

Long non-coding RNA small nucleolar RNA host gene 1 knockdown suppresses the proliferation, migration and invasion of osteosarcoma cells by regulating microRNA-424-5p/FGF2 *in vitro*

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Abstract. The aim of the present study was to clarify the effect of long non-coding RNA (lncRNA) small nucleolar RNA host gene 1 (SNHG1) on the proliferation, migration and invasion of osteosarcoma (OS) cells and to explore the potential underlying mechanisms. The expression levels of SNHG1, microRNA (miR)-424-5p and fibroblast growth factor 2 (FGF2) in OS tissues and cells were detected using reverse transcription-quantitative polymerase chain reaction. OS cell proliferation, migration and invasion were analysed by MTT, wound healing and Transwell invasion assays, respectively. The targeting relationships between SNHG1 and miR-424-5p, as well as between miR-424-5p and FGF2, were confirmed using RNA-binding protein immunoprecipitation and/or dual-luciferase reporter gene assays. The results demonstrated that the expression levels of SNHG1 and FGF2 were upregulated, whereas the expression of miR-424-5p was downregulated in OS tissues and cells. The silencing of SNHG1 significantly inhibited the proliferation, migration and invasion of OS cells. Additionally, FGF2 was shown to be a target of miR-424-5p, which in turn, was a target of SNHG1. miR-424-5p silencing and FGF2 overexpression both reversed the suppressive effects of SNHG1 knockdown on the proliferation, migration and invasion of OS cells. Thus, the silencing of

SNHG1 may inhibit the proliferation, migration and invasion of OS cells by regulating the miR-424-5p/FGF2 axis.

Introduction

Osteosarcoma (OS), a type of malignant tumour that is common in teenagers with a worldwide incidence of 3.4 per million people per year (1), typically originates from mesenchymal stem cells (2,3). In the past, amputation was primarily utilised to treat OS, but the curative effect of this remains limited (1). At present, various treatments for OS exist, including systemic chemotherapy, targeted drug therapy, immunotherapy and radiotherapy (4). However, many side effects occur from these processes, such as leukopenia and thrombocytopenia and the survival rate of OS patients remains less than 25% (5,6). Thus, understanding the mechanisms that underlie OS is critical for developing a new treatment strategy.

Long non-coding RNAs (lncRNAs) are RNAs that do not code for a protein but do serve important roles in cellular processes by regulating specific genes (7). Previous studies have shown that many lncRNAs are involved in the pathogenesis of OS, such as taurine upregulated 1 (TUG1), X-inactive specific transcript (XIST), long intergenic non-protein coding RNA 152 and FOXD2 adjacent opposite strand RNA 1 (FOXD2-AS1) (8-11). Zhang *et al* (8) found that downregulation of lncRNA TUG1 significantly inhibits OS cell proliferation and promotes apoptosis. Li *et al* (9) reported that XIST inhibition suppresses the proliferation and invasion of OS cells. Zhang *et al* (11) showed that FOXD2-AS1 downregulation limits the proliferation, migration and invasion of OS cells. The aforementioned lncRNAs serve as oncogenes in OS. Furthermore, lncRNA small nucleolar RNA host gene 1 (SNHG1) has been demonstrated to facilitate the progression of OS (12-14). Jiang *et al* (13) determined that upregulation of SNHG1 promotes OS cell proliferation and migration and inhibits apoptosis. In agreement with this, Wang *et al* (14) found that SNHG1 silencing restrains the proliferation, migration and invasion of OS cells. However, the detailed mechanisms of action of SNHG1 on OS still need to be deciphered.

MicroRNAs (miRNAs) are a kind of small endogenous RNA that can influence the post-transcriptional

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Abbreviations: FGF2, fibroblast growth factor 2; lncRNA, long noncoding RNA; miRNA, microRNA; RT-qPCR, reverse transcription-quantitative PCR; OS, osteosarcoma; SNHG1, small nucleolar RNA host gene 1

Key words: osteosarcoma, small nucleolar RNA host gene 1, microRNA-424-5p, invasion, migration, proliferation

regulation of specific genes (15). Increasing attention has been paid to the anti-tumoral roles of miRNAs in OS, such as miRNA (miR)-206 (16), miR-137 (9), and miR-193-3p (10). miR-424-5p is widely considered to be a suppressor in several types of human cancers, such as glioma (17), cholangiocarcinoma (18) and ovarian cancer (19). Notably, the inhibitory effect of miR-424 on the metastasis of OS cells has also been confirmed (20). lncRNAs can act as competitive endogenous RNAs or sponges of miRNAs. SNHG1 has been reported to facilitate the progression of OS by regulating many miRNAs, including miR-101-3p (12), miR-577 (13) and miR-326 (14). However, the regulatory relationship between SNHG1 and miR-424 remains unclear.

In the present study, the influence of SNHG1 inhibition on the viability, migratory ability and invasive ability of OS cells as well as the potential regulatory mechanisms of SNHG1/miR-424-5p/FGF2 were investigated with the goal of developing a new treatment strategy for OS.

Materials and methods

Sample collection. Between January 2016 and January 2018, 61 pairs of OS tissue samples and adjacent normal tissues were obtained from patients with OS (average age, 18.6 years old) at the ZhouPu Hospital Affiliated to Shanghai University of Medicine & Health Sciences. These patients did not receive radiotherapy or chemotherapy before the operation. The protocols of this study were reviewed and approved by the Ethical Committee of ZhouPu Hospital Affiliated to Shanghai University of Medicine & Health Sciences. All participants provided signed informed consent.

Cell grouping and transfection. The OS cell lines Saos-2, MG63, HOS and U2OS, as well as the human osteoblast cell line hFOB1.19 were purchased from Tongpai (Shanghai) Biotechnology Co., Ltd. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal bovine serum at 5% CO₂, 37°C and 95% humidity. Small interfering (si)RNA-negative control (si-NC; 5'-UUCUCCGAACGU GUCACGUTT-3'), siRNA-SNHG1-1 (si-SNHG1-1, 5'-CAG CAGTTGAGGGTTTGCTGTGTAT-3') and si-SNHG-2 (5'-TTCAACAGCTAGGTTGTCCTT-3') were purchased from Sangon Biotech Co., Ltd. Overexpression vectors pcDNA-FGF2, pcDNA-SNHG1 and empty vector (pcDNA-NC), along with miR-424-5p mimics, miRNA mimics-NC (miR-NC), miR-424-5p inhibitor and inhibitor NC were all procured from Guangzhou RiboBio Co., Ltd. The aforementioned agents (all, 50 nM) were transfected into the cells (6x10⁵ cells/well) using a Lipofectamine RNAiMAX kit (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37°C. Following transfection, the cells were harvested to perform the following experiments.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR). TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract the total RNA from tissues or cell lines. The GoScript reverse transcription system (Promega Corporation) was used to reverse transcribe the extracted RNA into cDNA. qPCR was performed using the SYBR Green PCR Master mix (Takara Biotechnology Co., Ltd.). The reaction conditions were as follows: 95°C for 10 min; followed

by 40 cycles at 94°C for 10 sec, 60°C for 20 sec and 72°C for 34 sec. The data were analysed by the 2^{-ΔΔC_q} method (21). For normalization, GAPDH was used as endogenous control to normalize lncRNA SNHG1 expression level and U6 was used as endogenous control to normalize miR-424-5p expression level. The sequences of the primers are as follows: SNHG1 forward, 5'-ACGTTGGAACCGAAGAGAGC-3' and reverse, 5'-GCA GCTGAATTCCTCCAGGAT-3'; miR-424-5p forward, 5'-GGC TAGTCAGCAGCAATTCATGT-3' and reverse, 5'-GTGCAG GGTCCGAGGT-3'; FGF2 forward, 5'-AGGAGAGCGACC CACACATCAA-3' and reverse, 5'-AGCCAGCAGTCTTCC ATCTTCC-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'; GAPDH forward, 5'-CCAGGTGGTCTCCTCTGACTT-3' and reverse, 5'-GTTGCTGTAGCCAAATTCGTTGT-3'.

MTT assay. Transfected MG63 and U2OS cells were seeded (2x10⁵ cells/well) into a 96-well plate and incubated for 24, 48, 72 and 96 h. MTT (5 mg/ml; 20 μl; Sigma-Aldrich; Merck KGaA) was added at different time points. After 2 h incubation at 37°C, cell viability (optical density at 450 nm) was analysed using a SpectraMax microplate spectrophotometer (Molecular Devices, LLC).

Wound healing assay. The transfected MG63 and U2OS cells (2x10⁵ cells/well) were seeded into 6-well plates. When the cells grew to a 100% confluence, wounds on the cell monolayer were created using a sterile p200 pipette tip, and the cells were incubated for 24 h in a serum-free medium. Subsequently, the cells were washed three times with PBS to wash away the floating cells. Images were captured at 0 and 24 h under a light microscope (magnification, x400; Olympus Corporation) and analysed with ImageJ software [version 1.46, National Institutes of Health (NIH)].

Transwell invasion assay. Cell invasion was assessed using Transwell chambers (Corning, Inc.) that were pre-coated (at 37°C for 30 min) with Matrigel[®] (BD Biosciences). Transfected MG63 and U2OS cells (2x10⁵ cells/well) were resuspended in serum-free medium and seeded into the Matrigel-coated upper chamber. A total of 600 μl DMEM containing 10% FBS was added into the lower chamber. After 24 h of culturing, the invasive cells were stained with 0.5% crystal violet. Invasive ability was evaluated by counting the number of invasive cells under a light microscope (magnification, x400; Olympus Corporation) in five randomly selected views.

Target prediction. StarBase version 2.0 (<http://starbase.sysu.edu.cn>), a software that decodes miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data, was used to predict the miRNA targets of SNHG1. A total of 144 putative targets was predicted. Among these miRNA targets, miR-424-5p was selected for the following assays owing to its important role in OS and the unknown regulatory relationship. In addition, TargetScan (<http://www.targetscan.org>), a software that predicts effective microRNA target sites in mammalian mRNAs, was used to predict the mRNA targets of miR-424-5p. Among the 1,515 target mRNAs, FGF2 was selected for the following assays owing to its important role in OS.

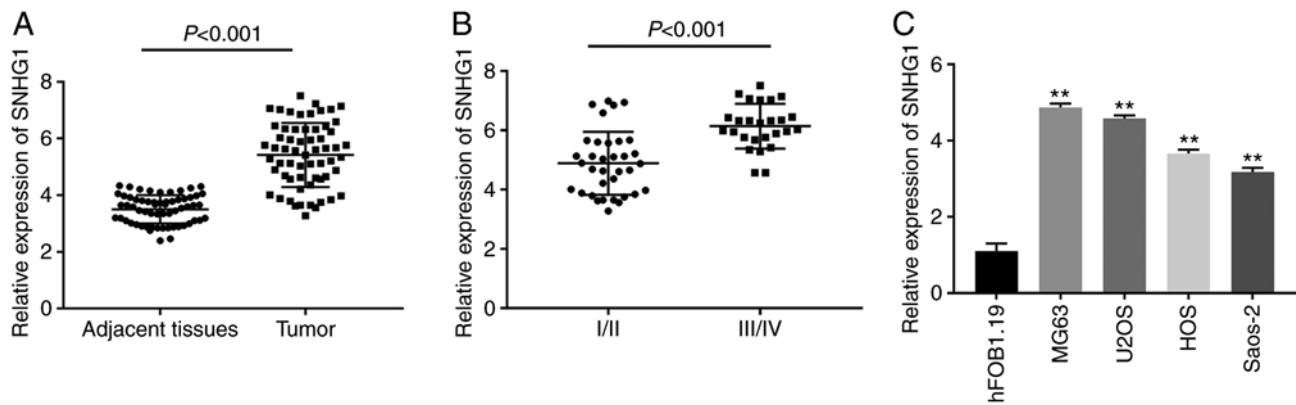


Figure 1. IncRNA SNHG1 expression is upregulated in OS tissues and cell lines. (A) RT-qPCR was used to detect the expression levels of IncRNA SNHG1 in OS and adjacent tissues. (B) The expression levels of SNHG1 were compared in patients with stage I/II and III/IV OS. (C) RT-qPCR was used to detect the expression levels of SNHG1 in hFOB1.19 and OS cell lines. The data were expressed as the mean \pm SD. ** $P < 0.01$ vs. hFOB1.19. LncRNA, long non-coding RNA; OS, osteosarcoma; RT-qPCR, reverse transcription-quantitative PCR; SNHG1, small nucleolar RNA host gene 1.

RNA-binding protein immunoprecipitation assay (RIP). RIP was conducted using the EZ-Magna RIP RNA-Binding Protein Immunoprecipitation Kit (EMD Millipore). MG63 and U2OS cells (5×10^5 cells/well) were lysed with RIPA lysis buffer (Beyotime Institute of Biotechnology). Subsequently, the cell extracts were incubated with RIPA buffer magnetic beads as well as anti-Argonaute2 (AGO2) and anti-immunoglobulin G (IgG) (Shanghai Kanglang Biotechnology Co., Ltd.) at 4°C overnight and then washed with RIPA buffer (Beyotime Institute of Biotechnology). The eluates were collected, and the expression levels of SNHG1 and miR-424-5p were detected by RT-qPCR, aforementioned.

Dual-luciferase reporter gene (DLR) assay. The predicted binding sequences of SNHG1 (binding sites, CCA GUGAUGAAUUGCUGCU) and corresponding mutation sequences (GCUUGUGUACUUAACGACGA) were inserted into the pGL3 vector to establish the SNHG1-wild-type (WT)/SNHG1-mutant-type (Mut). Similarly, The predicted binding sequences of FGF2 (binding sites, AAAAUA UUUUGCUGCU) and corresponding mutation sequences (UUUUUUUAUAACGACGA) were inserted into pGL3 vector to construct the FGF2-WT/FGF2-Mut. MG63 and U2OS cells (1×10^5 cells/well) were then co-transfected with SNHG1-Mut/FGF2-Mut or SNHG1-WT/FGF2-WT (80 ng) and miR-424-5p mimics/miR-NC (50 nM) at 37°C. After 48 h of culture, a Dual-Luciferase Reporter Assay System (Promega Corporation) was used to detect the luciferase activity. The activity of firefly luciferase was normalized to that of *Renilla* luciferase.

Western blot assay. The total protein from U2OS cells was extracted in RIPA buffer (Beyotime Institute of Biotechnology) containing 10 mmol/l phenylmethylsulfonyl fluoride (Beyotime Institute of Biotechnology); the protein concentration was detected by the BCA Protein Assay Kit (Abcam). All steps were conducted on ice. A total of $\sim 30 \mu\text{g}$ protein was separated by 10% SDS-PAGE and then transferred to a polyvinylidene fluoride membrane (EMD Millipore). Membrane blocking was performed using 5% bovine serum albumin (Thermo Fisher Scientific, Inc.) at room temperature.

Next, the membrane was incubated overnight at 4°C with primary antibodies against FGF2 (1:1,000; cat. no. ab208687; Abcam) and β -actin (1:1,000; cat. no. ab8226; Abcam). The membrane was washed with TBS + Tween-20 (0.05%) three times followed by incubation with the HRP-conjugated rabbit anti-mouse IgG secondary antibody (1:3,000; cat. no. ab6728; Abcam) for 1 h at 37°C. β -actin served as the internal loading control. Chemiluminescence was examined using the SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. ImageJ software (version 1.46; NIH) was utilised to semi-quantify the image.

Statistical analysis. SPSS Statistics 22.0 software (IBM Corp.) was used to analyse the data. The data are presented as the mean \pm SD. The comparisons between two groups were analysed by unpaired t-tests, matched samples were compared by paired t-test, and the one-way ANOVA was measured for more than two groups. After ANOVA analysis, pairwise comparisons were assessed using Tukey's multiple comparisons test. Pearson's correlation analysis was used to determine the correlations between the expression of SNHG1 and miR-424-5p, FGF2 and miR-424-5p, as well as SNHG1 and FGF2 in OS tissues. $P < 0.05$ was considered to indicate a statistically significant difference. All experiments were conducted in triplicate in at least three independent experiments.

Results

IncRNA SNHG1 expression is significantly increased in OS tissues and cell lines. To evaluate whether SNHG1 influences the development of OS, samples from 61 patients with OS were obtained and compared with the adjacent normal tissues. The expression levels of SNHG1 in OS tissues was found to be significantly higher compared with the adjacent tissues ($P < 0.001$; Fig. 1A). In addition, it was also determined that SNHG1 expression in OS is related to its clinical stages. The expression of SNHG1 in stage III/IV of OS was significantly higher compared with the expression levels in stage I/II, which indicated that SNHG1 expression was related to the severity of OS ($P < 0.001$; Fig. 1B). As presented in Table I, the patients

Table I. Clinicopathological characteristics of patients with OS, and lncRNA SNHG1 expression levels in OS tissues.

Clinicopathological characteristic	n=61	lncRNA SNHG1 expression		P-value
		Low (n=30)	High (n=31)	
Age, years				0.699
<20	31	16	15	
≥20	30	14	16	
Sex				0.885
Male	27	13	14	
Females	34	17	17	
Diameter, cm				0.683
<3	26	12	14	
≥3	35	18	17	
Resection degree				0.699
Total resection	31	16	15	
Subtotal resection	30	14	16	
WHO Grade				<0.001
I + II	35	24	11	
III + IV	26	6	20	

lncRNA SNHG1, long non-coding RNA small nucleolar RNA host gene 1; OS, osteosarcoma.

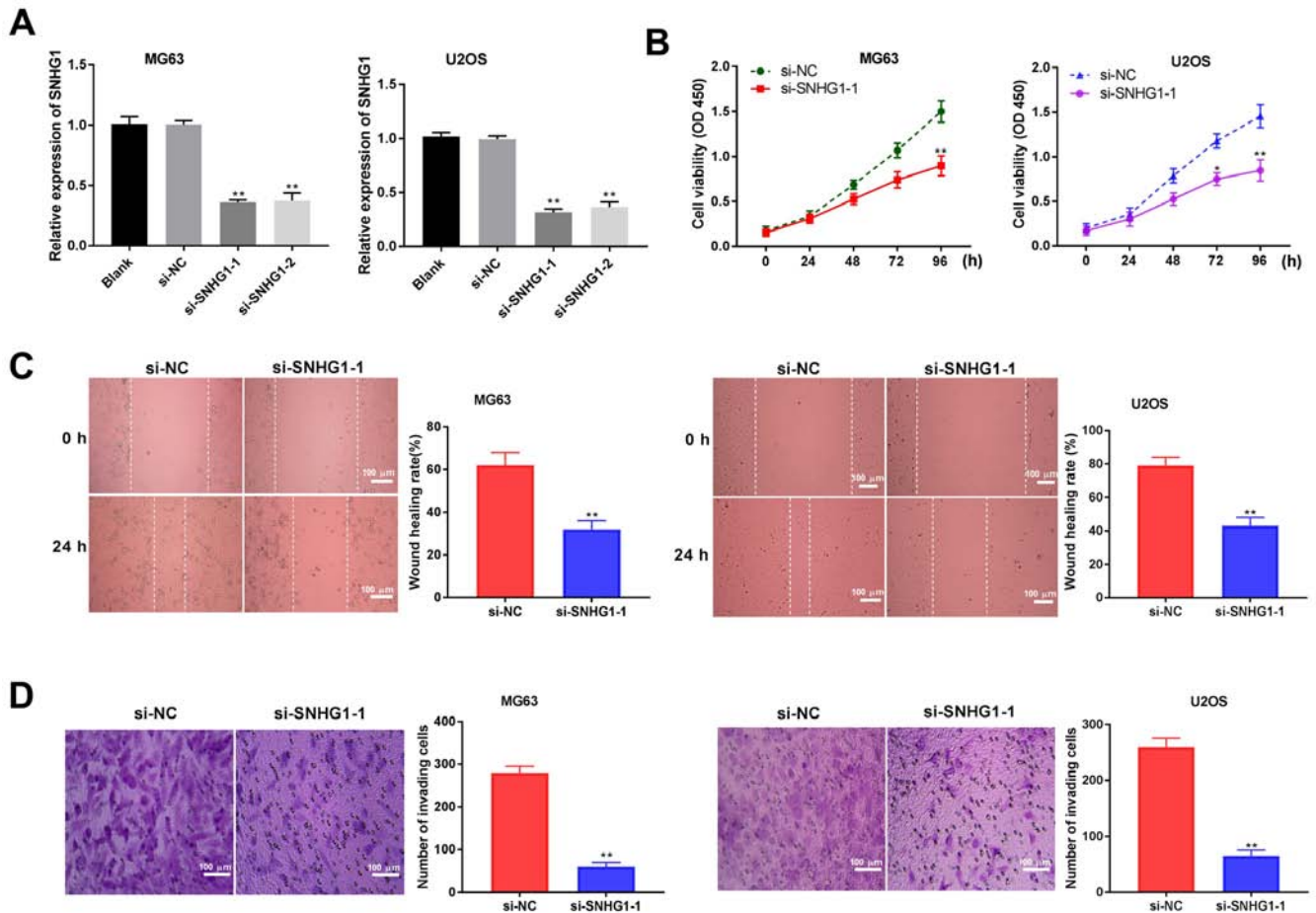


Figure 2. lncRNA SNHG1 knockdown inhibits proliferation, migration and invasion of OS cells. (A) Reverse transcription-quantitative PCR was used to detect the expression of lncRNA SNHG1 after transfection of si-SNHG1-1, si-SNHG1-2 and si-NC into MG63 and U2OS OS cells. (B) The viability of OS cells was detected by MTT assay. (C) The migratory ability of OS cells was determined by wound healing assay. (D) The invasive ability of OS cells was analyzed by Transwell invasion assay. Scale bar, 100 μ m; magnification, x400. The data are expressed as the mean \pm SD. * P <0.05, ** P <0.01 vs. si-NC. lncRNA, long non-coding RNA; NC, negative control; OD, optical density; OS, osteosarcoma; si-, small interfering RNA; SNHG1, small nucleolar RNA host gene 1.

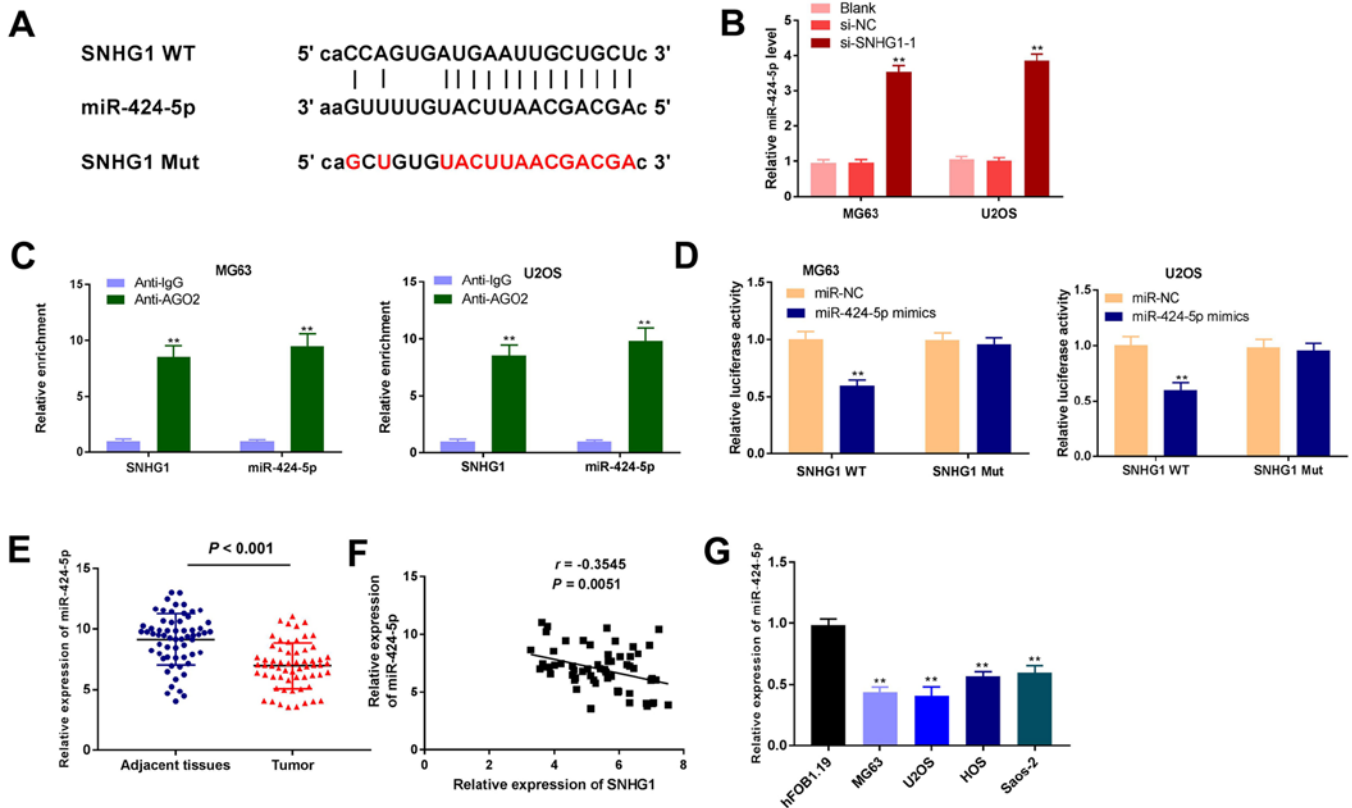


Figure 3. lncRNA SNHG1 targets miR-424-5p. (A) starBase was used to predict the binding between lncRNA SNHG1 and miR-424-5p. (B) RT-qPCR was used to detect the expression levels of miR-424-5p after transfection of si-SNHG1-1 into MG63 and U2OS OS cells. $^{**}P < 0.01$ vs. si-NC. (C) RNA-binding protein immunoprecipitation assay was performed in OS cells, and the expression of SNHG1 and miR-424-5p was detected by RT-qPCR. $^{**}P < 0.01$ vs. Anti-IgG. (D) Dual-luciferase reporter gene assays were used to confirm the targeting relationship between SNHG1 and miR-424-5p. $^{**}P < 0.01$ vs. miR-NC. (E) RT-qPCR was used to detect the expression of miR-424-5p in patient OS and adjacent normal tissues. (F) Correlation analysis between SNHG1 and miR-424-5p. (G) RT-qPCR was used to detect the expression of miR-424-5p in hFOB1.19 and OS cell lines. $^{**}P < 0.01$ vs. hFOB1.19. The data are expressed as the mean \pm SD. AGO2, argonaute2; IgG, immunoglobulin G; lncRNA, long non-coding RNA; miR, microRNA; Mut, mutant; NC, negative control; OS, osteosarcoma; RT-qPCR, reverse transcription-quantitative PCR; si, small interfering RNA; SNHG1, small nucleolar RNA host gene 1; WT, wild-type.

were divided into two groups: High and low lncRNA SNHG1 expression, using the median expression level as the cut-off point. High and low expression levels of SNHG1 were speculated to exhibit distinct differences based on tumour stage. SNHG1 expression levels were also detected in hFOB1.19 and a number of OS cell lines. SNHG1 was found to be expressed in the four OS cell lines at significantly higher levels compare with expression in the hFOB1.19 cells ($P < 0.01$; Fig. 1C). MG63 and U2OS cell lines were selected for the further experiments due to their relatively high expression of SNHG1. These results indicated that SNHG1 may be an onco-lncRNA in OS.

Silencing lncRNA SNHG1 inhibits the proliferation, migration and invasion of OS cells. Following transfection of si-SNHG1-1, si-SNHG1-2 and si-NC into OS cells, SNHG1 expression level was detected by RT-qPCR. The results indicated that SNHG1 expression in MG63 and U2OS cells were downregulated after transfection with si-SNHG1-1 and SNHG1-2 compared with the si-NC group ($P < 0.01$; Fig. 2A). Using the MTT assay, it was confirmed that the viability was significantly inhibited 96 and 72 h after si-SNHG1-1 transfection in MG63 and U2OS cells, respectively ($P < 0.05$; Fig. 2B). The migratory ability of the OS cell lines was also significantly inhibited after SNHG1 knock-down compared with si-NC ($P < 0.01$; Fig. 2C). The Transwell invasion assay showed similar results; the invasive ability of

OS cells was significantly inhibited after SNHG1 interference ($P < 0.01$; Fig. 2D). Together, these data demonstrated that SNHG1 silencing may limit the proliferation, migration and invasion of OS cells.

lncRNA SNHG1 targets miR-424-5p. Using starBase software, the binding region between miR-424-5p and SNHG1 was predicted (Fig. 3A). miR-424-5p expression levels were detected after transfection of si-SNHG1-1 into MG63 and U2OS OS cells, and the results revealed that the expression of miR-424-5p was significantly increased in the si-SNHG1-1 group compared with the si-NC group ($P < 0.01$; Fig. 3B). The RIP assay demonstrated that in OS cell lines, SNHG1 was enriched with anti-AGO2 compared with those of the anti-IgG control and that miR-424-5p exhibited similar results ($P < 0.01$; Fig. 3C). The DLR assays showed a marked decrease in luciferase activity in the SNHG1 WT + miR-424-5p mimics group compared with that of the SNHG1 WT + miR-NC group ($P < 0.01$; Fig. 3D). miR-424-5p expression in the patient tumour and adjacent tissues were also detected; RT-qPCR results demonstrated that miR-424-5p expression levels in OS tissues decreased significantly compared with expression levels in the adjacent tissues ($P < 0.001$; Fig. 3E). Correlation analysis between SNHG1 and miR-424-5p expression levels revealed that there was a negative correlation ($r = -0.3545$; $P = 0.0051$;

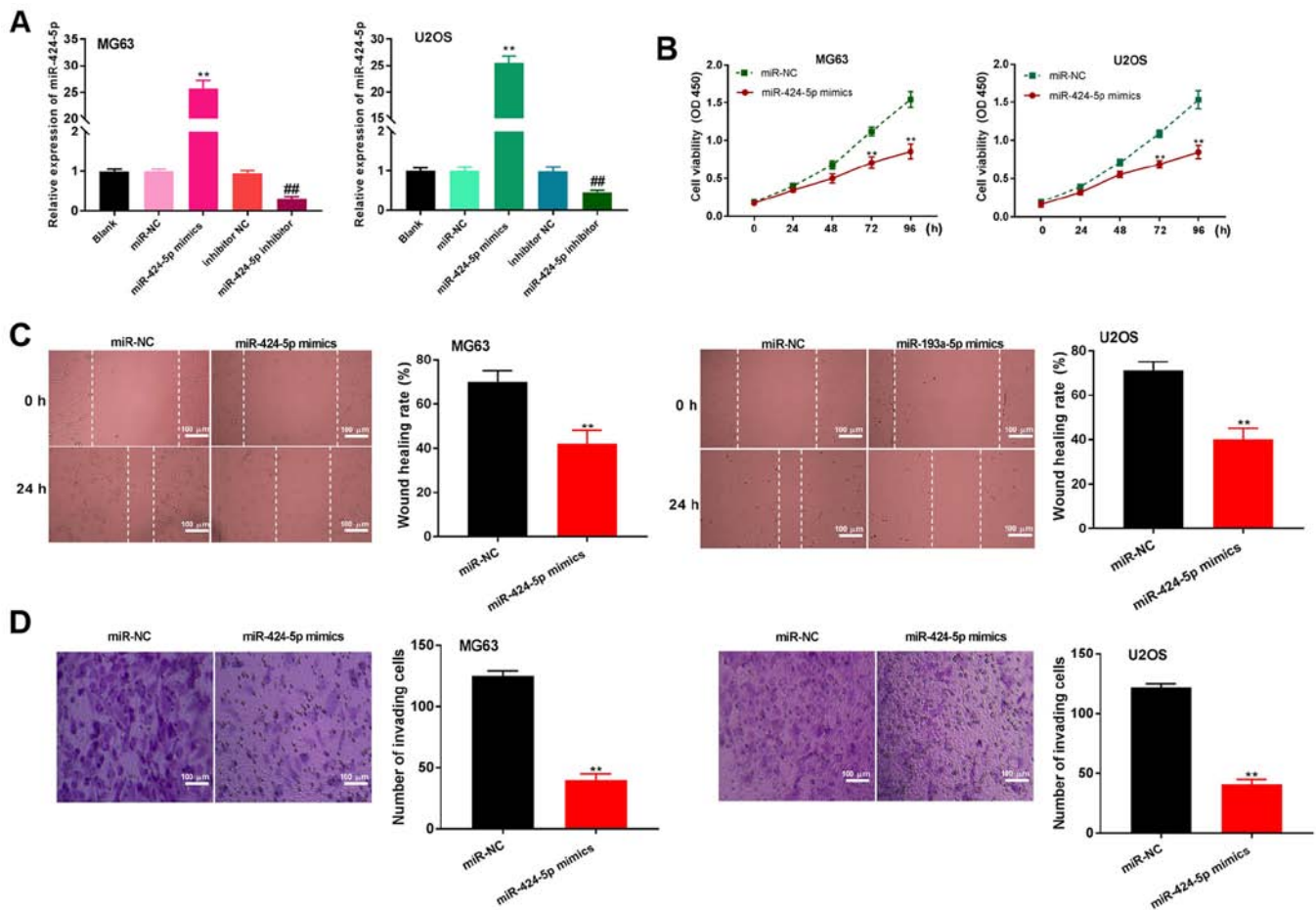


Figure 4. Overexpression of miR-424-5p inhibits the proliferation, migration and invasion of OS cells. (A) Reverse transcription-quantitative PCR was used to detect the expression levels of miR-424-5p after transfection of miR-424-5p mimics, miR-NC, miR-424-5p inhibitor or inhibitor NC into MG63 and U2OS OS cells. (B) The viability of OS cells was detected by MTT assay. (C) Wound healing assays were used to determine the migratory ability of OS cells. (D) Transwell invasion assays were used to analyze the invasion ability of OS cells. Scar bar, 100 μ m; magnification $\times 400$. The data are expressed as the mean \pm SD. ** $P < 0.01$ vs. miR-NC; ### $P < 0.01$ vs. inhibitor NC. miR, microRNA; NC, negative control; OD, optical density; OS, osteosarcoma.

Fig. 3F). Finally, the expression of miR-424-5p was detected in OS and normal human osteoblast cell lines. RT-qPCR results indicated that miR-424-5p expression levels in the OS cells were significantly reduced compared with that of hFOB1.19 ($P < 0.01$; Fig. 3G). These data indicated that miR-424-5p was the target of, and negatively modulated by, SNHG1.

miR-424-5p upregulation limits the proliferation, migration and invasion of OS cells. After transfection of miR-424-5p mimics, miR-NC, miR-424-5p inhibitor or inhibitor NC into MG63 and U2OS OS cells, miR-424-5p expression was detected. The results demonstrated that the expression of miR-424-5p was upregulated after transfection of miR-424-5p mimics and downregulated after transfection of the miR-424-5p inhibitor, compared with the respective controls ($P < 0.01$; Fig. 4A). The MTT assay revealed that miR-424-5p overexpression significantly suppressed the viability of OS cells at 72 h ($P < 0.01$; Fig. 4B). Furthermore, the wound healing assay confirmed that upregulated miR-424-5p expression significantly inhibited the migratory ability of OS cells ($P < 0.01$; Fig. 4C). miR-424-5p overexpression also limited the number of invasive OS cells ($P < 0.01$; Fig. 4D). These results indicated that overexpression of miR-424-5p inhibits the proliferation, migration and invasion of OS cells.

miR-424-5p targets FGF2. Using TargetScan software, FGF2 was predicted to be a downstream target of miR-424-5p (Fig. 5A). The DLR assay revealed that in MG63 and U2OS OS cell lines, the luciferase activity in the FGF2 WT + miR-424-5p mimics group was significantly decreased compared with that of the FGF2 WT + miR-NC group, an effect that was partially reversed when SNHG1 was overexpressed ($P < 0.01$; Fig. 5B). In addition, the expression levels of FGF2 in patient tissue samples were examined; FGF2 expression levels in the OS tissues were significantly higher compared with expression in the adjacent normal tissues ($P < 0.001$; Fig. 5C). Correlation analysis indicated that FGF2 expression was negatively correlated with miR-424-5p expression ($r = -0.5258$; $P < 0.0001$; Fig. 5D) and positively correlated with SNHG1 expression ($r = 0.5196$; $P < 0.0001$; Fig. 5E). Finally, compared with the hFOB1.19 cells group, the expression of FGF2 in the OS cell lines were found to be significantly higher ($P < 0.01$; Fig. 5F). The aforementioned data suggested that FGF2, which was highly expressed in OS, is a target of miR-424-5p.

SNHG1 knockdown inhibits the proliferation, migration and invasion of OS cells by regulating miR-424-5p/FGF2 in vitro. The transfection efficiency of pcDNA-SNHG1 or pcDNA-FGF2 in U2OS cells was initially determined; the

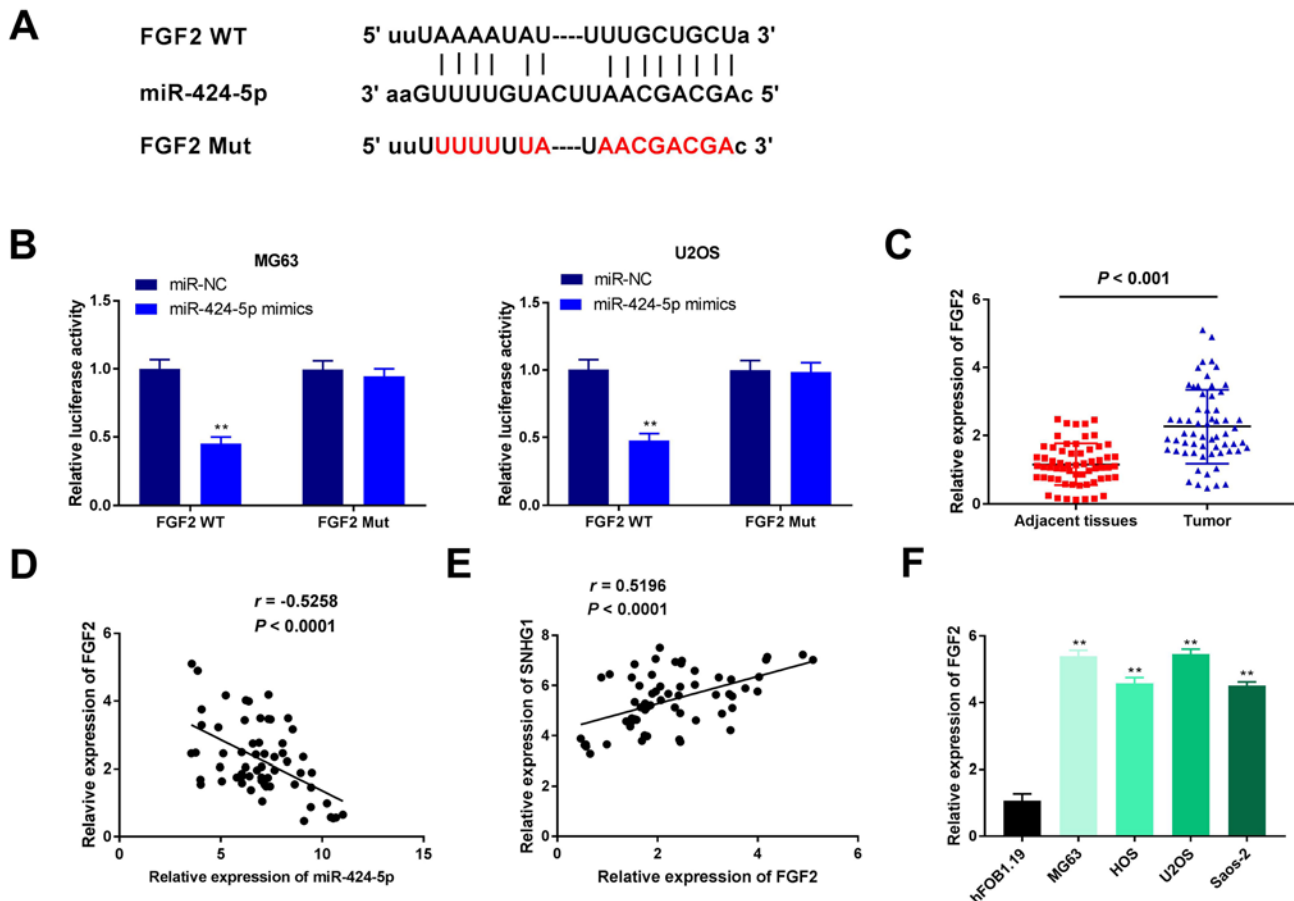


Figure 5. miR-424-5p targets FGF2. (A) TargetScan was used to predict target sites between the miR-424-5p and FGF2. (B) DLR assays were used to confirm the targeting relationship. ** $P < 0.01$ vs. miR-NC + pcDNA-NC group. (C) RT-qPCR was used to determine the expression levels of FGF2 in patient OS and adjacent tissues. (D) Correlation analysis between FGF2 and miR-424-5p. (E) Correlation analysis between SNHG1 and FGF2. (F) RT-qPCR was used to detect the expression levels of FGF2 in hFOB1.19 and OS cell lines. ** $P < 0.01$ vs. hFOB1.19. The data are expressed as the mean \pm SD. DLR, dual-luciferase reporter gene; FGF2, fibroblast growth factor-2; miR, microRNA; Mut, mutant; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR; SNHG1, small nucleolar RNA host gene 1; WT, wild-type.

expression levels of SNHG1 were significantly increased by transfection of pcDNA-SNHG1 in U2OS cells ($P < 0.01$; Fig. 6A), and FGF2 expression levels were significantly upregulated in U2OS cells transfected with pcDNA-FGF2 ($P < 0.01$; Fig. 6B). Western blot analysis results demonstrated that miR-424-5p overexpression in U2OS cells significantly inhibited FGF2 expression, which could be partially reversed by overexpression of SNHG1 ($P < 0.01$; Fig. 6C). The MTT assay revealed that cell viability was inhibited by depletion of SNHG1 at 96 h, but this effect could be partly reversed by miR-424-5p inhibition or overexpression of FGF2 ($P < 0.01$; Fig. 6D). Similarly, the wound healing and Transwell invasion assays demonstrated that inhibition of SNHG1 suppressed the migratory and invasive abilities of U2OS cells, both of which could be reversed by miR-424-5p downregulation and FGF2 upregulation ($P < 0.01$; Fig. 6E and F). These data indicated that SNHG1 knockdown may suppress the proliferation, migration and invasion of OS cells by regulating the miR-424-5p/FGF2 axis (Fig. 7).

Discussion

OS is one of the most common malignant bone tumours in adolescents (22). Several lncRNAs have been reported to be

involved in the regulation of OS. For example, Fei *et al* (16) discovered that expression of the lncRNA regulator of reprogramming (ROR) is significantly increased in OS tissues and in OS cell lines, and that upregulation of ROR appears strongly related to tumour stage. Yang *et al* (23) found that the expression of lncRNA XIST is dramatically elevated in OS tissues and also strongly associated with tumour stage. Similarly, results from the present study demonstrated that SNHG1 expression is elevated in OS tissues and cell lines, and presents a notable correlation with tumour stage. Thus, it was hypothesised that SNHG1 may act as a pathogenic factor in OS.

In the past decade, researchers have determined that lncRNAs function as crucial regulators in the progression of OS. For example, Li *et al* (9) reported that suppression of XIST limits the proliferation and invasion of OS cells. Zhao *et al* (24) discovered that ASBEL interference decreases viability and the migratory and invasive abilities of OS cells. Xu *et al* (25) found that SNHG4 inhibition suppresses the proliferation of OS cells. The present study found that knockdown of SNHG1 inhibited the viability, migratory and invasive abilities of OS cells. Similar findings in another study demonstrated that OS cell viability, migration and invasion is regulated by silencing SNHG1, thereby eventually halting the progression of OS (14).

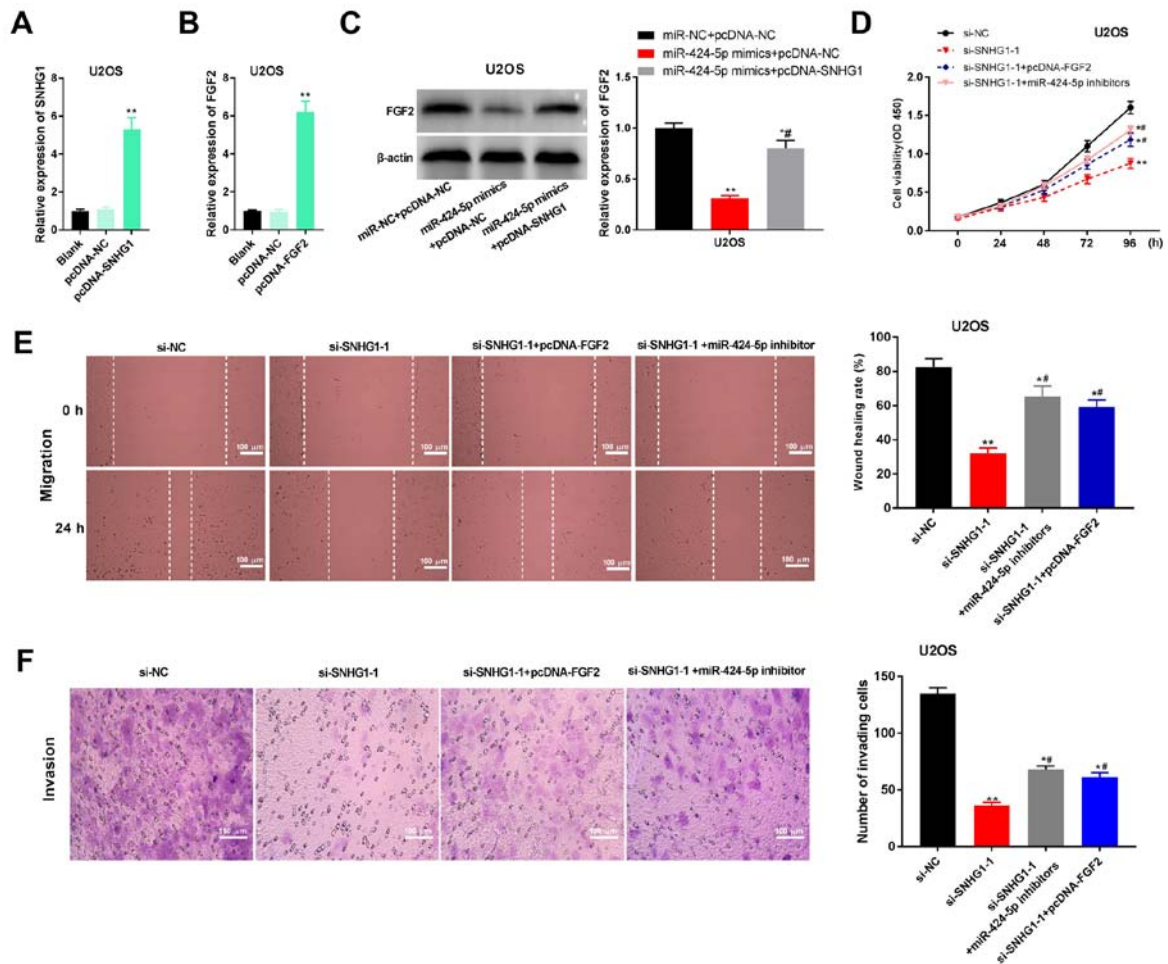


Figure 6. IncRNA SNHG1 knockdown inhibits proliferation, migration and invasion of U2OS cells by regulating miR-424-5p/FGF2. (A) RT-qPCR was used to detect the expression levels of SNHG1 after transfection of pcDNA-SNHG1. ** $P < 0.01$ vs. pcDNA-NC. (B) RT-qPCR was used to detect the expression of FGF2 after transfection of pcDNA-FGF2. ** $P < 0.01$ vs. pcDNA-NC. (C) Western blotting was used to detect the protein expression levels of FGF2 in transfected U2OS cells. * $P < 0.05$, ** $P < 0.01$ vs. miR-NC + pcDNA-NC; # $P < 0.05$ vs. miR-424-5p mimics + pcDNA-NC. (D) MTT assays were used to detect the viability of transfected U2OS cells. * $P < 0.05$, ** $P < 0.01$ vs. si-NC; # $P < 0.05$ vs. si-SNHG1-1. (E) Wound healing assays were used to determine the migratory ability of transfected U2OS cells. * $P < 0.05$, ** $P < 0.01$ vs. si-NC; # $P < 0.05$ vs. si-SNHG1-1. (F) Transwell invasion assays were used to analyze the invasive ability of transfected U2OS cells. Scar bar, 100 μ m; magnification, x400. * $P < 0.05$, ** $P < 0.01$ vs. si-NC; # $P < 0.05$ vs. si-SNHG1-1. The data are expressed as the mean \pm SD. FGF2, fibroblast growth factor-2; IncRNA, long non-coding RNA; miR, microRNA; NC, negative control; OD, optical density; OS, osteosarcoma; si-, small interfering RNA; SNHG1, small nucleolar RNA host gene 1.

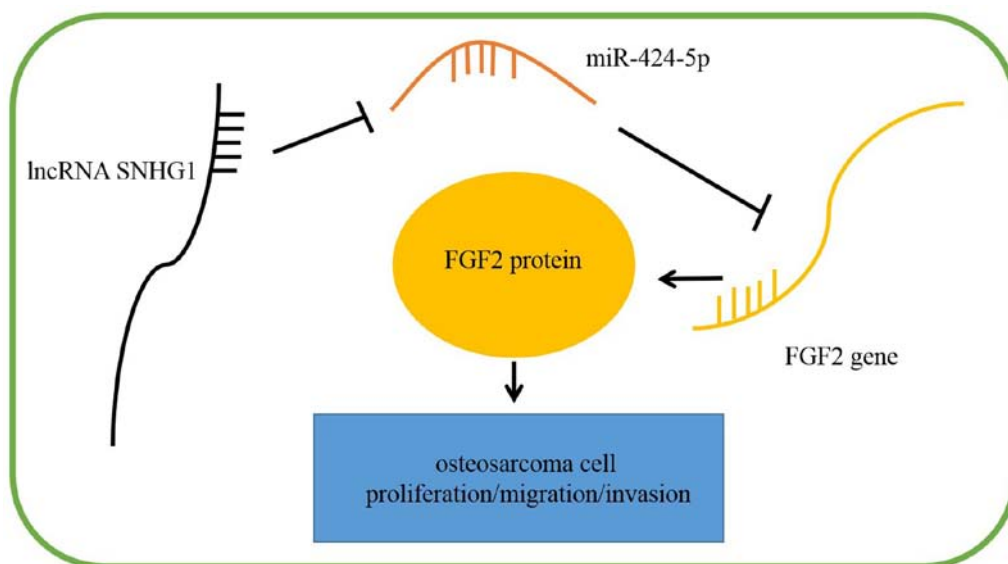


Figure 7. Schematic diagram of the regulatory mechanism of the SNHG1/miR-424-5p/FGF2 axis in osteosarcoma cells. FGF2, fibroblast growth factor-2; IncRNA, long non-coding RNA; miR, microRNA; SNHG1, small nucleolar RNA host gene 1.

Therefore, it was hypothesized that SNHG1 inhibition may attenuate the development of OS.

miR-424 has been shown to attenuate the progression of several cancers, including endometrial cancer (EC) (26), breast cancer (BC) (27) and colorectal cancer (CRC) (28). Dong *et al* (26) reported that miR-424 expression is decreased in EC tissues and cell lines, and that upregulation of miR-424 suppresses EC cell invasion. Wang *et al* (27) found that miR-424 is minimally expressed in BC tissues and cell lines, and miR-424 upregulation inhibits the development of BC. Fang *et al* (28) demonstrated that miR-424 expression in CRC tissues and cell lines is low, and overexpression of miR-424 eventually suppresses the growth of CRC. The present study discovered that miR-424-5p expression is significantly lower in OS tissues and cell lines. Furthermore, OS cell viability, migration and invasion are inhibited by miR-424-5p overexpression. Consistent with these results, previous studies have reported that miR-424 expression is decreased in OS tissues and that miR-424 overexpression limits the viability (29), as well as the migratory and invasive abilities (17) of OS cells. In addition, the present study further determined that miR-424-5p is the target of and negatively modulated by SNHG1. Therefore, it was hypothesized that miR-424-5p may be regulated by SNHG1 to inhibit the development of OS.

FGF2 is a member of the FGF family and has been thought to take part in the development of various cancers (30-32). Zhang *et al* (33) found that FGF2 is highly expressed in cervical carcinoma tissues, and Cheng *et al* (34) reported that FGF2 expression is elevated in non-small cell lung cancer tissues and cells. The present study showed that FGF2 expression is upregulated in OS tissues and cell lines. Similarly, Sun *et al* (35) observed that FGF2 expression in OS tissues is significantly upregulated. In addition, the present study demonstrated that miR-424-5p targeted FGF2, and there is a notable inverse correlation between their expressions. The results indicated that FGF2 may be negatively modulated by miR-424-5p. The present study also found that high expression of miR-424-5p and low expression of FGF2 both reverse the suppressive effects of SNHG1 knockdown on the viability, migratory and invasive abilities of OS cells. These data indicated that SNHG1 knockdown may inhibit the viability, migration and invasion of OS cells by regulating miR-424-5p/FGF2.

However, there are some limitations within the present study. For instance, only miR-424-5p mimics were used in cell culture; miR-424-5p inhibitor should be added in future studies to further support these results. Secondly, the regulatory effects of the SNHG1/miR-424-5p/FGF2 axis on the apoptosis of OS cells was not determined. Furthermore, the present study was limited to *in vitro* experiments, thus, additional research using animal models is required. Finally, the detailed mechanisms of the SNHG1/miR-424-5p/FGF2 axis on OS, such as upstream factors and related signalling pathways, need to be further explored.

Collectively, results from the presented study suggested that SNHG1 knockdown may suppress the progression of OS by regulating miR-424-5p/FGF2 *in vitro*. Thus, the SNHG1/miR-424-5p/FGF2 axis may present a new potential target for treating OS.

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Availability of data and materials

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

Authors' contributions

ZL made substantial contributions to the conception and design of the study. ZL, XW, and SL made substantial contributions to the acquisition, analysis and interpretation of data, as well as the drafting and revision of the manuscript. All authors confirmed the authenticity of all the raw data, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

The present study was conducted after obtaining local Ethical Committee approval of Zibo Zhoucun People's Hospital. Written informed consent was obtained from patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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