DOI: https://doi.org/10.5114/pjp.2024.143231 Pol J Pathol 2024; 75 (3): 215-227

Original paper

CircRNA TUBA1C promotes proliferation and glucose metabolism, and blocks apoptosis of osteosarcoma cells through sponging miR-143-3p

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Osteosarcoma (OS) is a malignant bone tumour that commonly occurs in paediatric and adolescent patients. Currently, effective therapy for OS remains elusive due to poor patient survival rates.
In this study, we observed significantly elevated expressions of circTUBA1C in OS tumours and cells.
Silencing circTUBA1C effectively suppressed proliferation and glucose metabolism, and promoted apoptosis of OS cells. Furthermore, we discovered that miR-143-3p played a reverse role to circTUBA1C in OS cells. Bioinformatics analysis, RNA pull-down assay, and luciferase assay demonstrated that circTUBA1C acted as a sponge for miR-143-3p, blocking its expression in OS cells. Finally, rescue experiments showed that inhibition of miR-143-3p in circTUBA1C-silenced OS cells significantly overrode the low-circTUBA1C-mediated miR-143-3p upregulation and OS cell progression *in vitro* and *in vivo*.
Our results demonstrate the critical roles and molecular targets of circTUBA1C in modulating OS progression, suggesting that circTUBA1C inhibition could serve as a new therapeutic strategy for treating OS.

Key words: osteosarcoma, circular RNA, circTUBA1C, miR-143-3p, cell proliferation, glucose metabolism, cell apoptosis.

Introduction

Osteosarcoma (OS) is a type of tumour derived from bone-forming mesenchymal stem cells. It is characterised by the production of osteoid tissue and stromal cells by tumour cells [1]. Osteosarcoma is a malignant primary bone tumour that is commonly found in paediatric and adolescent patients. It mainly originates from the metaphyseal regions of long bones during periods of rapid growth in children and adolescents [2, 3]. Current therapy approaches for OS include surgery, neoadjuvant and adjuvant chemotherapies such as doxorubicin, cisplatin, ifosfamide, and high-dose methotrexate [4]. Although the 5-year survival rate of OS patients has improved recently, many patients still do not survive longer than 10 years due to recurrence and chemoresistance [5]. Therefore, it is crucial to explore new diagnostic targets and investigate therapeutic strategies for the treatment of this potentially fatal disease.

Circular RNAs (circRNAs) are a type of non-coding RNA molecule that plays essential roles in the origination and progression of cancer through various molecular mechanisms [6]. CircRNAs commonly exhibit tissue and cancer-specific expression patterns and functions, making them potentially clinically relevant [7]. CircRNAs have been identified as potential diagnostic and prognostic biomarkers [8].

For example, circRNA_102231 has been shown to promote cancer cell proliferation in lung cancer [9]. Circ_0025202 suppresses breast cancer cell proliferation and tumour growth, thereby inhibiting breast cancer development [10]. In lung cancer, circRNA TUBA1C (circ_0026134) has been found to play oncogenic roles [11], suggesting that circRNA TUBA1C could potentially serve as a diagnostic and therapeutic marker for OS. However, the molecular targets and regulatory mechanisms of circTUBA1C in OS remain unclear.

This study aimed to investigate the biological roles and molecular mechanisms of circTUBA1C in mediating OS progressions. We examined the effects of silencing circTUBA1C on cell proliferation, glucose metabolism rate, and apoptosis. Additionally, we identified and validated the miRNA target of circTUBA1C. Our study revealed that circTUBA1C promotes OS progression by sponging miR-143-3p. This finding suggests that targeting the circTUBA1C-miR-143-3p axis could serve as a novel therapeutic approach for treating OS.

Material and methods

Osteosarcoma tissue collections from patients

Osteosarcoma tissue collections were obtained from patients with OS. Between May 2019 – March 2021, forty-five OS specimens and matched adjacent normal bone tissues were obtained from patients at the Department of Orthopaedics, Changzhou TCM Hospital of Nanjing University of Chinese Medicine. Among the OS patients, 23 were males and 22 were females, ranging in age 12–33 years. This study was approved by the Ethics Committee of Changzhou TCM Hospital of Nanjing University of Chinese Medicine and conducted in accordance with the Declaration of Helsinki. After dissection, the tissues were immediately frozen in liquid nitrogen and transferred to a –80°C freezer. Written informed consent was obtained from all patients.

Cell culture and reagents

Four human OS cell lines (MG-63, U2OS, Saos2, and HOS) and one human normal osteoblast cell line (hFOB 1.19) were purchased from the Chinese Academy of Sciences. Cells were cultured in DMEM medium (Thermo Fisher Scientific, USA) supplemented with 10% foetal bovine serum (Thermo Fisher Scientific, USA) and 1 × streptomycin (10 mg/ml) (Thermo Fisher Scientific, USA) at 37°C with 5% CO_2.

Transfections of siRNA and miRNAs

Osteosarcoma cells were transfected using Lipofectamine 2000 (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. In brief, MG-63 and U2OS cells (5 × 10^6 cells/well) were plated onto a 6-well plate for 24 hours. CircTUBA1C siRNA, miRNA-143-3p precursor and inhibitor and their negative controls were synthesized (GenePharma, China). Forty-eight hours after transfection, cells were collected for analysis.

Quantitative reverse transcription-polymerase chain reaction

Total RNAs from OS cells were extracted using the TRIzol® reagent (Thermo Fisher Scientific, USA). Complementary DNA (cDNA) was synthesised using the Prime Script RT Master Mix kit (Takara Bio, Japan). Polymerase chain reaction (PCR) reactions were performed using SYBR Premix Ex Taq™ II and SYBR PrimeScript miRNA RT-PCR kit (Takara Bio, Japan). Polymerase chain reaction thermocycling conditions were set as follows: 95°C, 10 min, then 40 cycles at 95°C, 10 sec, and 60°C, 1 min. The primer sequences were as follows: circTUBA1C, forward 5'-ACTCCTTTGTCTTGGAACTGTCT-3', reverse 5´-GAAGGGACGGCAACAAGGAT-3´; β-actin: Forward: 5'-CTGAGAGGGAAATCGTGCGT-3', Reverse: 5'-CCACAGGATTCCATACCCAAGA-3'; miR-143-3p: Forward: 5'-TGAGATGAAGCACTG-3', Reverse: 5'-GTGCAGGGTCCGAGGT-3'; U6: Forward: 5'-CTCGCTTCGGCAGCACA-3', Reverse: 5'-AACGCTTCACGAATTTGCGT-3'. Expression of circRNA was normalised to β-actin. miRNA expression was normalised to human U6. Relative expressions were calculated using the $2^{-\Delta\Delta Ct}$ method.

RNA pull-down assay

Binding of circTUBA1C on miR-143-3p was verified by RNA pull-down assay. Briefly, control, sense and antisense circTUBA1C probe were synthesised and biotin-labelled. Osteosarcoma cell lysates were extracted using RIPA buffer (Thermo Fisher Scientific, USA). Cells were incubated with each probe at 4°C for 2 hours. Cells were then incubated with streptavidin-coupled agarose beads for 2 more hours at 4°C. The binding of miR-143-3p with circTUBA1C was evaluated by the amount of miR-143-3p in the RNA-RNA complex by quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Luciferase reporter assay

The association between circTUBA1C and miR-143-3p was predicted using the starBase 2.0 database (http://starbase.sysu.edu.cn/). The wild-type (WT) or mutant (MUT) sequence of circTUBA1C miR-143-3p binding site was inserted into the luciferase reporter vector pGL3 (Promega, USA). The construct mentioned above was then co-transfected with a control miRNA or miR-143-3p into OS cells for a duration of 48 hours. Subsequently, luciferase activity was detected using the Dual-Luciferase Reporter Assay System (Promega, USA).

Measurement of glucose metabolism

Glucose metabolism of OS cells was measured using glucose uptake assay (#ab136955, Abcam, UK) and lactate product assay (#ab65331, Abcam, UK) according to the manufacturer's instructions. Experiments were performed in triplicate.

Cell proliferation assay

Cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. MTT solution (Sigma-Aldrich, USA) was added into each cell plate at 37°C for 4 hours. After washing, DMSO (200 μl) was added for one hour of incubation. Absorption value was measured using a spectrophotometer (Agilent Technologies, Inc., USA) at 590 nm.

Caspase-3 activity assay

Cell apoptosis rate was assessed by Caspase-3 activity assay using the Caspase-3 Assay Kit, Colorimetric (#CASP3C-1KT, Sigma-Aldrich, USA) according to the manufacturer's instructions. Results were normalised to the cell number of each experiment group.

Cell apoptosis

Cell apoptosis in OS cells was assessed using the Annexin V-FITC/PI apoptosis kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. After transfection, OS cells were collected and washed with cold PBS. Then, 5 μl of FITC-Annexin V solution (100 μg/ml) and 1 μl of PI solution (100 μg/ml) were added to the suspension. The suspension was incubated for 15 min at room temperature in the dark, and the fluorescence intensity was measured using a FACScan flow cytometer (BD Biosciences, USA).

In vivo xenograft experiments

The protocols for xenograft mouse experiments were approved by the Ethics Committee of Changzhou TCM Hospital of Nanjing University of Chinese Medicine. The experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). In this study, 30 BALB/c nude mice were used. U2OS cells were transfected with control shRNA, sh circTUBA1C alone, or sh circTUBA1C plus miR-143-3p inhibitor for 48 hours. The mice were randomly divided into 3 groups (10 mice per group) and subcutaneously injected with the aforementioned transfected cells (1×10^7). The survival rate was monitored for 7 weeks, and tumour volumes were examined every 5 days for a total of 35 days. Tumour volumes were calculated using the formula V (mm^3) = 1/2ab^2.

Statistical analysis

Statistical analysis was conducted using Prism 7.0 software (GraphPad Software, USA). Differences between 2 experiment groups were analysed using Student's *t*-test. Comparison of 3 or more groups was analysed using a one-way analysis of variance (ANOVA) *post hoc* test. Data were shown as mean ± standard deviation (SD). $P < 0.05$ was considered as statistical significance.

Results

CircRNA TUBA1C is upregulated in osteosarcoma tumours and cell lines

Previous studies have suggested that circTUBA1C is positively associated with various types of cancer [11, 12]. In this study, we aimed to investigate the clinical significance of circTUBA1C in OS. We compared the expression levels of circTUBA1C in OS tissues and matched normal bone tissues. As expected, the results from qRT-PCR showed a significant upregulation of circTUBA1C in OS tissues (Fig. 1A). Furthermore, when compared to a normal osteoblast cell line, hFOB1.19, we observed a notably higher expression level of circTUBA1C in 4 OS cell lines (Fig. 1B). These findings suggest that circTUBA1C plays an oncogenic role in OS and could potentially serve as a biomarker for OS.

Expression of miR-143-3p is attenuated in osteosarcoma tumours and cell lines

We evaluated the downstream molecular targets of circTUBA1C in OS cells. Accumulating studies have revealed that circRNAs form a competing endogenous RNA (ceRNA) network with target miRNAs by sponging them, which results in the blocking of downstream miRNA expression [13]. Bioinformatics analysis has indicated that miR-143-3p, which has been reported to function as a tumour suppressor in various cancers [14–16], contains binding sites for circTUBA1C (Fig. 2A) according to non-coding RNA service, starBase 2.0. Subsequently, the expression of miR-143-3p was compared in OS tissues and matched normal bone tissues, and the expected results showed that miR-143-3p was significantly attenuated in OS tissues (Fig. 2B). Consistently, compared to the normal osteoblast cell line hFOB1.19, miR-143-3p was remarkably downregulated in 4 OS cell lines (Fig. 2C). Taken together, these results indicate that circTUBA1C could bind with miR-143-3p to downregulate its expression in OS.

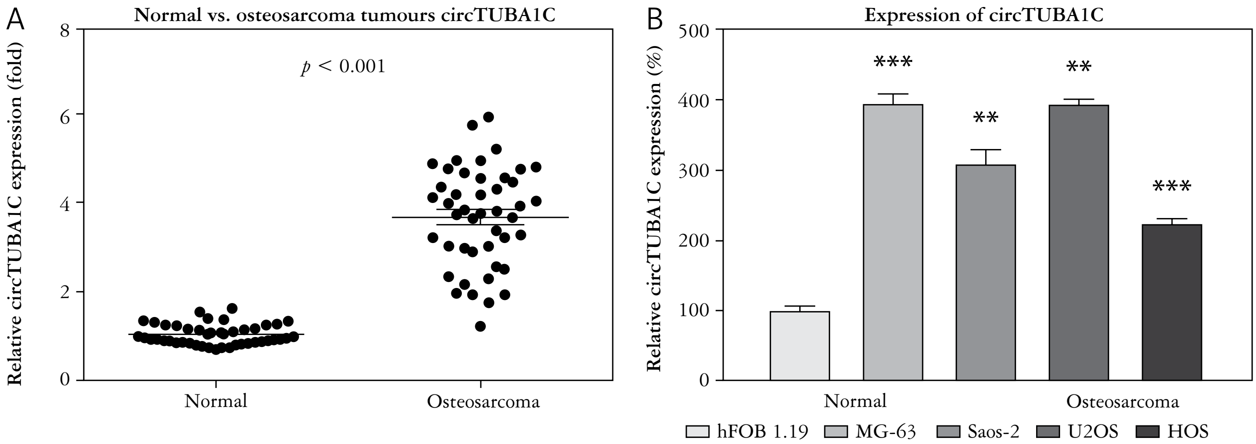


Fig. 1. CircTUBA1 is upregulated in osteosarcoma tumours and cell lines. A) Expressions of circTUBA1C from 45 osteosarcoma (OS) tumours tissues and their adjacent normal bone tissues were examined by quantitative reverse transcription-polymerase chain reaction. **B**) Expressions of circTUBA1C were examined in human normal osteoblast cell line and 4 OS cell lines

*** *p* < 0.01*
**** *p* < 0.001, each cell line vs. hFOB 1.19*

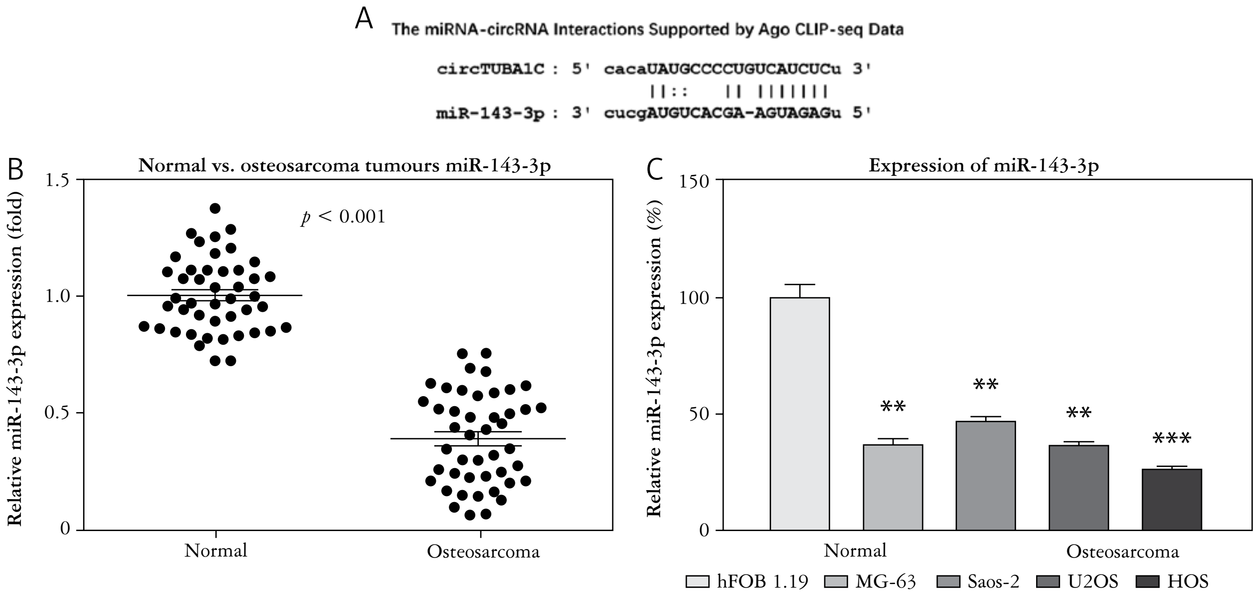


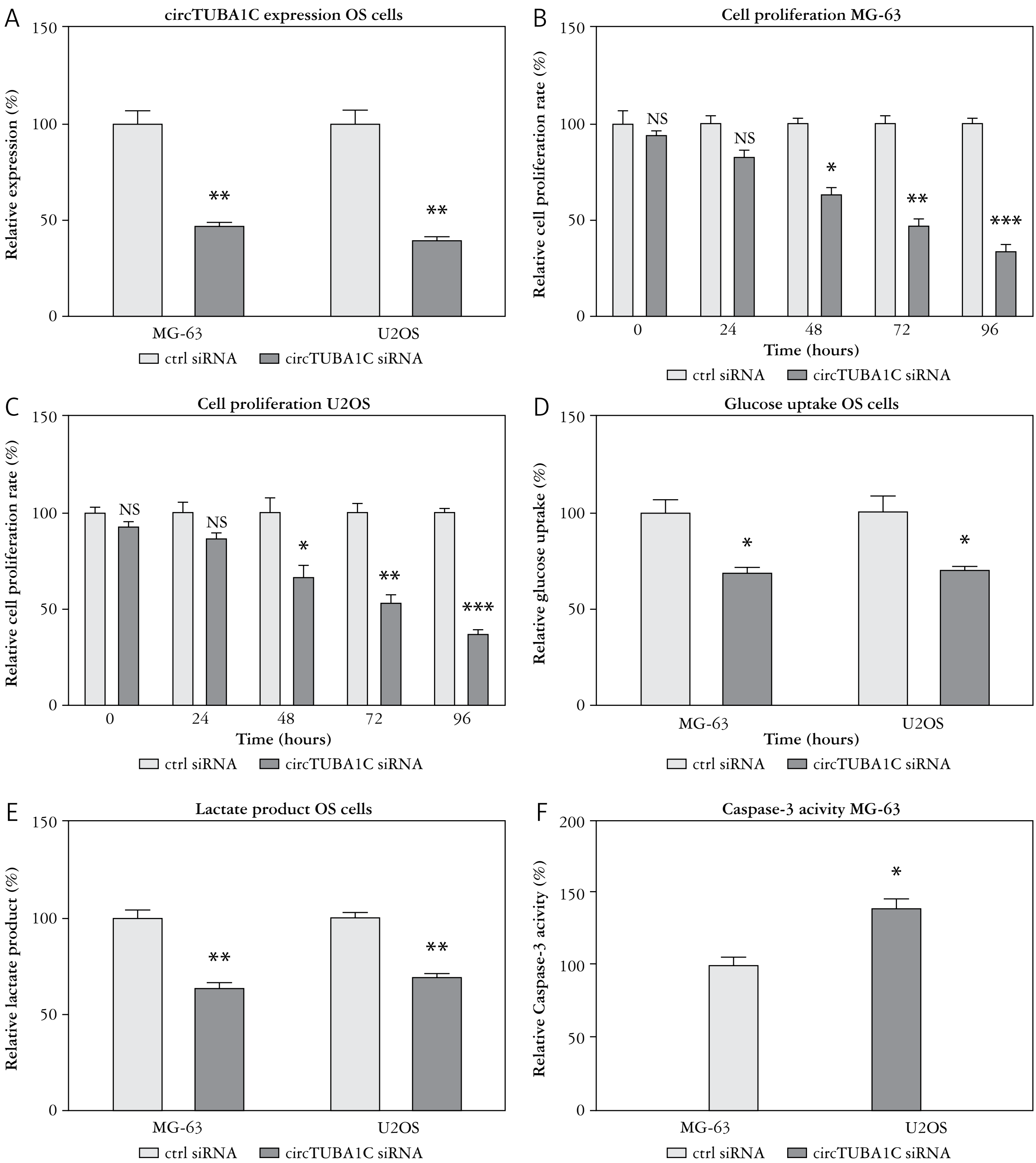
Fig. 2. miR-143-3p is down regulated in osteosarcoma tumours and cell lines. A) Prediction of the miR-143-3p/circTUBA1C association from starBase 2.0. B) Expressions of miR-143-3p from 45 osteosarcoma (OS) tumours tissues and their adjacent normal bone tissues were examined by quantitative reverse transcription-polymerase chain reaction. C) Expressions of miR-143-3p were examined in human normal osteoblast cell line and 4 OS cell lines

*** *p* < 0.01*
**** *p* < 0.001, each cell line vs. hFOB 1.19*

CircTUBA1C and miR-143-3p play reverse functions in proliferation, glucose metabolism, and apoptosis of osteosarcoma cells

Given the results above, which demonstrated reverse expression patterns of circTUBA1C and miR-143-3p in OS cells, we assessed the biological roles of circTUBA1C and miR-143-3p in OS cellular processes. We silenced circTUBA1C using siRNA in MG-63 and U2OS cells (Fig. 3A). The cell proliferation assay showed that silencing circTUBA1C effectively suppressed cell proliferation rates at 48, 72, and 96 hours (Fig. 3B, C). Consistently, OS cells with circTUBA1C knockdown exhibited significantly suppressed glucose uptake (Fig. 3D) and lactate production (Fig. 3E), indicating that circTUBA1C contributes to the glucose metabolism of OS cells. Conversely, the Caspase-3 assay and Annexin V assay showed that blocking circTUBA1C effectively promoted cell apoptosis of MG-63 and U2OS cells (Fig. 3F, G, H).

Subsequently, we examined the biological roles of miR-143-3p in OS. As expected, overexpression



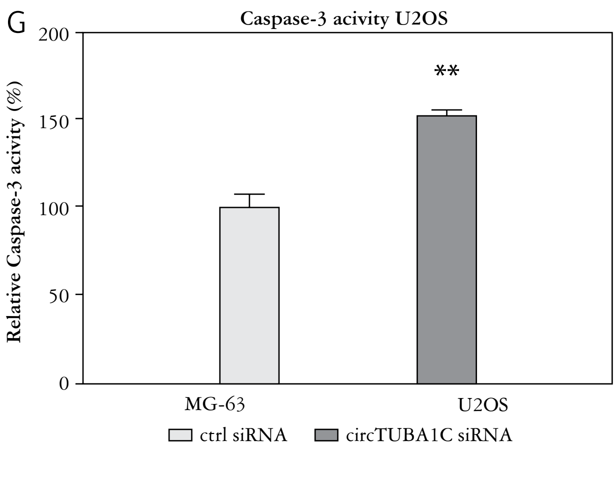


Fig. 3. CircTUBA1C promotes proliferation and glucose metabolism, and inhibits apoptosis of osteosarcoma cells. A) MG-63 and U2OS cells were transfected with control siRNA or circTUBA1C siRNA. Expressions of circTUBA1C were detected. B, C) Cell proliferation rates from the above transfected cells were measured at 0, 24, 48, 72, and 96 hours by MTT assay. Glucose uptake (D), lactate product (E), and cell apoptosis rates (F, G, H) from the above transfected cells were examined

** $p < 0.05$*
*** $p < 0.01$*
**** $p < 0.001$, si circTUBA1C vs. Control siRNA*

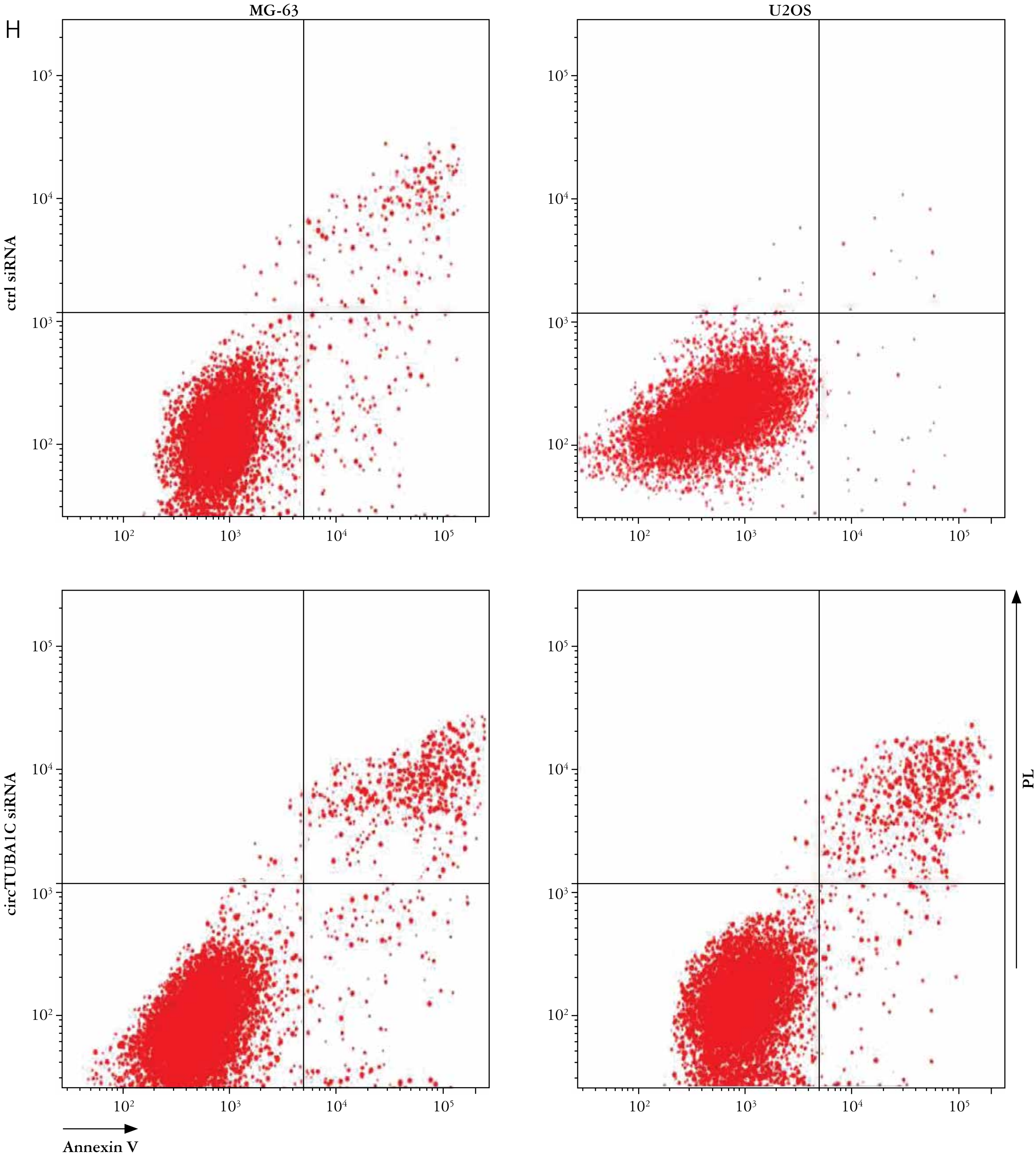
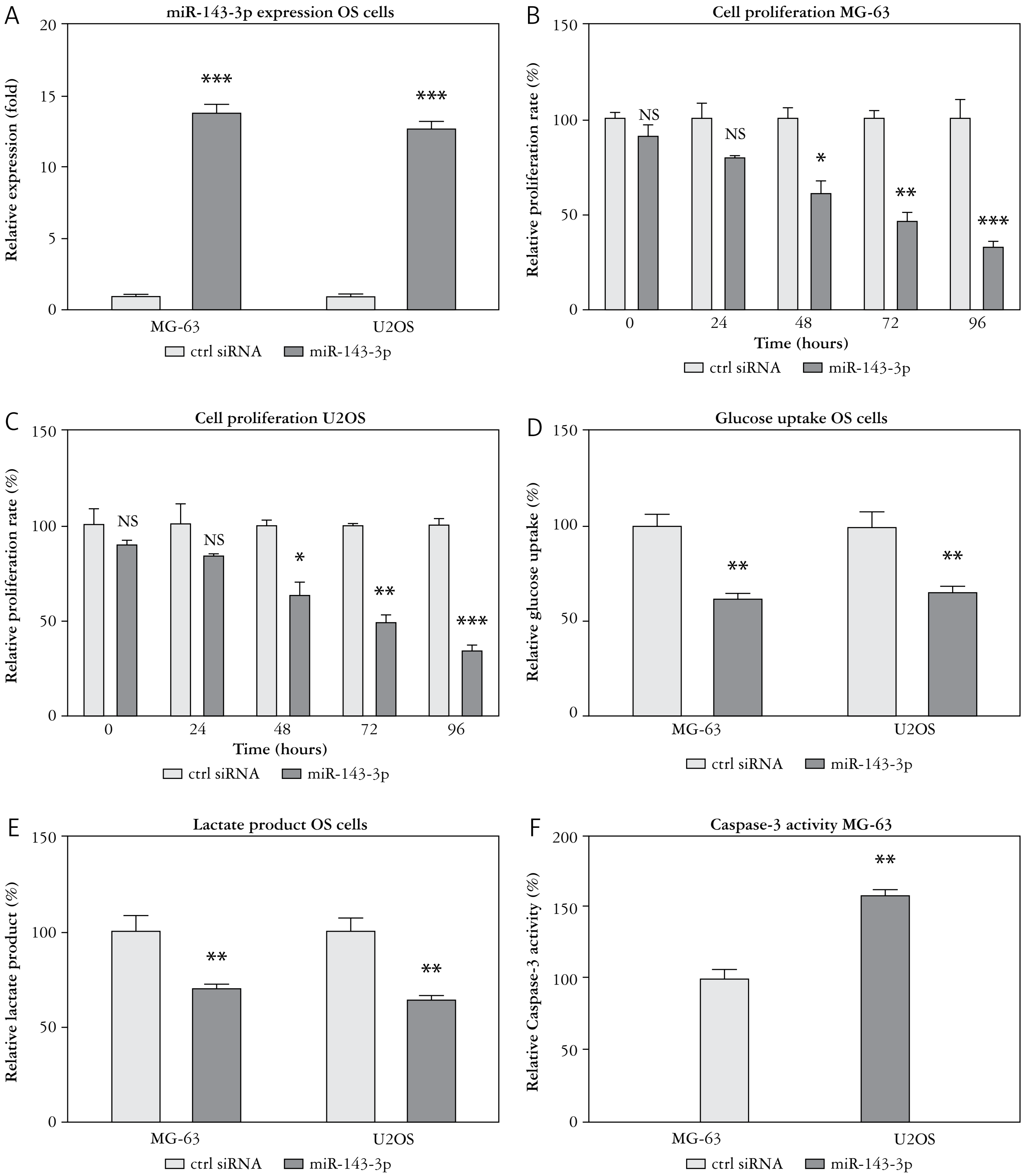


Fig. 3. Cont.

of miR-143-3p (Fig. 4A) suppressed cell proliferation rates (Fig. 4B, C) and glucose metabolism (Fig. 4D, E) in OS cells. Moreover, MG-63 and U2OS cells with miR-143-3p overexpression displayed a significant increase (p < 0.05) in cell apoptosis rates according to the Caspase-3 activity assay and Annexin V assay (Fig. 4F, G, H). In summary, these functional assays suggested that circTUBA1C plays oncogenic roles and miR-143-3p acts as a tumour suppressor in OS.

CircTUBA1C sponges miR-143-3p in osteosarcoma cells

Since miR-143-3p was predicted as a target of circTUBA1C (Fig. 2A), we evaluated whether circTUBA1 associates with miR-143-3p in OS cells. Pearson's correlation coefficient analysis showed a negative correlation between circTUBA1 and miR-143-3p in OS tumours (p < 0.001) (Fig. 5A). We tested whether circTUBA1C could inhibit



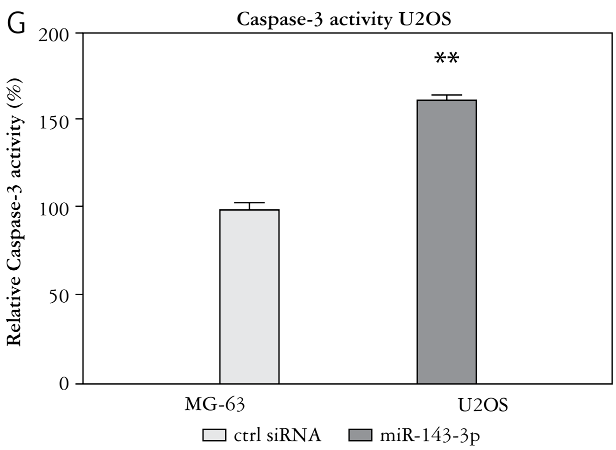


Fig. 4. miR-143-3p suppresses proliferation, glucose metabolism and promotes apoptosis of osteosarcoma cells. **A**) MG-63 and U2OS cells were transfected with control miRNA or miR-143-3p precursor. Expressions of miR-143-3p were detected. **B, C**) Cell proliferation rates from the above transfected cells were measured at 0, 24, 48, 72, and 96 hours by MTT assay. Glucose uptake (**D**) lactate product (**E**) and cell apoptosis rates (**F, G, H**) from the above transfected cells were examined

** $p < 0.05$*
*** $p < 0.01$*
**** $p < 0.001$, miR-143-3p vs. Control miRNA*

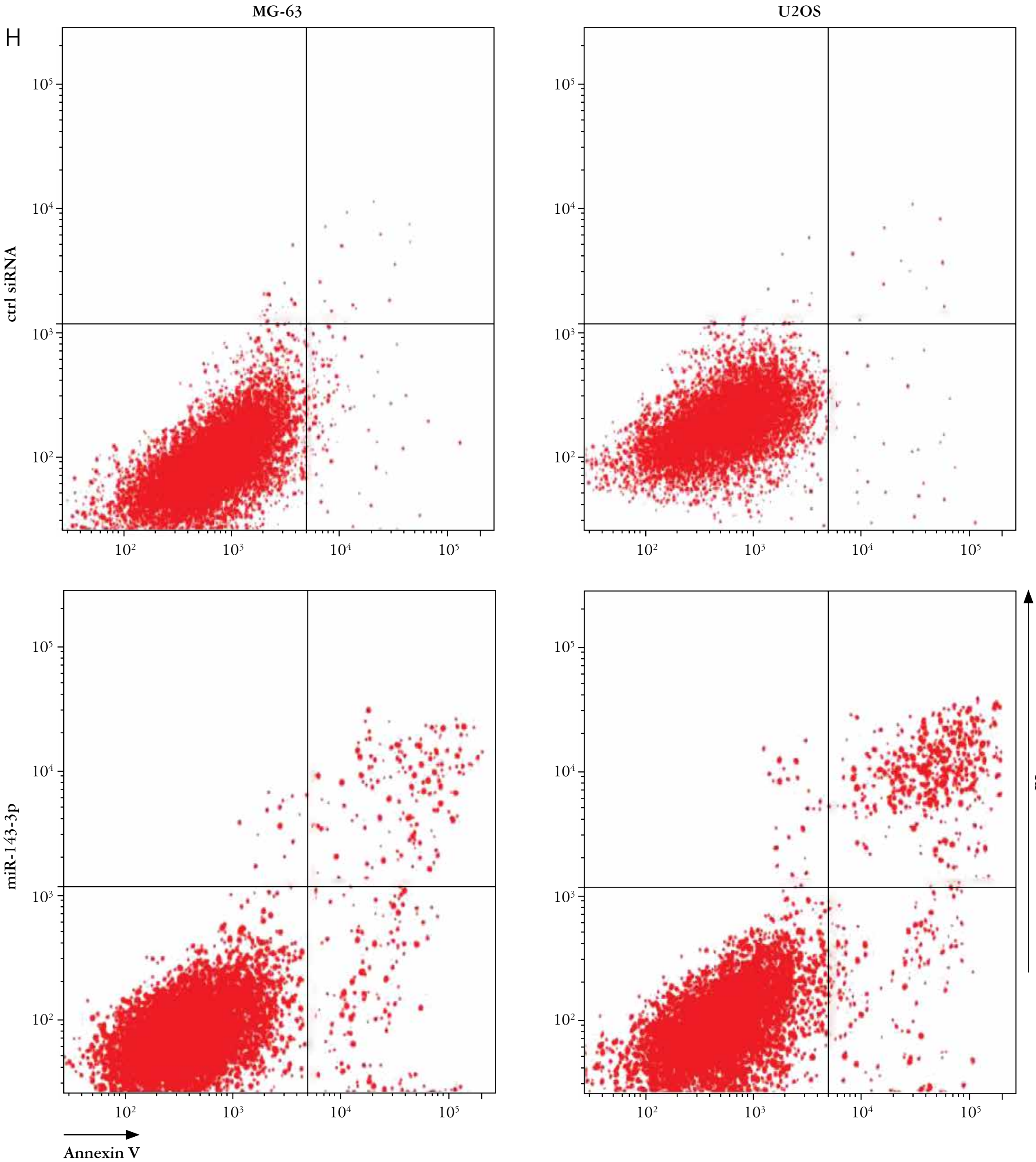
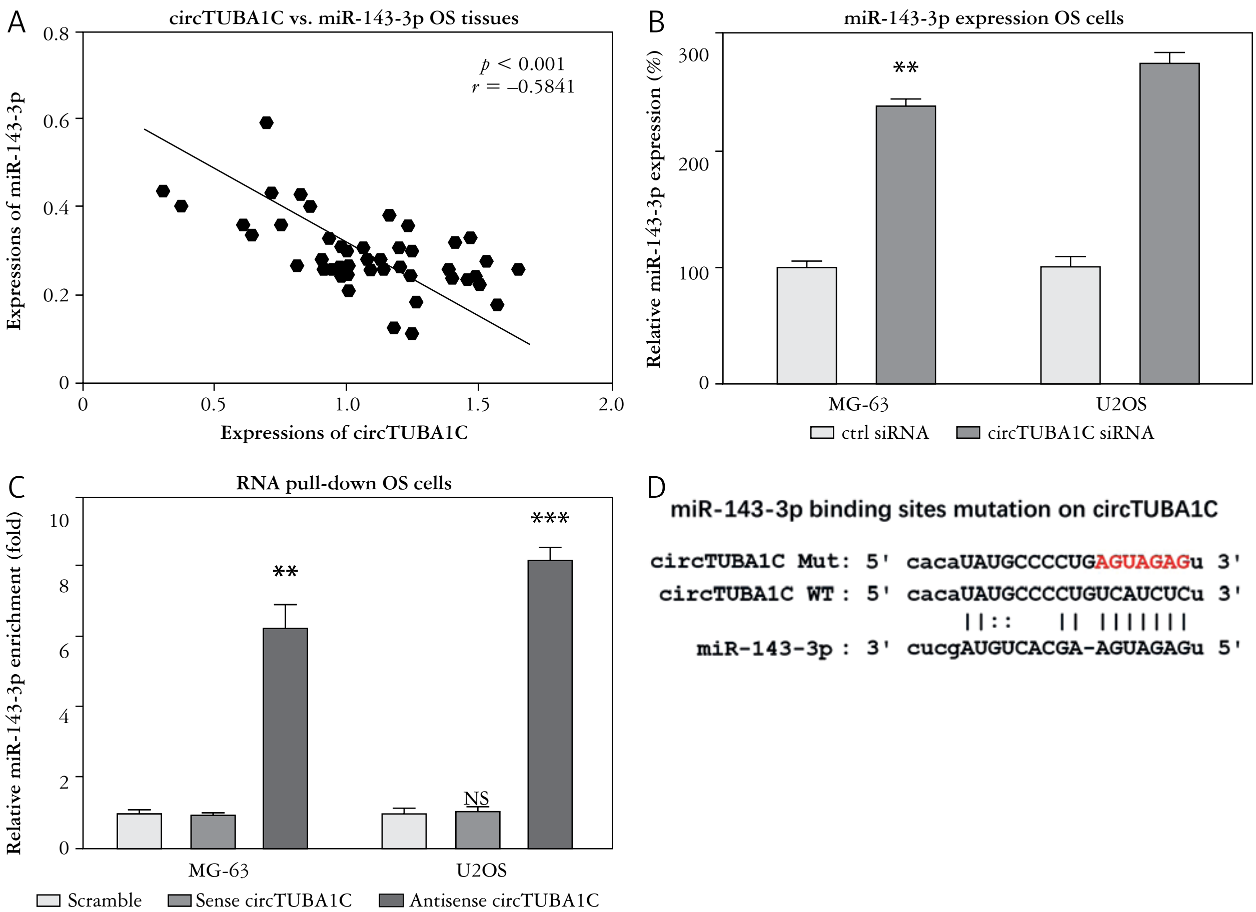


Fig. 4. Cont.

miR-143-3p expression. Blocking circTUBA1C significantly increased miR-143-3p expression in OS cells (Fig. 5B). To verify the predicted association between circTUBA1 and miR-143-3p, a RNA pull-down assay was conducted. Quantitative reverse transcription PCR results showed that a sufficient amount of miR-143-3p could bind with circTUBA1C and be precipitated using the antisense circTUBA1C probe (Fig. 5C). However, miR-143-3p could not be effectively pulled down by the circTUBA1 sense or scramble control probe (Fig. 5C). To validate the specific binding of miR-143-3p to the predicted sites of circTUBA1, a luciferase assay was performed in OS cells by co-transfecting the control miRNA or miR-143-3p with luciferase vector containing the WT or binding site MUT-circTUBA1C (Fig. 5D). As expected, the luciferase activities of MG-63 and U2OS cells co-transfected with miR-143-3p and luciferase vector containing WT-circTUBA1C were significantly reduced (Fig. 5E). However, OS cells co-transfected with the control miRNA or miR-143-3p and luciferase vector containing MUT-circTUBA1 did not show



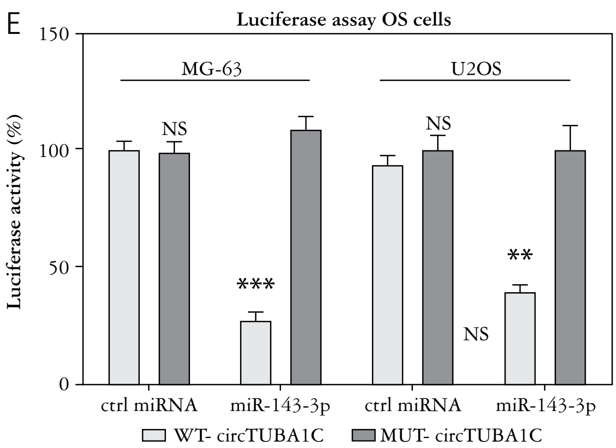


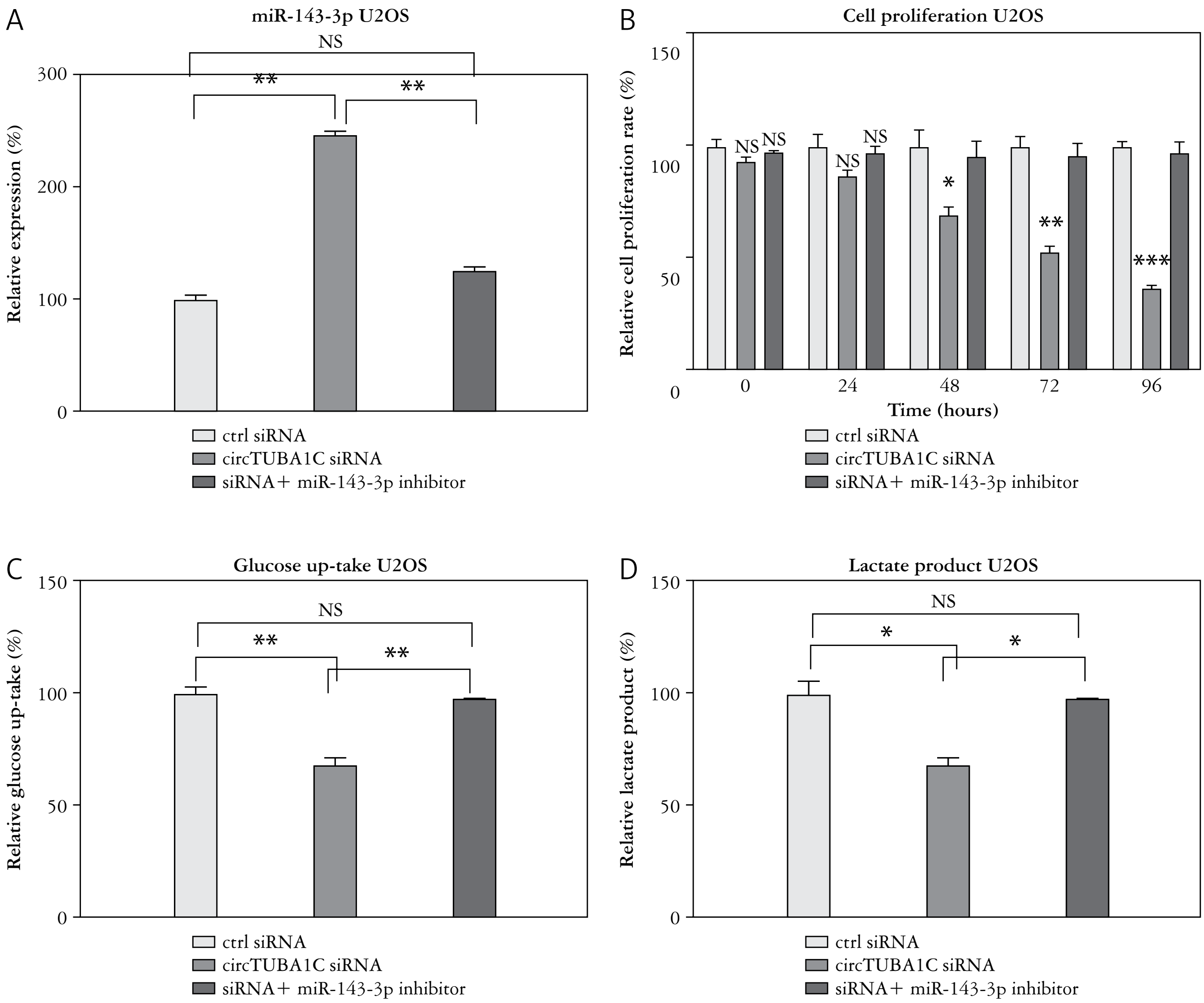
Fig. 5. CircTUBA1C sponges miR-143-3p in osteosarcoma cells. **A**) Spearman's correlation analysis indicated a negative correlation between circTUBA1C and miR-143-3p in osteosarcoma (OS) tissues. **B**) MG-63 and U2OS cells were transfected with control miRNA or circTUBA1C siRNA. miR-143-3p expression was examined. **C**) RNA pull-down assay was performed to show that miR-143-3p was precipitated only with antisense circTUBA1C probe in OS cells (** $p < 0.01$; *** $p < 0.001$, antisense circTUBA1C vs. scramble and sense circTUBA1C). **D**) Binding site MUT on circTUBA1C was shown in the sequence alignment of circTUBA1C-miR-143-3p. **E**) Luciferase assay was performed to show miR-143-3p only suppressed luciferase activity of vector containing WT-circTUBA1C (** $p < 0.01$; *** $p < 0.001$. WT-circTUBA1C/miR-143-3p vs. MUT-circTUBA1C/miR-143-3p)

significant changes in luciferase activities (Fig. 5E). In conclusion, these results demonstrate that circTUBA1 inhibits miR-143-3p expression by directly associating with it in OS cells.

CircTUBA1C promotes the cellular processes of osteosarcoma cell through direct sponging of miR-143-3p

Subsequently, we assessed whether circTUBA1C promotes proliferation, glucose metabolism, and inhibits apoptosis of OS cells by targeting miR-143-3p. U2OS cells were transfected with control siRNA, circTUBA1C siRNA alone, or circTUBA1C siRNA plus miR-143-3p inhibitor. Knocking down of circTUBA1C significantly upregulated miR-143-3p expression, which was further overridden by miR-143-3p inhibition (Fig. 6A). Consequently, co-transfection of circTUBA1C siRNA and miR-143-3p inhibitor significantly restored cell proliferation (Fig. 6B) and glucose metabolism rates (Fig. 6C, D) of U2OS cells compared to circTUBA1C siRNA transfection alone. Consistently, Caspase-3 activity assay (Fig. 6E) and apoptosis assay (Fig. 6F) demonstrated that rescuing miR-143-3p in circtTUBA1C silencing OS cells successfully restored the cell apoptosis rate. In summary, the results from rescue experiments consistently supported that circTUBA1C promotes cellular processes of OS cells by targeting miR-143-3p.

We then validated the *in vitro* results using an *in vivo* xenograft mouse model. U2OS cells were transfected with control shRNA, sh circTUBA1C alone, or sh circTUBA1C plus miR-143-3p inhibitor for 48 hours. Cells from each group were subcutaneously injected into nude mice (10 mice/group). Survival analysis from Figure 7A demonstrated that most mice whose xenograft tumours were developed from the control shRNA and sh circTUBA1C plus miR-143-3p inhibitor groups died within 6 weeks. As expected, silencing circTUBA1C resulted in a significantly prolonged survival rate for mice that developed



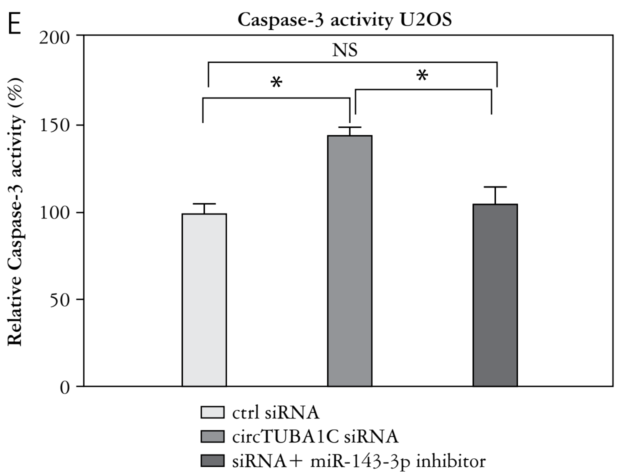


Fig. 6. The roles of circTUBA1C-miR-143-3p association in proliferation, glucose metabolism, and apoptosis of osteosarcoma cells. **A**) U2OS cells were transfected with control siRNA, or circTUBA1 siRNA alone or plus miR-143-3p inhibitor for 48 hours. Expression of miR-143-3p was examined. **B**) Cell proliferation rates from the above transfected cells were measured at 0, 24, 48, 72, and 96 hours by MTT assay. Glucose uptake (**C**), lactate product (**D**), and cell apoptosis rate (**E, F**) from the above transfected cells were examined

*$p < 0.05$
**$p < 0.01$
***$p < 0.001$, *si circTUBA1C vs. control siRNA and si circTUBA1C+miR-143-3p inhibitor*

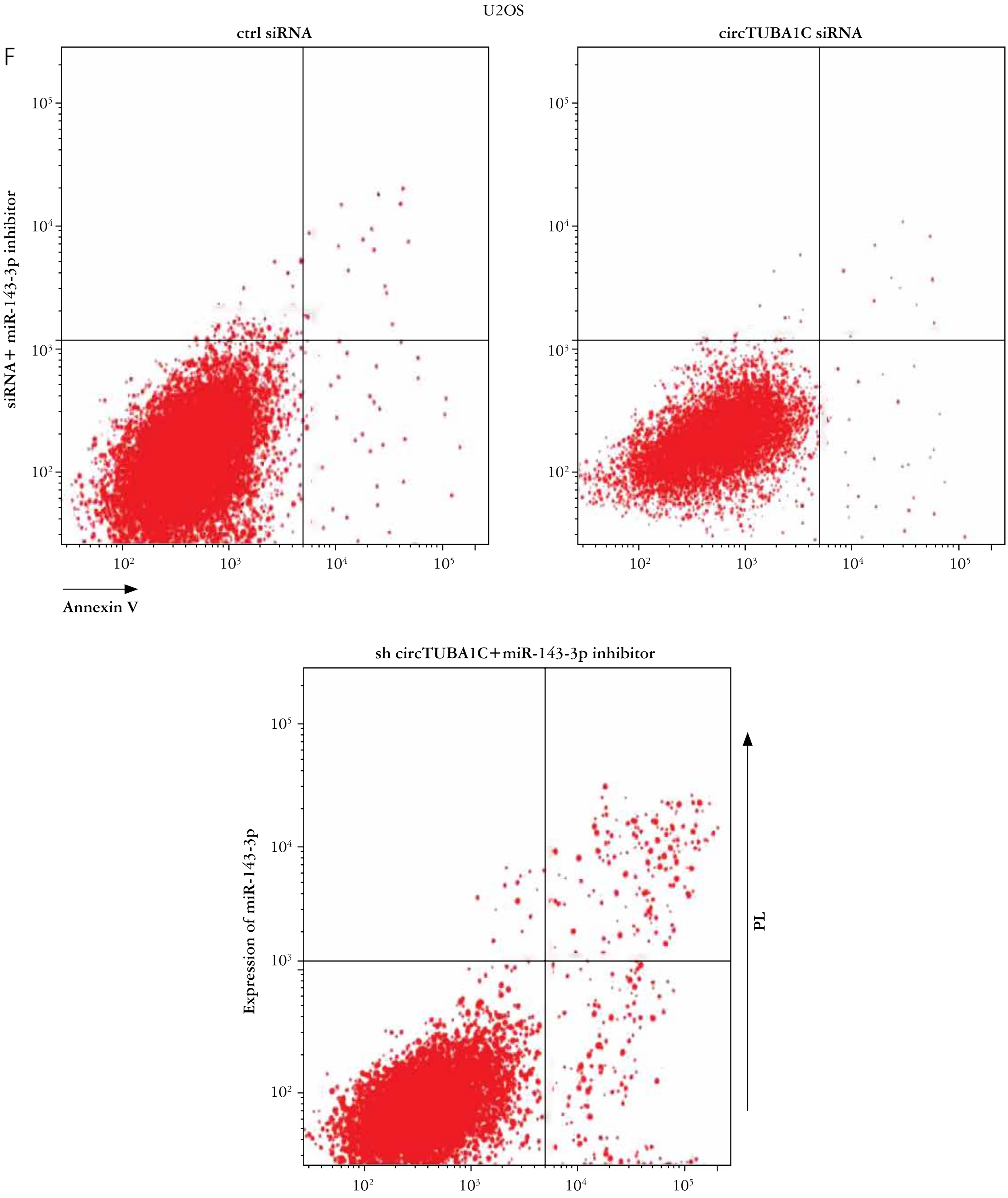


Fig. 6. Cont.

xenograft tumours from circTUBA1C knockdown cells (Fig. 7A) and reduced tumour sizes (Fig. 7B). The expression of miR-143-3p was significantly upregulated in xenograft tumours derived from circTUBA1C-silenced OS cells (Fig. 7C). These *in vivo* results verified that circTUBA1C promotes OS cell growth by modulating miR-143-3p, indicating that blocking circTUBA1C could contribute to the development of new therapeutic approaches against OS.

Discussion

Osteosarcoma is a malignant bone tumour that originates from the bone-forming mesenchymal stem cells. It commonly occurs in paediatric and adolescent patients [1]. The current therapy approaches for OS include surgery, and neoadjuvant and adjuvant chemotherapies [2, 3]. However, the 10-year survival rate is still underestimated due to recurrence and

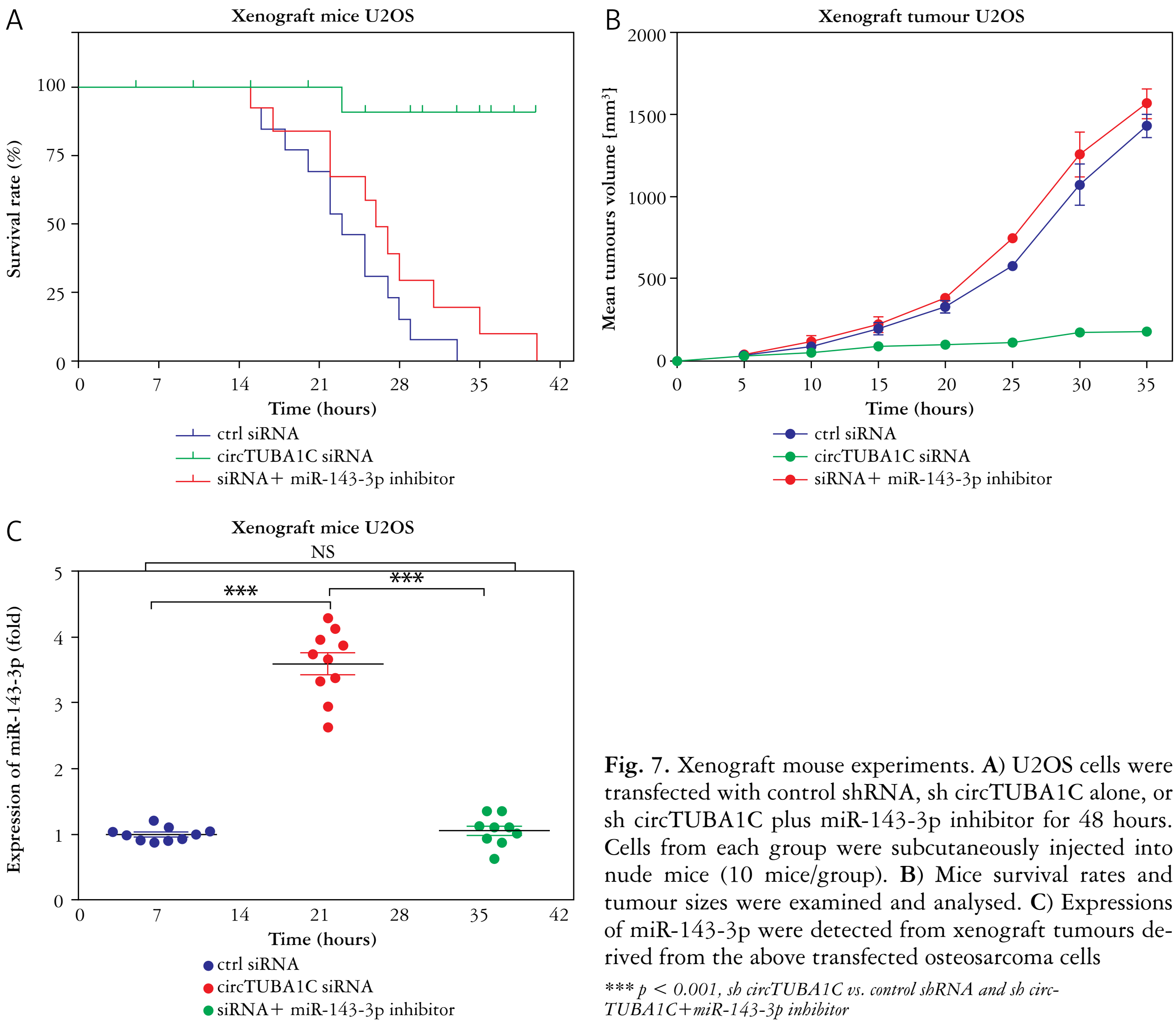


Fig. 7. Xenograft mouse experiments. **A)** U2OS cells were transfected with control shRNA, sh circTUBA1C alone, or sh circTUBA1C plus miR-143-3p inhibitor for 48 hours. Cells from each group were subcutaneously injected into nude mice (10 mice/group). **B)** Mice survival rates and tumour sizes were examined and analysed. **C)** Expressions of miR-143-3p were detected from xenograft tumours derived from the above transfected osteosarcoma cells

**** $p < 0.001$, sh circTUBA1C vs. control shRNA and sh circTUBA1C+miR-143-3p inhibitor*

chemoresistance [4]. This study aimed to investigate the roles and molecular mechanisms involved in the development of OS. We have shown that circTUBA1C was significantly upregulated in OS tumour tissues and cell lines. Blocking circTUBA1C effectively inhibited cell growth and glucose metabolism, and accelerated apoptosis of OS cells by sponging miR-143-3p. Our results conclude that circTUBA1C serves as a new biomarker and potential therapeutic target for OS.

Reprogramming cancer cell metabolism, such as glucose and glutamine metabolisms, has been revealed as an essential characteristic of cancer cells, a phenomenon known as the "Warburg effect" [17]. Increased glucose metabolism is essential for the generation of both bioenergy and biosynthesis materials for cancer cell growth, metastasis, and anti-apoptosis [17]. Additionally, blocking glucose metabolism effectively inhibits tumourigenesis and progression [18]. In this study, we discovered that circTUBA1C and miR-143-3p play opposite roles in regulating the glucose metabolism of OS cells, with circTUBA1C promoting and miR-143-3p suppressing glucose metabolism. This suggests that modifying the circTUBA1C-miR-143-3p-mediated glucose metabolism could be an effective strategy for treating OS.

Increasing evidence has revealed that circRNAs function as either tumour suppressors or promoters in OS. For example, it has been shown that CircRNA_103801 promotes the proliferation of OS cells by sponging miR-338-3p [19]. Similarly, CircECE1 accelerates energy metabolism in OS cells by stabilising c-Myc [20]. Additionally, circRNA_001422 is positively associated with the progression and metastasis of OS through the regulation of the miR-195-5p/FGF2/PI3K/Akt axis [21]. However, the roles and mechanisms of circTUBA1C in OS have not been reported.

MicroRNAs (miRNAs) are a class of endogenous, small (20–24 nts), non-coding RNA molecules that play essential roles in the biological processes of various cancers [22]. In OS, miRNA-151a-3p regulates the invasion and migration of OS by directly targeting RAB22A [23]. Furthermore, miR-143 has been shown to suppress the progression of ovarian cancer [24] and colon cancer [25]. In our study, we demonstrate a tumour suppressive role for miR-143-3p in OS. Overexpression of miR-143-3p effectively suppressed cell proliferation and glucose metabolism and facilitated apoptosis in OS cells. Several studies have shown that circRNAs can directly bind to miRNAs on their endogenous miRNA response element [13]. In this study, we showed a negative correlation between the expressions of circTUBA1C and miR-143-3p in OS tumours. Bioinformatics analysis predicts that circTUBA1C contains an miR-143-3p response element, suggesting a circTUBA1C-miR-143-3p ceRNA network in OS cells. This association was further verified by RNA pull-down and luciferase assays. Importantly, rescue experiments confirmed that the circTUBA1C-regulated cell proliferation, glucose metabolism, and apoptosis were mediated by sponging miR-143-3p in OS cells both *in vitro* and *in vivo*. Although the potential roles of miR-143-3p have been reported in OS [26], our results reveal a specific circTUBA1C-miR-143-3p axis in regulating OS progression.

Conclusions

Our study uncovers a new circTUBA1C-miR-143-3p axis in the cell proliferation, glucose metabolism, and apoptosis of OS. This suggests that targeting circTUBA1C could be an effective therapeutic strategy for the treatment of OS.

Disclosures

1. This study was approved by the Ethics Committee of the Changzhou TCM Hospital of Nanjing University of Chinese Medicine. All methods involving human participants were performed in accordance with the Declaration of Helsinki.
2. Assistance with the article: None.
3. This study was supported by Department of Orthopaedics, Changzhou TCM Hospital of Nanjing University of Chinese Medicine, China.
4. Conflicts of interest: None.

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