antibodies (1:1000) and GAPDH (1:5000) overnight at 4˚C. All of above antibodies were purchased from Cell Signaling Technology (MA, USA). Then the blots were washed

and probed with HRP-conjugated secondary antibodies for 1.5 h, respectively, and were visualized using an ECL detection system (Thermo Fisher Scientific). The relative densities of protein bands were analyzed with NIH Image J software.

2.11 Statistical analysis

All data shown in the graphs were analyzed with Prism 6.0 (GraphPad Software, CA, USA), and presented as mean± standard deviation of at least three independent experiments. Statistical analysis was performed using the Student's t-test for comparing two independent groups or one-way analysis of variance for multiple comparisons among three or more independent groups. $p<0.05$ was considered statistically significant.

3. Results

3.1 The expression of lncRNA DUXAP8 was upregulated in osteosarcoma tissues and cell lines.

To explore the role of lncRNA DUXAP8 in osteosarcoma, we examined the relative expression level of lncRNA DUXAP8 in 35 pairs of osteosarcoma tissues and adjacent non-tumor tissues was examined by RT-qPCR analysis. As presented in Fig. 1A, the level of lncRNA DUXAP8 was higher in OS tissues than in non-cancerous samples. Additionally, we also examined the expression of DUXAP8 in 5 human osteosarcoma cell lines (KHOS, SOSP-9607, U2OS, MG-63 and SaOS-2) and normal osteoblastic cell line hFOB1.19 by qRT-PCR. The results revealed that lncRNA DUXAP8 expression was remarkably enhanced in five OS cell lines compared with the hFOB1.19 cell line (Fig. 1B). To further figure out the relationship between DUXAP8 expression and longterm prognosis of patients, we performed Kaplan-Meier analysis based on TCGA patients using GEPIA. As shown, patients with higher DUXAP8 expression levels had shorter overall survival time than patients with lower DUXAP8 expressions (Fig. 1C). These results suggested that DUXAP8 was involved in the progression of OS.

3.2 DUXAP8 promoted the proliferation, migration, and invasion of osteosarcoma cells.

To determine the potential biological role of lncRNA DUXAP8 in OS cells, we selected two OS cell lines, U2OS and SaOS2 cells, with higher expression of lncRNA DUXAP8 to assess the effects of siRNA-mediated knockdown of lncRNA DUXAP8 on cell proliferation and colony formation. After transfection with lncRNA DUXAP8-specific siRNAs or a control siRNA, lncRNA DUXAP8 expression was efficiently decreased by qRT-PCR analysis (Fig. 2A). We observed that knockdown of lncRNA DUXAP8 caused a significant decrease in cell proliferation, as measured using CCK8 assays (Fig. 2B and C). We further examined the migration ability of U2OS and SaOS2 cells by scratch assay. The results showed that the migration ability of U2OS and SaOS2 cells were significantly decreased after knockdown of DUXAP8 (Fig. 2D). We also examined the effects of DUXAP8 on invasion of OS cells by Transwell assay. Consistently, the results demonstrated that knockdown of DUXAP8 inhibited the invasion of U2OS and SaOS2 cells (Fig. 2E). Colony formation assay further indicated that lncRNA DUXAP8 knockdown reduced

the colony numbers of U2OS and SaOS2 cells (Fig. 2F). Collectively, these results confirmed that DUXAP8 was an oncogenic lncRNA in OS.

3.3 DUXAP8 targeted miR-635 in osteosarcoma cells.

LncRNA can act as a molecular sponge to regulate the expression of downstream genes through absorbing miRNA. By searching for the potential binding miRNA of DUXAP8 in the StarBase online database (www.starbase.sysu. edu.cn), miR-635 was selected as a predictive target for DUXAP8 because of its high binding potential (Fig. 3A). Importantly, the expression correlation between DUXAP8 and miR-635 was analyzed in OS tissue samples and their expression was found to be negatively correlated (Fig. 3C). Furthermore, the expression of miR-635 was significantly downregulated in OS tissues and cells (Fig. 3B, D). Additionally, an increase in miR-635 expression was observed in U2OS and SaOS2 cells with DUXAP8 knockdown, suggesting that DUXAP8 can negatively regulate miR-635 expressions in OS (Fig. 3E). To further validate the binding relationship between miR-635 and DUXAP8, dual-luciferase reporter assay was performed and it showed that luciferase activity decreased significantly after the U2OS or SaOS2 cells were co-transfected with miR-635 and DUXAP8-WT reporter plasmids; however, the luciferase activity did not decrease significantly when the cells were co-transfected with miR-635 and DUXAP8-MUT reporter plasmids (Fig. 3F, G). Taken together, these results indicated that DUXAP8 negatively regulated miR-635 in OS.

3.4 miR-635 could reverse the function of DUXAP8 on osteosarcoma cells.

Next, we transfected the miR-635 mimics into U2OS or SaOS2 cells of OS cells with DUXAP8 knockdown. We found that overexpression of miR-635 attenuated the effect of overexpressing DUXAP8 on proliferation by CCK-8 and Colony formation assays (Fig. 4A, B and E). Then scratch and Transwell assays were conducted. As shown, overexpression of miR-635 attenuated the effect of overexpressing DUXAP8 on migration, and invasion of OS cells (Fig. 4C, D). These findings indicated that miR-635 reversed the function of DUXAP8 in OS cells.

3.5 Regulation of DUXAP8/ miR-635 on TOP2A expression in osteosarcoma cells.

After confirming that DUXAP8 can regulate miR-635 expression, we attempted to explore the downstream targets of miR-635 in OS. A recent study demonstrates that TOP2A, an oncogene, is a target of miR-635, so we investigated whether DUXAP8 can regulate TOP2A expression in OS. As shown, DUXAP8 knockdown significantly reduced mRNA and protein expressions of TOP2A (Fig. 5A, B). Furthermore, our results revealed that overexpression of miR-635 reduced the TOP2A expression and abolished the DUXAP8-induced upregulation of TOP2A (Fig. 5C). Furthermore, we also found that the expression of TOP2A mRNA and DUXAP8 were positively correlated in OS tissue samples, while the expression of TOP2A mRNA was negatively correlated with the expression of miR-635 (Fig. 5D, E). These results indicated that DUXAP8 could upregulate TOP2A expression in OS and promote OS progression, probably by modulating miR-635.

4. Discussion

Osteosarcoma (OS) is thought to be one of most common cause of cancer-related mortality worldwide, with only 8% overall 5-year survival[20]. Increasing evidence suggests that lncRNAs are involved in the development and progression of a diverse range of cancer types, including gastric cancer, hepatocellular carcinoma, clear cell renal cell carcinoma, colorectal cancer, breast cancer, non-small cell lung cancer and osteosarcoma[21–24]. For example, the lncRNA OSA3, which is specifically upregulated in prostate cancer, has been approved by the Food and Drug Administration (FDA) for the diagnosis of prostate cancer[25]. Downregulation of lncRNA HOST2 represses cell proliferation and promotes cell apoptosis in osteosarcoma, which may offer a potential therapeutic target for osteosarcoma[26]. The regulation of lncRNA on OS proliferation and metastasis has been studied, but not for LncRNA DUXAP8. LncRNA DUXAP8 has been previously reported to be upregulated and may serve as a potential therapeutic target in several types of cancer[27]. In a recent study, it was reported that LncRNA DUXAP8 enhances renal cell carcinoma progression via downregulating miR-126[28]. In our present study, we found that LncRNA DUXAP8 was expressed significantly higher in OS cell lines and tissues, suggesting that LncRNA DUXAP8 might contribute to the progression of OS. Consistent with previous studies, our study showed that LncRNA DUXAP8 silencing significantly inhibited OS cell growth, cell migration and invasion ability, implying that LncRNA DUXAP8 played an important role in OS progression.

MicroRNAs (miRNAs) are members of non-coding RNAs that play a spectacular regulatory role through acting as tumor promoters or suppressors in various cancers. For example, miRNA-214 suppression contributes to cell migration, invasion, and EMT in gastric cancer by targeting FGF9[29]. Various studies have focused on the role of miRs in OS cells. However, few reports have demonstrated the effect of miR-635 in OS. A recent report demonstrated that miR-635 may accelerate invasion of A375 melanoma cells[30]. The present study demonstrated that miR-635 may function as a tumor suppressor in OS, as determined by experiments with human specimens and OS cell lines in vitro study. In this study, we showed that miR-635 could be sponged by LncRNA DUXAP8 in OS. We validated the binding relationship between DUXAP8 and miR-635 by dual-luciferase reporter gene assay. Additionally, knockdown of DUXAP8 significantly induced the expression of miR-635 in OS. Importantly, with functional experiments, we demonstrated that miR-635 could reverse the function of DUXAP8 on OS cells. Our results not only explained the mechanism of miR-635 dysregulation in OS, but also proved that miR-635 was a crucial effector during DUXAP8 regulating the malignant phenotypes of OS cells. TOP2A has been found to be upregulated in hepatocellular carcinoma based on several studies [31–32]. Most studies have focused on TOP2A and reported involvement of tumor chemoresistance to DNA-damaging agents in AML as well as other tumors [33–35]. We found that TOP2A can be enrolled in the development of OS cells as a carcinogenic factor and may be one of the candidate targets for miR-635. Knockdown of DUXAP8 significantly reduced TOP2A mRNA and protein expression, and DUXAP8 was positively correlated with TOP2A expression in OS tissues. Interestingly, expression of miR-635 partially reversed the promotion of TOP2A expression caused by DUXAP8. These results manifested that DUXAP8 can target miR-635 to indirectly regulate TOP2A and thus affect the development of OS cells.

To conclude, our study demonstrated that DUXAP8 is upregulated in OS tissues and cells. lncRNA DUXAP8 knockdown can suppress cell proliferation, cell migration, cell invasion in U2OS or SaOS2 cells. This study also elucidates the mechanism of DUXAP8/miR-635/ TOP2A axis in the development of OS. With the deepening of research, DUXAP8 is likely to become a marker for clinical diagnosis and prognosis, and even be a therapeutic target of OS.

Declarations

Ethics approval and consent to participate

All research protocols in this study was approved by the Ethics Committee of the the Affiliated Hospital of Bei hua University. Signed informed consent was got from every patient.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests:

The authors declare no competing financial interests.

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Authors' contributions:

Conceptualization: Yang ting. Data curation: Yang ting, Guo jian ping, Li fan. Formal analysis: Yang ting. Investigation: Yang ting, Duan xian liang. Methodology: Yang ting, Duan xian liang. Resources: Wang hua, Xiu chao. Software: Wang hua, Xiu chao. Supervision: Duan xian liang. Validation: Yang ting. Visualization: Guo jian ping, Li fan. Writing-original draft: Yang ting. Writing-review & editing: Duan xian liang. Approval of final manuscript: all authors.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018, CA Cancer J. Clin. 2018;68(1):7–30.
2. Harrison DJ, Geller DS, Gill JD, Lewis VO, Gorlick R. Current and future therapeutic approaches for osteosarcoma. Expert Rev Anticancer Ther. 2018;18(1):39–50.
3. Strobel O, Neoptolemos J, Jager D, Buchler MW. Optimizing the outcomes of osteosarcoma surgery. Nat Rev Clin Oncol. 2018.
4. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: current treatment and a collaborative pathway to success. J Clin Oncol. 2015;33(27):3029–35.
5. Chen LL, Carmichael GG. Long noncoding RNAs in mammalian cells: what, where, and why? Wiley Interdiscip Rev RNA. 2010;1(1):2–21.
6. Iyer MK, Niknafs YS, Malik R. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47:199–208.
7. Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer Discov. 2011;1:391–407.
8. Rafiee A, Riazi-Rad F, Havaskary M, Nuri F. Long noncoding RNAs: regulation, function and cancer. Biotechnol Genet Eng Rev. 2018;34:153-180.
9. Mei D, Song H, Wang K, Lou Y, Sun W, Liu Z, et al. Up-regulation of SUMO1 pseudogene 3 (SUMO1P3) in gastric cancer and its clinical association. Med Oncol. 2013;30:709.
10. Lian Y, Xiong F, Yang L, Bo H, Gong Z, Wang Y. Long noncoding RNA AFAP1-AS1 acts AS a competing endogenous RNA of miR-423-5p to facilitate nasopharyngeal carcinoma metastasis through regulating the Rho/Rac pathway. J Exp Clin Cancer Res. 2018;37:25310.
11. Xu LJ, Yu XJ, Wei B, et al. Long non-coding RNA DUXAP8 regulates proliferation and invasion of esophageal squamous cell cancer. Eur Rev Med Pharmacol Sci. 2018;22(9):2646–52.
12. Ma HW, Xie M, Sun M, et al. The pseudogene derived long noncoding RNA DUXAP8 promotes gastric cancer cell proliferation and migration via epigenetically silencing PLEKHO1 expression.
13. Oncotarget. 2016;8(32):52211–52224.
14. Jiang H, Shi X, Ye G, et al. Up-regulated long non-coding RNA DUXAP8 promotes cell growth through repressing Krüppel-like factor 2 expression in human hepatocellular carcinoma. Onco.
15. Targets Ther. 2019;11(12):7429–7436.
16. ambRoS v. The functions of animal microRNAs. Nature. 2004;431:350–5.
17. Cummins JM, He Y, Leary RJ, Pagliarini R, Diaz LA Jr, Sjoblom T, et al., The colorectal microRNAome, Proc. Natl. Acad. Sci. U. S. A. 2006; 103: 3687–3692.
18. Weber CE, Luo C, Hotz-Wagenblatt A, Gardyan A, Kordaß T, Holland-Letz T, et al. miR- is a tumor suppressor in melanoma. Cancer Res. 2016;76(12):3562–71.
19. TOP2A is overexpressed and is a
Jain M, Zhang L, He M, Zhang YQ, Shen M, Kebebew E. TOP2A is overexpressed and is a.
20. therapeutic target for adrenocortical carcinoma. Endocr Relat Cancer. 2013;20:361–70.

21. Ejlertsen B, Jensen MB, Nielsen KV, Balslev E, Rasmussen BB, Willemoe GL, et al. HER2, TOP2A, and TIMP-1 and responsiveness to adjuvant anthracycline-containing chemotherapy in high-risk breast cancer patients. J Clin Oncol. 2010;28:984–90.
22. The resistance of esophageal cancer cells to paclitaxel
Zhang S, Jiang H, Xu Z. The resistance of esophageal cancer cells to paclitaxel.
23. can be reduced. by the knockdown of long noncoding RNA DDX11-AS1 through TAF1/TOP2A inhibition. Am J Cancer Res. 2019;9(10):2233–48.
24. Lulla RR, Costa FF, Bischof JM, Chou PM, de F Bonaldo, Vanin M. EF, et al. Identification of differentially expressed MicroRNAs in osteosarcoma. Sarcoma. 2011: 732690.
25. Yang C, Wu K, Wang S, Wei G. Long non–coding RNA XIST promotes osteosarcoma progression by targeting YAP via miR–195–5p. J Cell Biochem. 2018;119:5646–56.
26. Liu X, Wang Y, Sun L, Min J, Liu J, Chen D, et al. Long noncoding RNA BC005927 upregulates EPHB4 and promotes gastric cancer metastasis.
27. under hypoxia. Cancer Sci. 2018;109:988–1000.
28. Liang Y, Ma Y, Li L, Shen X, Xin T, Zhao Y, Ma R. Effect of long non–coding RNA LINC01116 on biological behaviors of non–small cell lung cancer cells via the hippo signaling pathway. J Cell Biochem. 2018;119:6310.
29. Zhang Y, Tao Y, Li Y, Zhao J, Zhang L, Zhang X, et al. The regulatory network analysis of long noncoding RNAs in human colorectal cancer. Funct Integr Genomics. 2018;18:261–75.
30. an n, cHeng d. The long noncoding RNA HOST2 promotes gemcitabine resistance in human osteosarcoma cells. Pathol Oncol Res. 2018;13:211–25.
31. Lu W, Zhang H, Niu Y, Wu Y, Sun W, Li H, et al. Long non-coding RNA lincDUXAP8 regulated non-small cell lung cancer proliferation, migration, invasion and epithelial mesenchymal transition by sponging miR-150-5p. Mol Cancer. 2017;16:118.
32. Lin MG, Hong YK, Zhang Y, Lin BB, He XJ. Mechanism of lncRNA DUXAP8 in promoting proliferation of bladder cancer cells by regulating PTEN. Eur Rev Med Pharmacol Sci. 2018;22(11):3370–7.
33. Huang T, Wang X, Yang X, et al. Long non-coding RNA DUXAP8 enhances renal cell carcinoma progression via downregulating miR-126. Med Sci Monit. 2018;14(24):7340–7.
34. Wang R, Sun Y, Yu W, Yan Y, Qiao M, Jiang R, et al. Downregulation of miRNA-214 in cancer-associated fibroblasts contributes to migration and invasion of gastric cancer cells through targeting FGF9 and inducing EMT. J Exp Clin Cancer Res. 2019;38:20.
35. Weber CE, Luo C, Hotz-Wagenblatt A, Gardyan A, Kordaß T, Holland-Letz T, et al. miR-339-3p is a tumor suppressor in melanoma. Cancer Res. 2019;76:3562–71.
36. Wong N, Yeo W, Wong WL, Wong NL, Chan KY, Mo FK, et al. TOP2A overexpression in hepatocellular carcinoma correlates with early age onset, shorter patients' survival and chemoresistance. Int J Cancer. 2009;124:644–52.

37. Panvichian R, Tantiwetrueangdet A, Angkathunyakul N, Leelaudomlipi S. TOP2A amplification and overexpression in hepatocellular carcinoma tissues. Biomed Res Int. 2015;2015:381602.
38. Chekerov R, Klaman I, Zafrakas M, Ko¨nsgen D, Mustea A, Petschke B, et al. Altered expression pattern of topoisomerase IIalpha in ovarian tumor epithelial and stromal cells after platinum-based chemotherapy. Neoplasia. 2006;8:38–45.
39. Chen CC, Gau JP, You JY, Lee KD, Yu YB, Lu CH, et al. Prognostic significance of beta-catenin and topoisomerase IIalpha in de novo acute myeloid leukemia. Am J Hematol. 2009;84:87–92.
40. Chen CF, He X, Arslan AD, Mo YY, Reinhold WC, Pommier Y, et al. Novel regulation of nuclear factor-YB by miR-485-3p affects the expression of DNA topoisomerase IIa and drug responsiveness. Mol Pharmacol. 2011;79:735–41.
41. **1 The expression of lncRNA DUXAP8 was upregulated in osteosarcoma tissues and cell lines**.

Figures

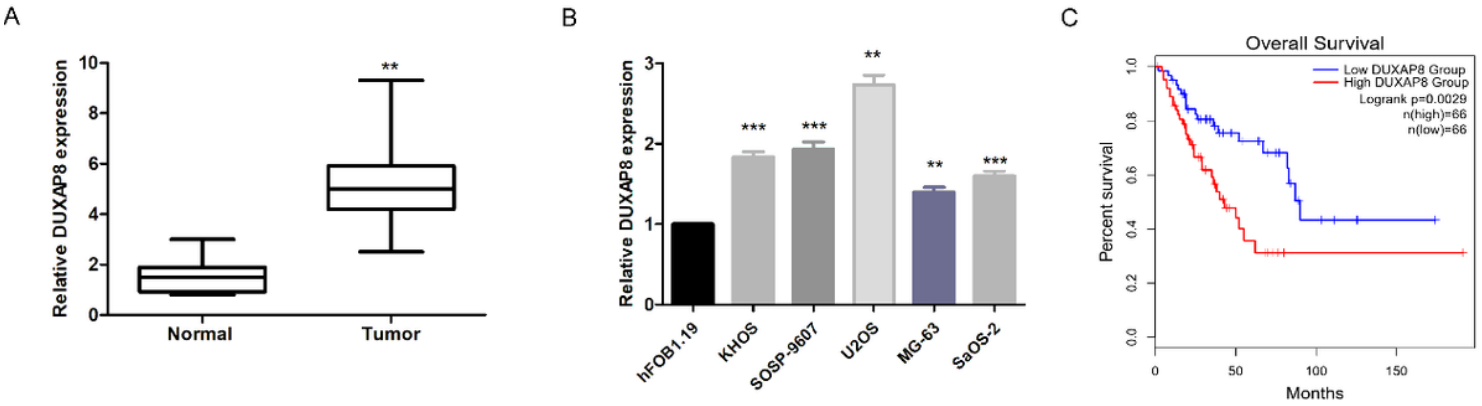

Figure 1

lncRNA DUXAP8 expression is increased in OS tissues and cell lines. A, Expression of lncRNA DUXAP8 was measured using qRT-PCR in OS tissues and healthy adjacent tissues. Data were expressed as the mean ± SD, Student's t-test. B, qRT-PCR analysis was used to determine lncRNA DUXAP8 expression in KHOS, SOSP-9607, U2OS, MG-63, SaOS-2 and normal osteoblastic cell line hFOB1.19. C, Survival analysis of OS patients with high and low expression of DUXAP8. Data are presented as mean ± SD of fold change, One-way ANOVA. **P < .01, ***P < .001.

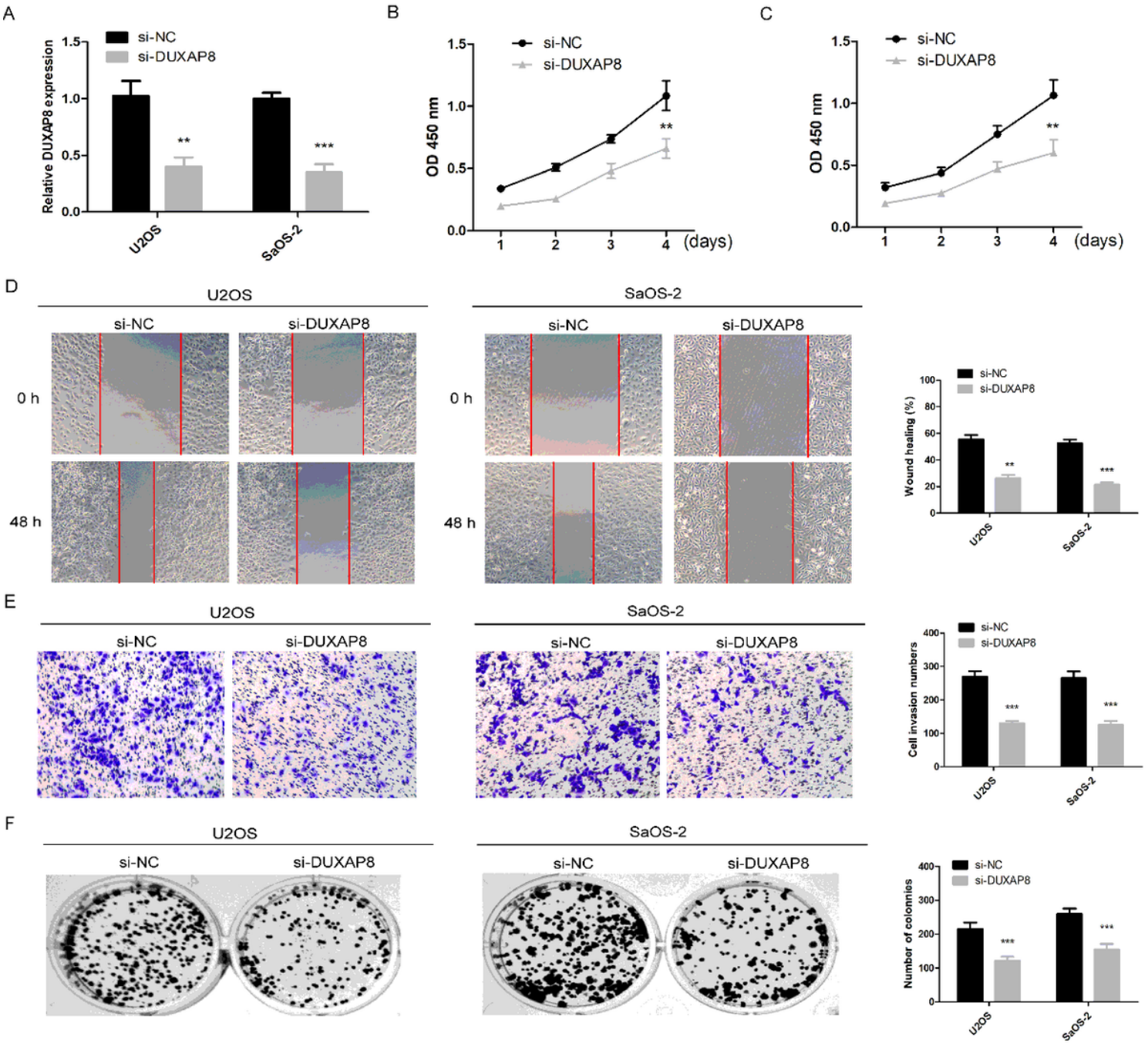

Figure 2

DUXAP8 promoted osteosarcoma proliferation, migration, and invasion. A, lncRNA DUXAP8 expression was detected in U2OS and SaOS-2 cells transduced with a lncRNA DUXAP8 siRNA vector (si lncRNA DUXAP8) or negative control siRNA vector (si NC). B, C and F, Cell viability was measured using an CCK8 assay and colony formation assay at the indicated times after transfection. D, the migration of OS cells after DUXAP8 knockdown was detected by scratch healing assay. E, the invasion of OS cells after DUXAP8 knockdown was detected by Transwell assay. Data were expressed as the mean ± SD, Student's t-test. **P < .01, ***P < .001.

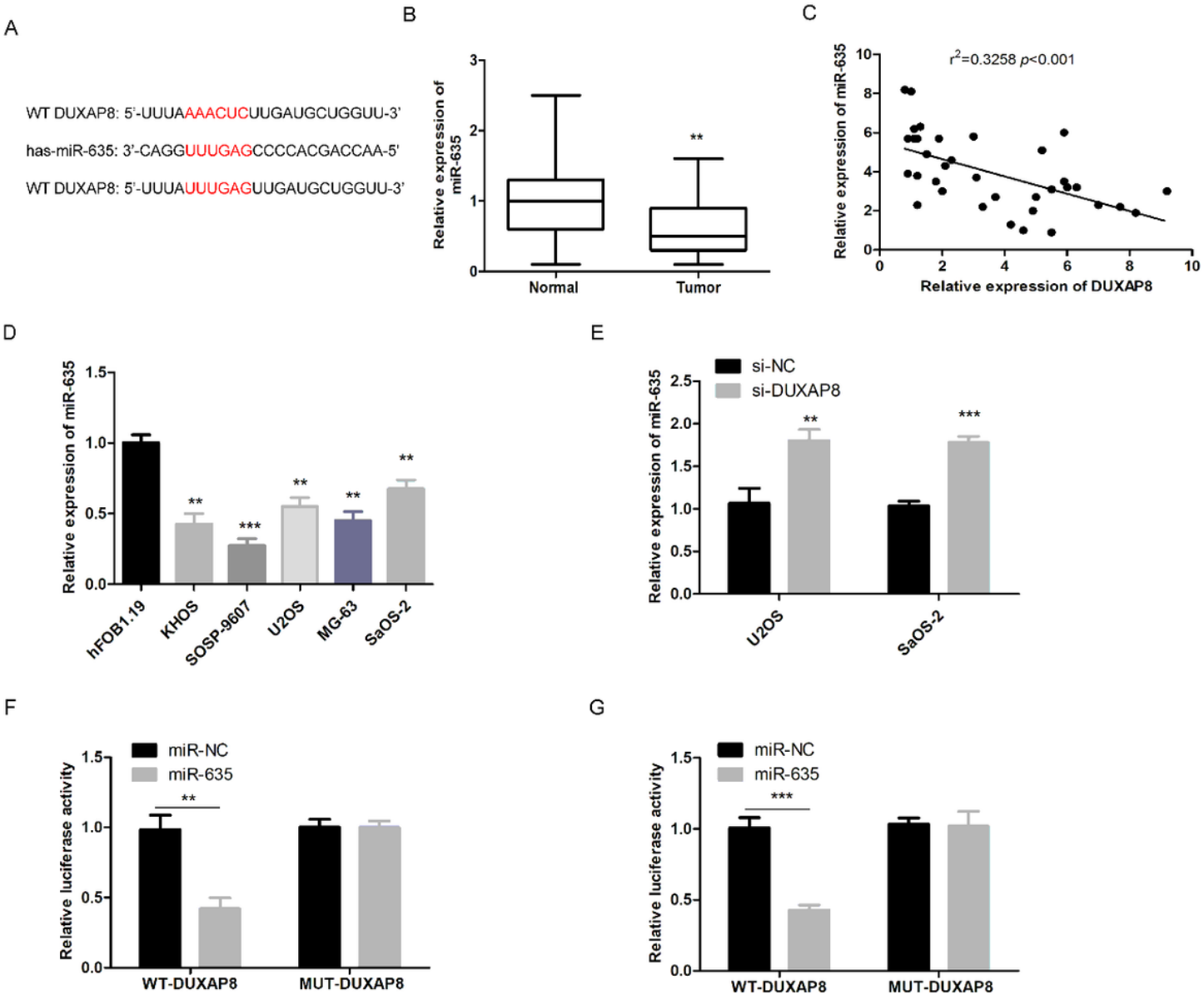

Figure 3

DUXAP8 targeted miR-635 in osteosarcoma. A, Prediction of binding sites between miR-635 and DUXAP8. B, the expression levels of miR-635 in 35 matched OSs and adjacent nontumor controls were detected by qRT-PCR. C, the correlation between the expression levels of DUXAP8 and miR-635 in OS samples was analyzed. D, the expression of miR-635 in OS cell lines and normal osteoblastic cell line hFOB1.19 was detected by qRT-PCR. E, the expression levels of miR-635 in OS cell lines were detected by qRT-PCR after DUXAP8 knockdown. F, G, Dual-luciferase reporter assay indicated that miR-635 mimics could reduce the luciferase activity of wild-type DUXAP8 reporter in U2OS and SaOS-2 cells. **P < .01, ***P < .001.

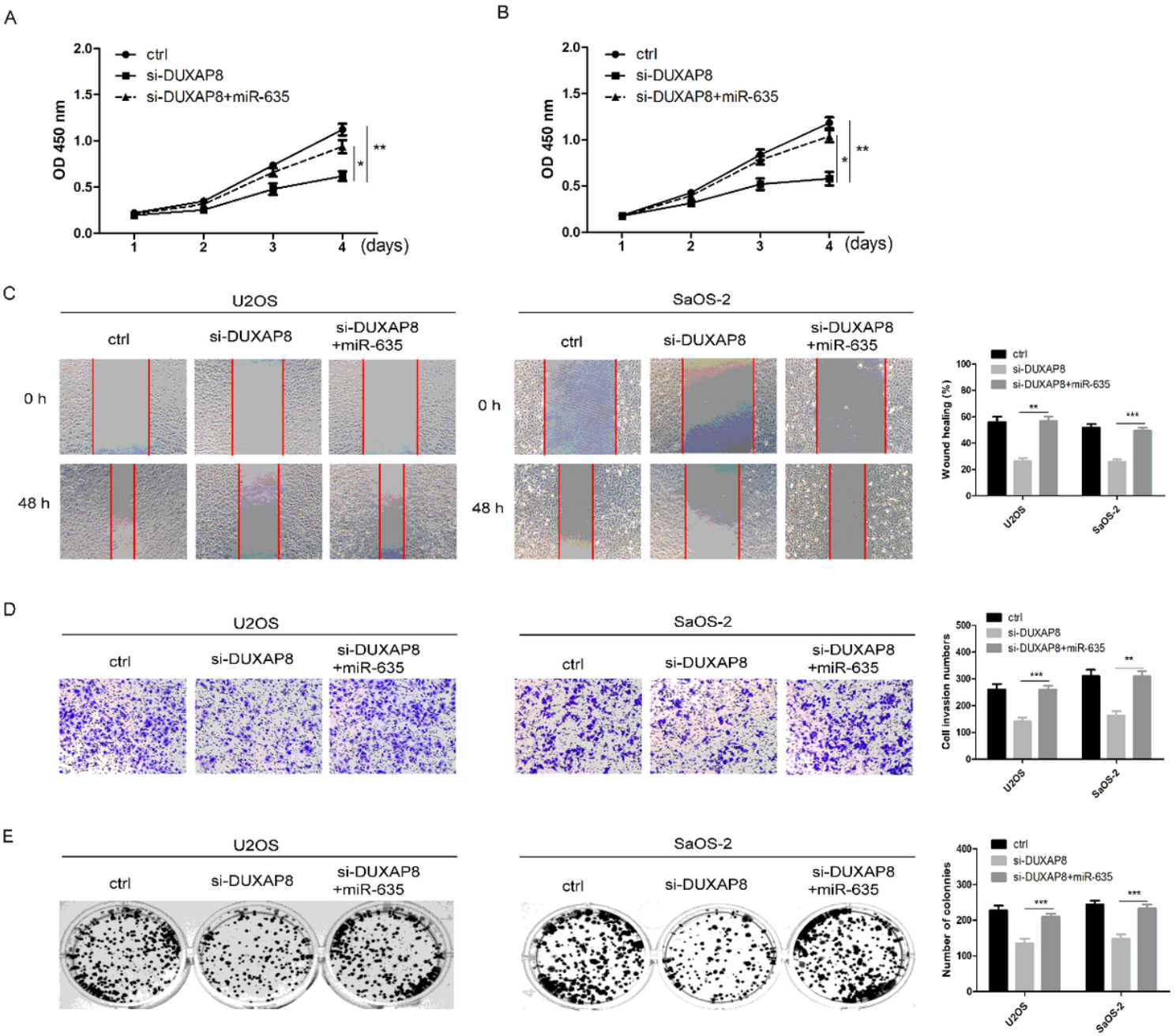

Figure 4

miR-635 could reverse the function of DUXAP8 in osteosarcoma. A, B, miR-635 mimics and DUXAP8 siRNA were co-transfected into OS cells, the proliferation of OS cells in each group was detected by CCK-8 assay. C, the migration in each group were evaluated by scratch healing assay. D, the invasion of OS cells in each group were evaluated by Transwell assay. E, the cell viability in each group were measured using colony formation assay. *P < .05, **P < .01, ***P < .001.

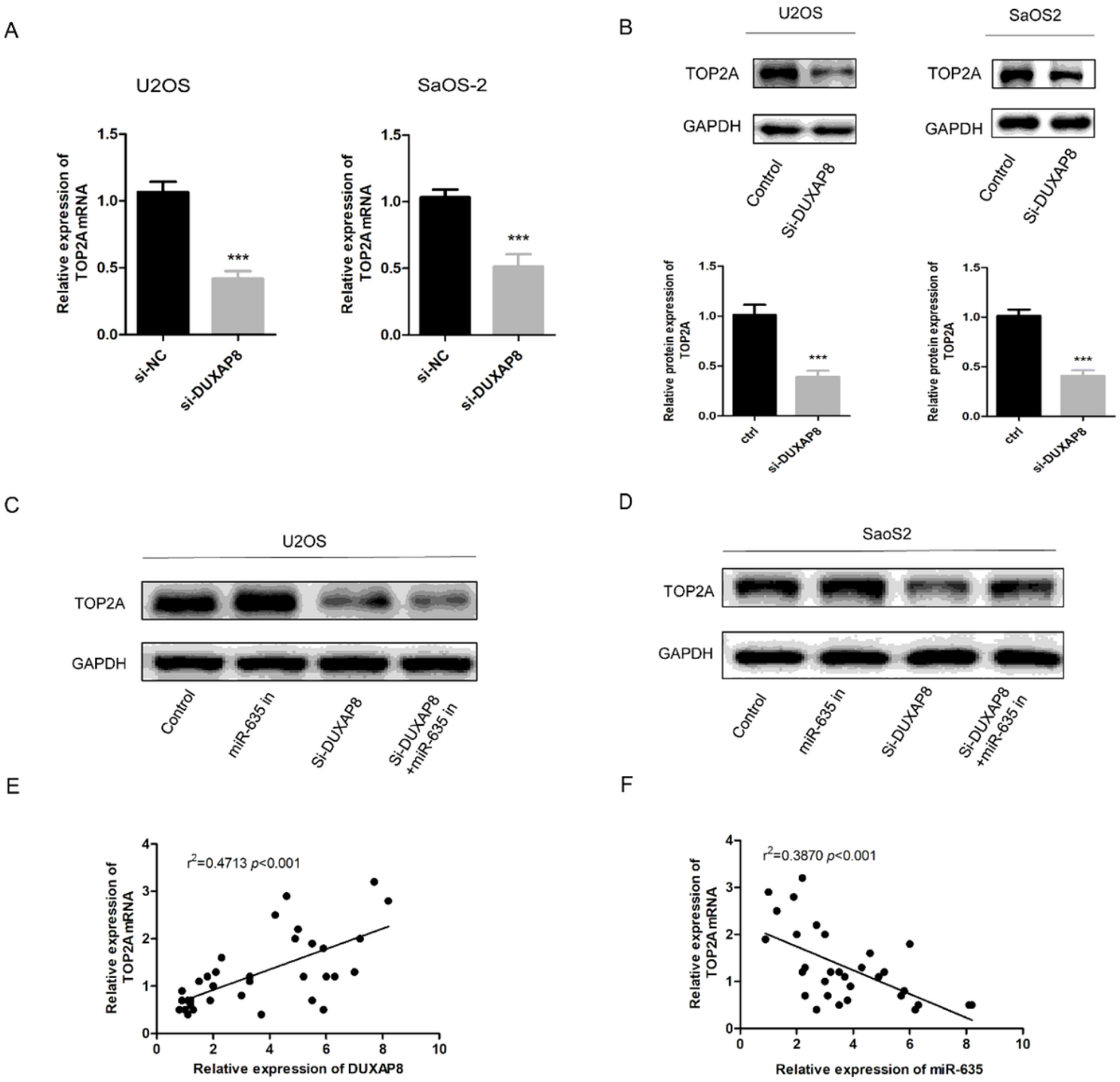

Figure 5

Regulation of DUXAP8/miR-635 on TOP2A. A, qRT-PCR was used to detect the effect of knockdown of DUXAP8 on TOP2A mRNA expressions in OS cell lines. B, Western blot was used to detect the effect of knockdown or overexpression of DUXAP8 on TOP2A protein expressions in OS cell lines. C, D, Western blot was used to detect the effect of DUXAP8 and miR-635 on TOP2A protein expressions in U2OS and SaoS2 cell lines. E, the correlation between the expression levels of miR-635 and TOP2A mRNA in OS samples was analyzed. ***P < .001.