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SNHG9/miR-214-5p/SOX4 feedback loop regulates osteosarcoma progression

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Osteosarcoma (OS) is a high-grade, aggressive bone sarcoma. LncRNAs play a key regulatory role in controlling biological and pathological processes. The expression of lncRNA SNHG9 varies among different cancer tissues, and the role of SNHG9 in OS progression is unclear. In this study, we found SNHG9 overexpression in OS tissues and cells. In addition, downregulated SNHG9 expression impaired the proliferation, migration, and invasion abilities of OS cells. SNHG9 expression was positively regulated by the transcription factor SOX4. SNHG9 interacted with miR-214-5p as a molecular sponge and SOX4 was identified as the target of miR-214-5p. The interaction affected the expression of SNHG9, miR-214-5p, and SOX4, and regulated OS cell proliferation, migration, and invasion. Therefore, the SNHG9/miR-214-5p/SOX4 feedback loop performs an important role in OS progression and might be used as a new potential therapeutic target for the treatment of OS.

Key words: SNHG9, miR-214-5p, SOX4, feedback loop, osteosarcoma

Osteosarcoma (OS) is a highly malignant primary bone tumor that originates from mesenchymal tissue. It has a rapid progression and a high fatality rate and tends to occur in adolescence [1]. OS is highly malignant, frequently invades the surrounding soft tissues, causes distant metastasis [2], and is the leading cause of cancer-related deaths in adolescents and young people [3]. At present, the 5-year survival rate of patients with the metastatic OS is less than 25%, which indicates that it has a poor response to treatment [4, 5]. Therefore, it is important to develop targeted drugs that effectively prevent OS occurrence and progression.

Only 2% of the genes in the human genome encode proteins and at least 75% of the remaining genes are transcribed into non-coding RNA, including long-chain non-coding RNA (lncRNA) [5]. LncRNA is a regulatory non-coding RNA with a length of more than 200 nucleotides, which can be used as a key regulatory factor to control biological and pathological processes [6, 7]. Some studies have reported that lncRNA interacts with proteins, RNA, and DNA, which play an important role in regulating transcription, translation, and post-translational regulation [8–11]. The expression level of lncRNA changes during the development of cancers, which directly or indirectly regulates cancer-related signals and, subsequently, the occurrence and progression of a variety of tumors [12]. Multiple lncRNAs play a role in OS, such as lncRNA TTN-AS1, which regulates OS cell apoptosis and drug resistance [13], and lncRNA SNHG3, which regulates OS invasion and migration [14].

Small nucleolar RNA host gene 9 (SNHG9) is a membrane lipid-related lncRNA [15] that regulates the proliferation of pancreatic cancer [16], glioblastoma [17], and lung cancer [18] cells. In contrast, SNHG9 serves as a tumor suppressor gene in ovarian cancer and inhibits its progression [19]. In the present study, we aimed to determine the biological role and potential molecular mechanism of SNHG9 in OS.

Patients and methods

Patients and clinical samples. 20 pair OS tumor tissues and adjacent normal tissues were collected from Beijing Cancer Hospital, informed consents were signed by patients before this study. The study obtained the approval of the Research Ethics Committee of Beijing Cancer Hospital (No. 2019yzj78). All mandatory laboratory health and safety procedures have been followed during any experimental work. The clinical characteristics are shown in Table 1.

Cell culture and transfection. Human osteoblasts hFOB1.19 and three OS cell lines (U2OS, Saos-2, and HOS) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and the Institute of

Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in DMEM medium supplemented with 10% fetal bovine serum (ProSpec-Tany TechnoGene, Ltd., Israel) and 100 mg/ml penicillin/streptomycin (ProSpec-Tany TechnoGene, Ltd.) maintained at 37 °C in a humidified incubator with 5% CO_2. In addition, 200 mM glutamine and 2 mg/ml sodium bicarbonate were added to hFOB1.19 cells, 1.0 g/l glucose and 1 mM sodium pyruvate were added to HOS and U2OS cell lines, and 2 mM L-glutamine was added to for Saos-2 cells.

Short interfering RNA (siRNA) specifically targeting SNHG9 and negative control (NC) were purchased from Invitrogen (Carlsbad, CA, USA). For overexpression of SNHG9 and SOX4, the full-length SNHG9 and SOX4 sequence were transfected into pcDNA-3.1 vector (Thermo Fisher) respectively, called pcDNA-SNHG9 and pcDNA-Sox4. pcDNA-3.1 was used as a blank control. Luciferase reporter vectors (pGL3/SNHG9-WT, pGL3/SNHG9-MUT, pGL3/SOX4-WT, and pGL3/SOX4-MUT) were designed and constructed by Genechem (Shanghai, China). miR-214-5p (HMI0378) were purchased from Sigma Aldrich (St. Louis, MO, USA). Transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

RNA extraction and qRT-PCR. Total RNA of cells or tissues extracted with TRIzol reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA) and purified with an RNeasy mini kit (Qiagen GmbH). The quality and quantity of RNA were analyzed using a NanoDrop spectrophotometer. A reverse transcription kit (Cat: RR036A; Takara, Japan) was performed to reverse transcribe 1,000 ng total RNA to cDNA in a final volume of 20 μl. miRNA reverse transcription was performed using miRNA 1st Strand cDNA Synthesis Kit (by stem-loop) (Vazyme, China). Real-time PCR used SYBR Select Master mix (cat. no. 4472908; Applied Biosystems; Thermo Fisher Scientific, Inc.), and QuantStudio™ 6 Flex Real-Time PCR system (Thermo Fisher Scientific, Inc.) was used to collect RT-qPCR data. Each sample was carried out in triplicate, and relative expression was calculated and normalized using the $2^{-\Delta\Delta Cq}$ method relative to GAPDH. The primers used in this study are listed in Table 2.

Western blotting. Cells were harvested and processed in lysis reagents (Thermo Scientific, Waltham, MA, USA) on ice, and a BCA protein assay kit (Nanjing KeyGen Biotech Co., Ltd.) was utilized to quantify protein concentrations. Proteins were boiled for 5 min in a loading buffer before equal amounts of protein (6–10 μg) were separated via 10% SDS-PAGE, and then transferred onto polyvinylidene fluoride membranes. The membranes were blocked in 5% skimmed milk powder in Tris-buffered saline with 0.1%Tween 20 (TBST) and subsequently incubated with primary antibodies against N-cadherin (ab76057), E-cadherin (ab76055), Vimentin (ab137321), SOX4 (ab86809), and GAPDH (ab245357). After washing with TBST three times, the membrane was incubated with an HRP-conjugated goat anti-rabbit secondary antibody (ab7090). Enhanced ECL Chemiluminescent Substrate Kit (YEASEN, Shanghai, China) was used to visualize the bands on the membrane. The primary and secondary antibodies were purchased from Abcam.

Luciferase assay. Luciferase reporter vectors (pGL3/SNHG9-WT, pGL3/SNHG9-MUT, pGL3/SOX4-WT, and pGL3/SOX4-MUT), miRNA mimics or NC mimics were co-transfected in HOS and U2OS cells using Lipofectamine 2000 according to the manufacturer's protocol. Luciferase activity was measured using the dual-luciferase reporter kit (Promega, USA) after 48 h of transfection. Relative luciferase activity was expressed as the ratio of firefly luciferase to Renilla luciferase activity.

Cell viability assay. The viability of cells was examined using Cell Counting Kit-8 (CCK-8) assays (Solarbio, Beijing, China). Cells were seeded at a density of 1,000 cells/well in a 96-well plate and were cultured in humidified 5% CO_2

Table 1. Clinical characteristics of patients with osteosarcoma.

Clinical characteristics	Cases (n=20)
Sex	
Male	11
Female	9
Age	
≥18	13
<18	7
TNM stage	
I–II	14
III	6
Tumor site	
Proximal tibia	8
Distal femur	11
Other	1
Distant metastasis	
Yes	6
No	14

Table 2. The primers of gene for qRT-PCR.

Gene	Forward	Reverse
SNHG9	GGAATCTACGTCACCCGAAA	TTGAAAGACGTGGGACAGC
miR-214-5p	CTCCAGCTGGGCGTGTCGTTCACAT	CTACAAAGGGAAGCGACAGGCA
SOX4	CCAGCAAGAAGGCGAGTTAG	CGGAGCCTTCTGTCTTCATC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	ATCACTGCCACCCAGAAGAC	TTTCTAGACGGCAGGTCAGG

atmosphere at 37 °C. The optical density value was measured at 450 nm using a microplate reader (Multimode Reader; PerkinElmer, Inc.). Cell numbers were analyzed and a growth curve was drawn.

Cell migration and invasion assays. Cell migration and invasion assays were performed in 6.5 mm Transwell inserts (pore size, 8.0 μm; cat. no. 3422; Corning, Inc.). Cells (2×10^5) were suspended in 100 μl serum-free DMEM medium, whilst the lower chamber was filled with a complete DMEM medium with 10% serum. For migration assay, the cells were allowed to migrate for 18 h. For the invasion assay, the Transwell inserts were first coated with 2 mg/ml Matrigel (BD Biosciences) and cells were allowed to invade for 24 h. After removing the non-migratory cells, the membranes were fixed in 20% methanol and stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) solution (Thermo Fisher Scientific, Inc.). The number of stained cells was captured by a Nikon E800 microscope.

Chromatin immunoprecipitation (ChIP). Cells were fixed with a cross-linking solution to cross-link proteins to DNA. Chromatin immunoprecipitation (ChIP) analysis was performed as reported previously [20]. Free DNA was reversed from the cross-linking of protein/DNA complexes. qPCR primers for the SOX4 promoter regions are listed in Table 2.

Animal experiment. Ten BALB/c NSG mice (4–6 weeks old) were randomly divided into 2 groups, each group was subcutaneously injected with HOS cells transfected with si-SNHG9 or si-NC. Tumor volume and weight were measured every week using calipers and balance. The animal experiment was approved by the Research Ethics Committee of Beijing Cancer Hospital.

Statistical analysis. Data are presented as mean ± standard error of the mean. Statistical significance was evaluated with a one-way analysis of variance (ANOVA). LSD test was used for the statistical analysis of two groups of independent data using statistical GraphPad Prism software. A value of $p<0.05$ was considered statistically significant.

Results

SNHG9 is overexpressed in OS tissues and cells. The differential expression of SNHG9 in OS tissues, adjacent tissues, immortalized human osteoblasts hFOB1.19, and three OS cell lines (HOS, U2OS, and Saos-2) was detected using quantitative polymerase chain reaction (qPCR). We found that SNHG9 was highly expressed in OS tissues compared to the adjacent tissues (Figure 1A). Similarly, SNHG9 expression was 1.5–3-fold higher in OS cells than in hFOB1.19 cells (Figure 1B). In addition, the survival rate of OS patients was lower in those with higher SNHG9 expression than those with lower SNHG9 expression (Figure 1C).

SNHG9 knockdown affects the proliferation, migration, and invasion of OS cells. To explore the role of SNHG9 in OS, we reduced the content of SNHG9 in U2OS and HOS cells using siRNA. The interference efficiency of si-SNHG9 was examined using reverse transcription polymerase chain reaction (RT-PCR). The expression of SNHG9 in the si-SNHG9 groups was less than 50% of that in the si-NC groups (Figure 2A). The differences between the number of cells in the si-SNHG9 and si-NC groups gradually increased with a longer culture time (Figure 2B). Transwell assays were performed to investigate the effects of SNHG9 on OS cell migration and invasion. As shown in Figure 2C, the migration and invasion of si-SNHG9 cells were significantly decreased. Furthermore, detection of the expression of EMT-related proteins revealed higher E-cadherin level and lower N-cadherin and vimentin levels, in si-SNGH9 cells than in si-NC cells (Figure 2D). These results indicated that the lncRNA SNHG9 promotes proliferation, migration, and invasion of OS cells.

SNHG9 interacts with miR-214-5p as a molecular sponge. The function of lncRNAs is related to their subcellular distribution [20]. The online tools lncLocator (http://www.csbio.sjtu.edu.cn/bioinf/lncLocator/) and Locate-R (http://locate-r.azurewebsites.net/) were used to predict the subcellar location of SNHG9; both tools showed that SNHG9 was mainly located in the cytoplasm (Figure 3A).

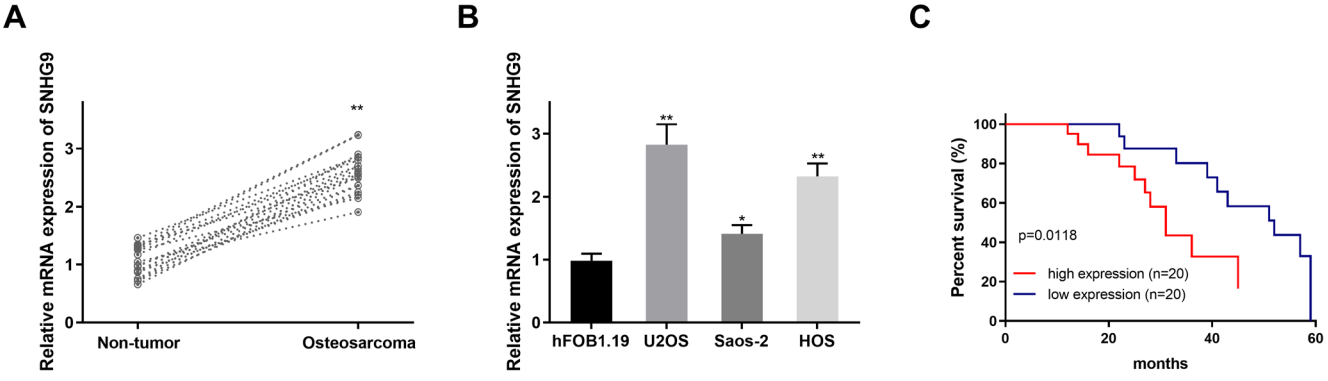

Figure 1. SNHG9 was overexpressed in OS and related to the overall survival. A) The expression of SNHG9 in OS tissues and adjacent normal tissues from the patients with OS. B) The expression of SNHG9 in human osteoblasts hFOB1.19 and OS cell lines U2OS, Saos-2, and HOS. C) The overall survival of OS patients with the expression of SNHG9. *p<0.05; **p<0.01

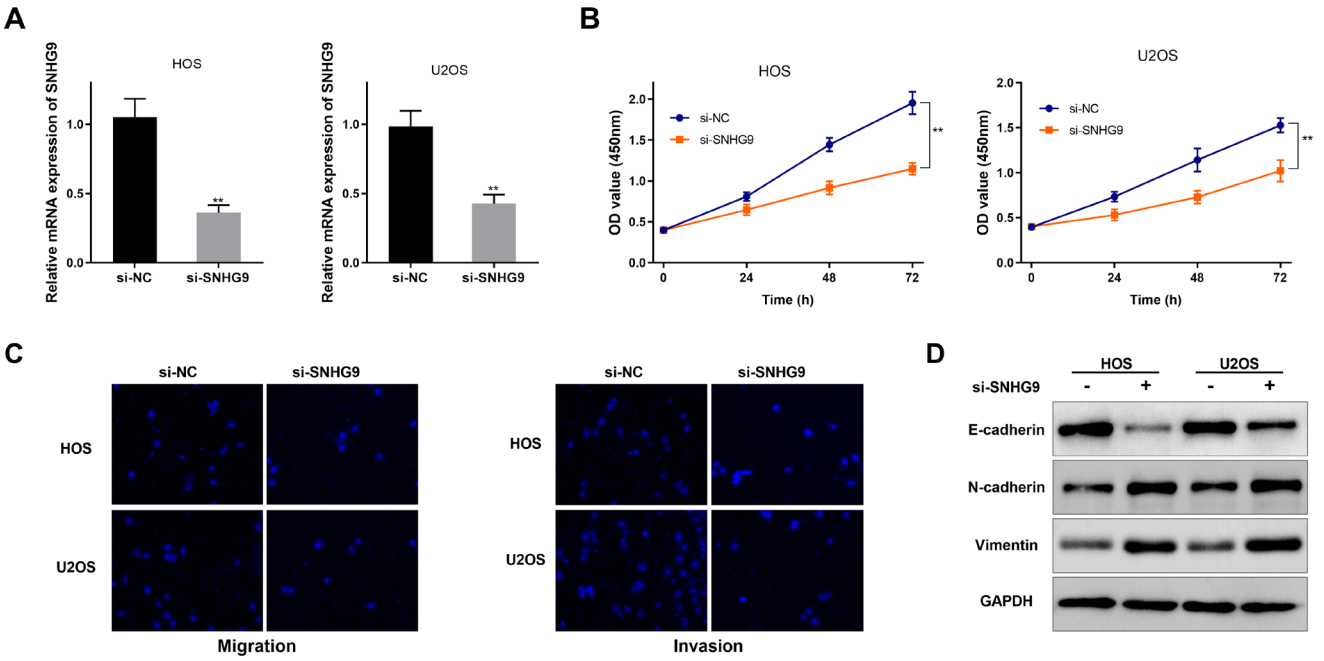

Figure 2. Knockdown of SNHG9 impairs proliferation, migration, and invasion of OS cells. A) The expression of SNHG9 in HOS and U2OS cells transfected with si-SNHG9. B) Growth curve derived from the OD values. C) Transwell assay images of migratory and invasive. D) The expression of EMT-related proteins. *p<0.05; **p<0.01

Then, qRT-PCR was performed to detect the distribution of SNHG9 in the nucleus and cytoplasm of HOS cells. SNHG9 was mainly expressed in the cytoplasm of HOS cells (Figures 3B, 3C), suggesting that SNHG9 may be involved in OS development through post-transcriptional regulation; therefore, SNHG9 is considered to be a competitive endogenous RNA in OS progression. LncRNA interacts with miRNA to perform their functions. We used starBase and mirBD to predict the candidate miRNA of SNHG9 and determined that miR-214-5p is targeted to SNHG9. In addition, SNHG9 knockdown significantly upregulated the expression of miR-214-5p in OS cells (Figure 3D). Our results showed two interactive targets between SNHG9 and miR-214-5p sequences (Figure 3E). We mutated these two target sites to obtain a dual-luciferase reporter gene vector SNHG9 MU and performed the dual-luciferase reporter assays, which showed that when miR-214-5p is overexpressed, luciferase activity was significantly inhibited in the SNHG9 WT group than the SNHG9 MU group (Figures 3F, 3G). We further analyzed the expression of miR-214-5p in OS tissues and cells, and found that the expression of miR-214-5p was significantly lower in both OS tissues and cells (HOS and U2OS) compared to the adjacent normal tissues and immortalized human osteoblasts hFOB1.19 cells, respectively (Figures 3H, 3I).

***SOX4* is the target of miR-214-5p.** Target Scan software and miRDB database were used to screen the target genes of miR-214-5p. We found that *SOX4* was the target gene of miR-214-5p. The 3'-UTR of *SOX4* contains a binding sequence complementary to miR-214-5p (Figure 4A). Next, we mutated this sequence to construct a dual-luciferase reporter gene vector SOX4 MU and performed the dual-luciferase reporter assays. When the overexpressed miR-214-5p and SOX4 WT were co-transfected into HOS and U2OS cells, the fluorescence activity was significantly reduced compared to the control group. However, there was no difference between the group with overexpressed miR-214-5p co-transformed with SOX4 MU and the control group (Figure 4B). It was found that the SOX4 expression was significantly reduced in miR-214-5p mimic cell lines (Figure 4C).

SOX4 is the transcription factor of SNHG9. Surprisingly, the miR-214-5 was extremely significantly downregulated in SOX overexpressing cell lines (Figure 5A). We found that SNHG9 expression was significantly upregulated along with the increase in *SOX4* expression (Figure 5B). According to the prediction based on the UCSC database (http://genome.ucsc.edu/) and JASPAR (http://jaspar.genereg.net/), SOX4 may be the potential transcription factor for SNHG9. As shown in Figure 5C, there are three binding sites in the promoter region of SNHG9 banded with SOX4. ChIP analysis showed that *SOX4* regulated the expression of SNHG9 by binding to the P2 region (–151 to –142) of the SNHG9 promoter (Figure 5D). The three sequences of the SNHG9 promoter (P1, P2, and P3) were connected to a fluorescent reporter gene, as well as to the SOX4 WT plasmid for co-transformation into the OS cells. The results showed that the fluorescence activity was highest for SOX4 combined with the P2 region

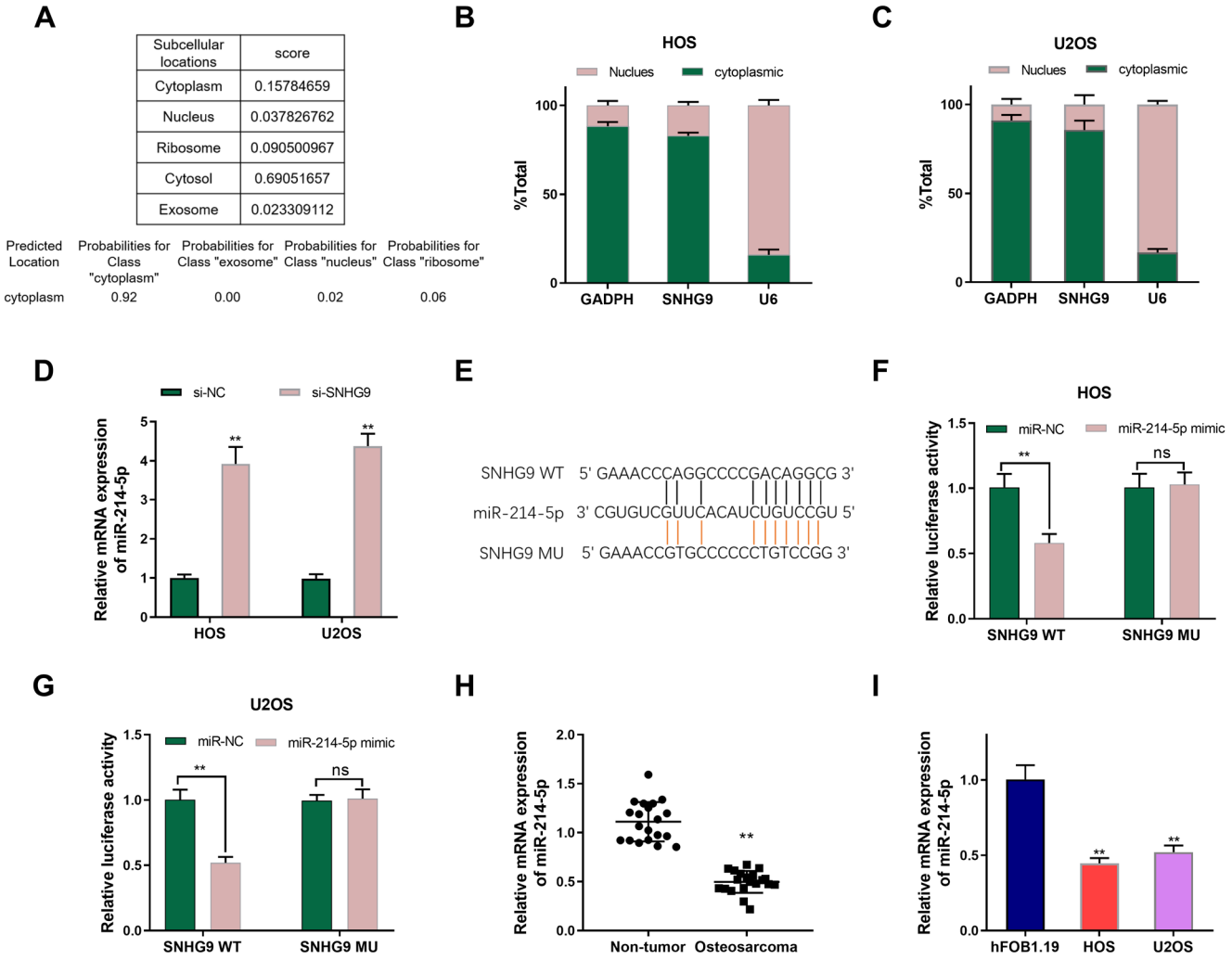

Figure 3. SNHG9 interacts with miR-214-5p as a molecular sponge. A) Prediction localization of SNHG9 online. B, C) SNHG9 expression in the nucleus and cytoplasm of OS cells. GAPDH as the cytoplasmic control and U6 as the nuclear control. D) The expression of miR-214-5p in OS cells transfected with si-SNHG9. E) The predicted binding sites between SNHG9 and miR-214-5p. F, G) The relative luciferase activities in OS cells co-transfected with luciferase reporter gene vectors containing the wild-type and mutant-type predicted target SNHG9 of miR-214-5p. H) The expression of miR-214-5p in OS tissues and adjacent normal tissues from the patients with OS. I) The expression of SNHG9 in human osteoblasts hFOB1.19 and OS cell lines HOS and U2OS. *p<0.05; **p<0.01

(Figure 5E), which verified that the P2 region of the SNHG9 promoter combines with the transcription factor SOX4.

Interaction effect on the expression of SNHG9, miR-214-5p, and SOX4. We tested the expression of SNHG9, miR-214-5p, and SOX4 in OS cells. When the expression of two of these genes changed, the expression of the third gene was altered. SOX4 expression was detected in SNHG9 or miR-214-5p overexpressed cell lines (LV-SNHG9, miR-214-5p mimic, LV-SNHG9+ miR-214-5p mimic, and control). We found that SOX4 was overexpressed in LV-SNHG9 cells and underexpressed in miR-214-5p cells, whereas the expression level in LV-SNHG9+miR-214-5p mimic cells was comparable to that in control cells (Figure 6A). The expression of miR-214-5p was analyzed in si-SNHG9, LV-SOX4, si-SNHG9+LV-SOX4, and control groups using qRT-PCR, which showed that miR-214-5p was overexpressed in the si-SNHG9 group and underexpressed in the LV-SOX4 group, whereas the expression of miR-214-5p in si-SNHG9+LV-SOX4 was comparable to that in the control group (Figure 6B). The SNHG9 expression, which was highly expressed in the LV-SOX4 cell line, was reduced in the miR-214-5p mimic cell line, whereas it was comparable to that in the control cell line (Figure 6C).

Effect of interaction of SNHG9, miR-214-5p, and SOX4 on the proliferation, migration, and invasion of OS cells. To explore the effect of the interaction of SNHG9, miR-214-5p, and SOX4 on the progression of OS cells, we evaluated the proliferation, migration, and invasion abilities of several cell

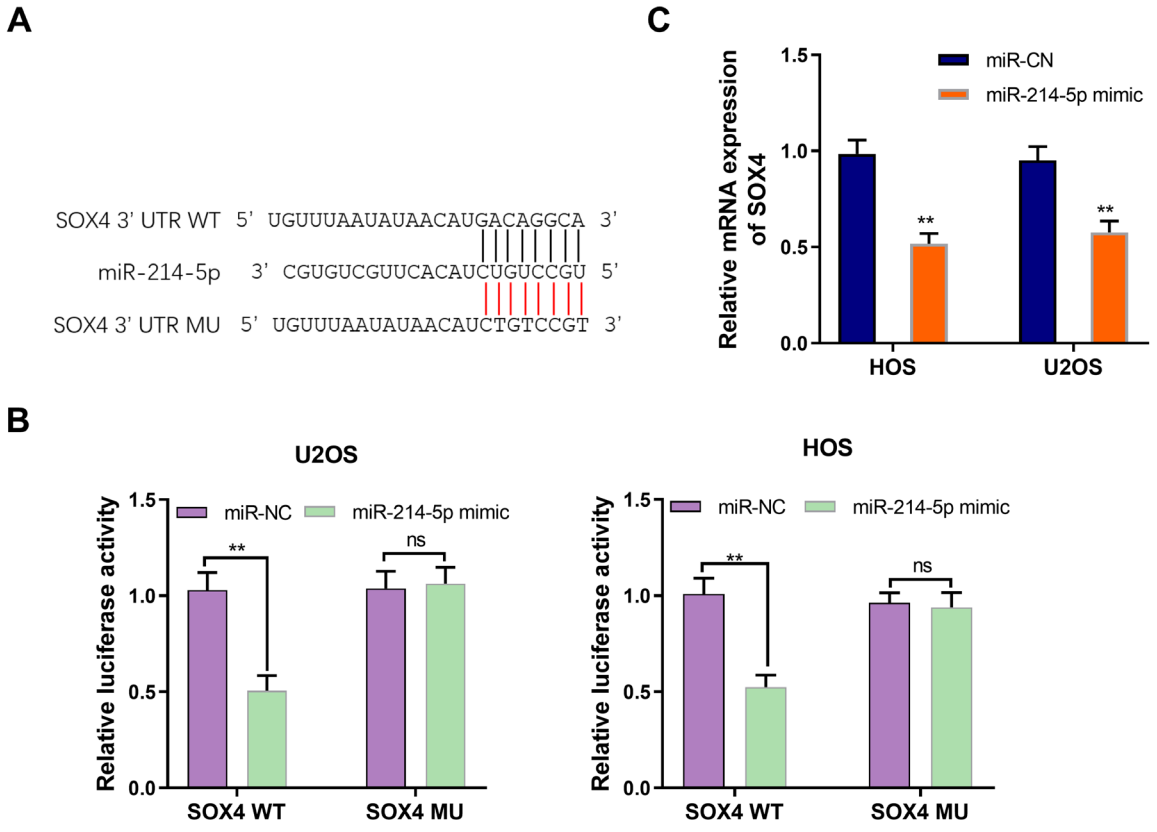

Figure 4. The gene SOX4 is a target of miR-214-5p. A) The potential target sites between 3' UTR of SOX4 and miR-214-5p. B) The relative luciferase activities in OS cells co-transfected with luciferase reporter gene vectors containing the wild-type and mutant-type predicted target SOX4 of miR-214-5p. C) The mRNA expression of SOX4 in OS cells transfected with miR-214-5p mimic. *p<0.05; **p<0.01

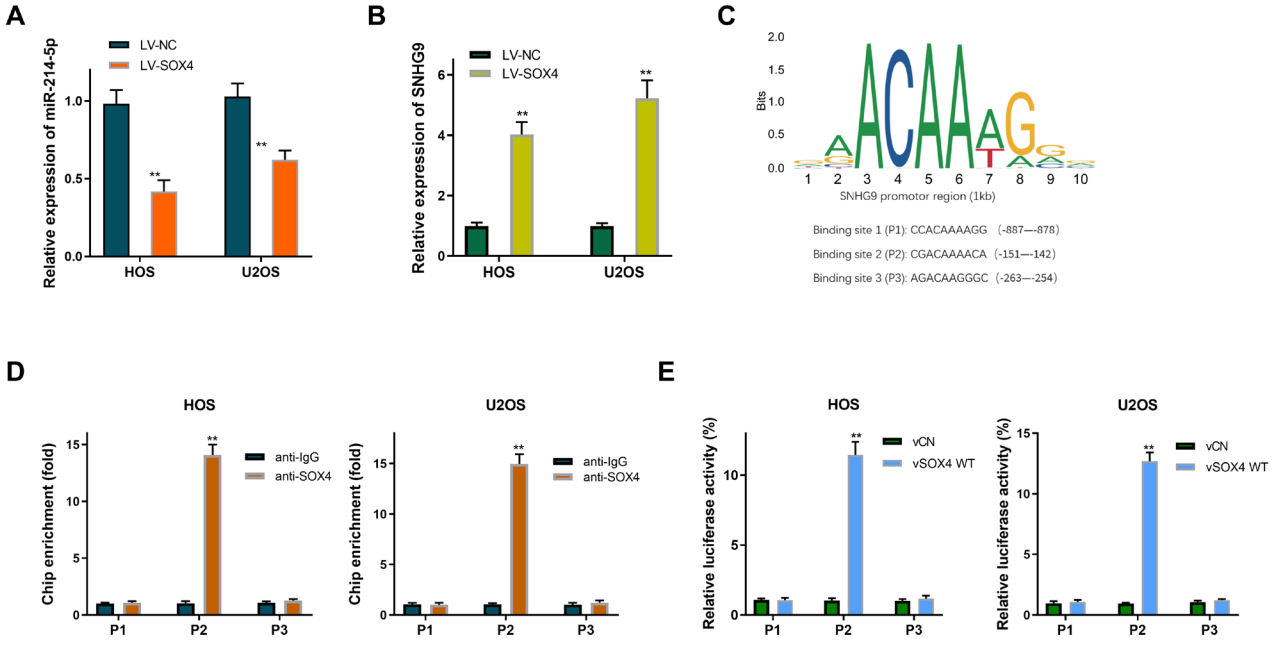

Figure 5. SOX4 is a transcription factor of SNHG9. A) The expression of miR-214-5p in LV-SOX4 and LV-NC OS cells. B) The expression of SNHG9 in LV-SOX4 and LV-NC OS cells. C) The predicted binding motif of SNHG9 promoter and the transcription factor SOX4. D) The binding sites between SOX4 and three SNHG9 promoter regions (P1, P2, or P3) were analyzed by ChIP assays with antibodies against SOX4. E) The relative luciferase activity of SOX4 binding to SNHG9 promoter regions (P1, P2, or P3) in OS cells. *p<0.05; **p<0.01

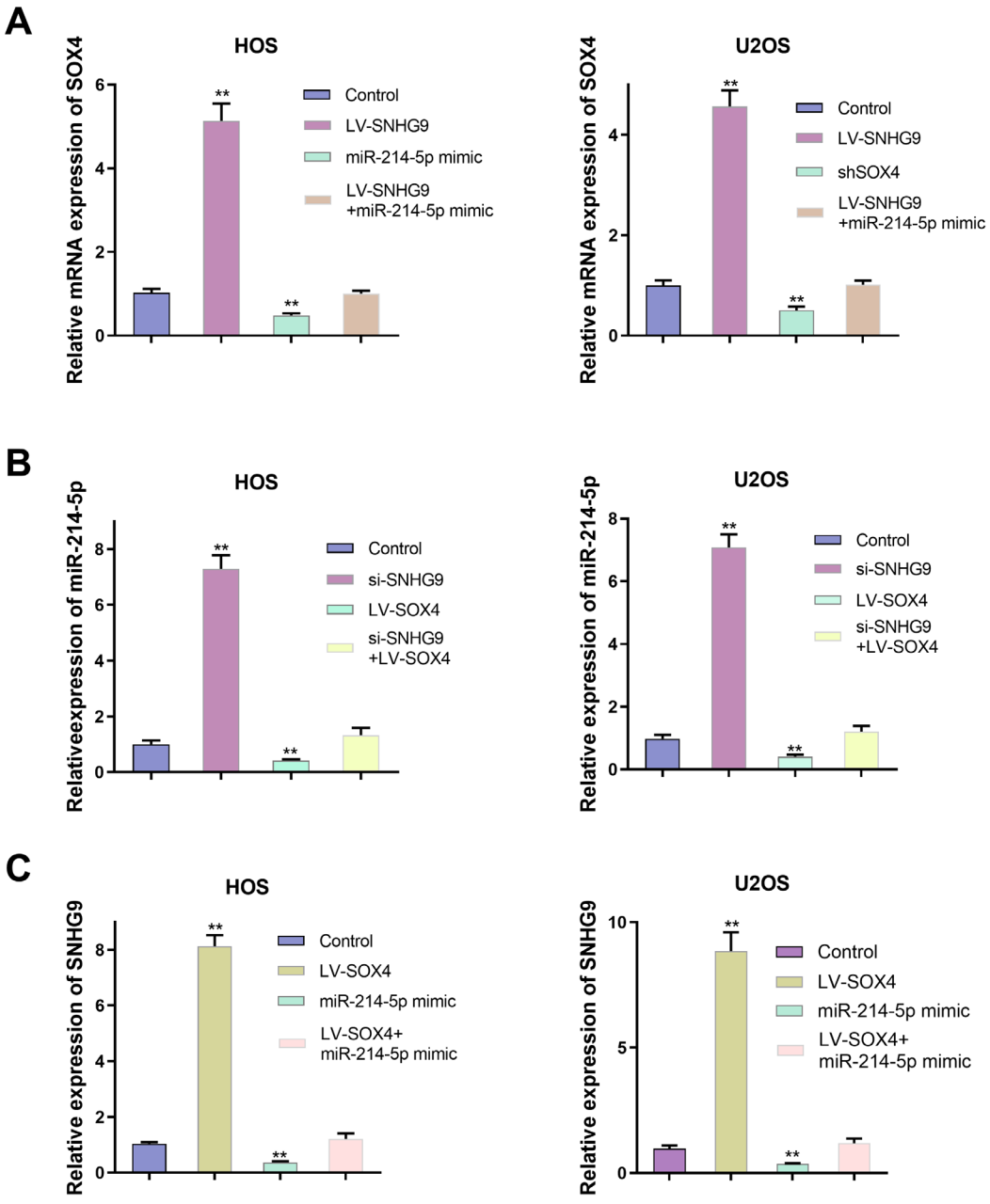

Figure 6. The interaction affects the expression of SNHG9, miR-214-5p, and SOX4. A) The expression of SOX4 in LV-SNHG9, miR-214-5p mimic, LV-SNHG9+miR-214-5p mimic, and control groups. B) The expression of miR-214-5p in si-SNHG9, LV-SOX4, LV-SOX4+si-SNHG9, and control groups. C) The expression of SNHG9 in LV-SOX4, miR-214-5p mimic, LV-SOX4+miR-214-5p mimic, and control groups. *p<0.05, **p<0.01

lines with different expression levels of the three genes. The cell lines with overexpressed SOX4 and SNHG9, especially in LV-SOX4 cells, had a high rate of cell proliferation. SNHG9 knockdown and miR-214-5p overexpression inhibited cell proliferation. The cell proliferation rate of LV-SNHG9+miR-214-5p mimic cells, LV-SOX4+miR-214-5p mimic cells, and LV-SOX4+si-SNHG9 cells recovered to that of the control cells (Figure 7A). Similar trends were observed in the invasion and migration abilities of cells: the migration and invasion abilities of cells were increased by overexpression of SOX4 and SNHG9, whereas the migration and invasion abilities were reduced by SNHG9 downregulation or miR-214-5p upregulation in OS cells. The migration and invasion abilities of LV-SNHG9+miR-214-5p mimic cells, LV-SOX4+miR-214-5p mimic cells, and LV-SOX4+si-SNHG9 cells were restored to the level of control cells (Figure 7B). The expres-

sion of EMT-related proteins in these cell lines was in line with the results of cell proliferation, migration, and invasion (Figure 7C).

Downregulated SNHG9 expression suppressed *in vivo* tumor growth. To verify the effect of SNHG9 on tumors *in vivo*, we established a mouse xenograft model using HOS cells. The tumors in mice injected with si-SNHG9 were significantly smaller than those in mice injected with si-NC cells (Figure 8A). The tumor volume and weight were lower in mice injected with si-SNHG9 OS cells than with si-NC OS cells (Figures 8B, 8C).

Discussion

Non-coding RNAs longer than 200 nucleotides are called long non-coding RNAs (lncRNAs). LncRNAs perform important roles in a variety of biological processes, such as cell signal transduction, development, tumor development, embryonic stem cell pluripotency, and stress response [21]. The lncRNA SNHG9 is related to a variety of cancers, such as ovarian cancer [19] and glioblastoma [17]. In this study, we found that SNHG9 was overexpressed in OS tissues and cells. SNHG9 performed a vital role in cell proliferation,

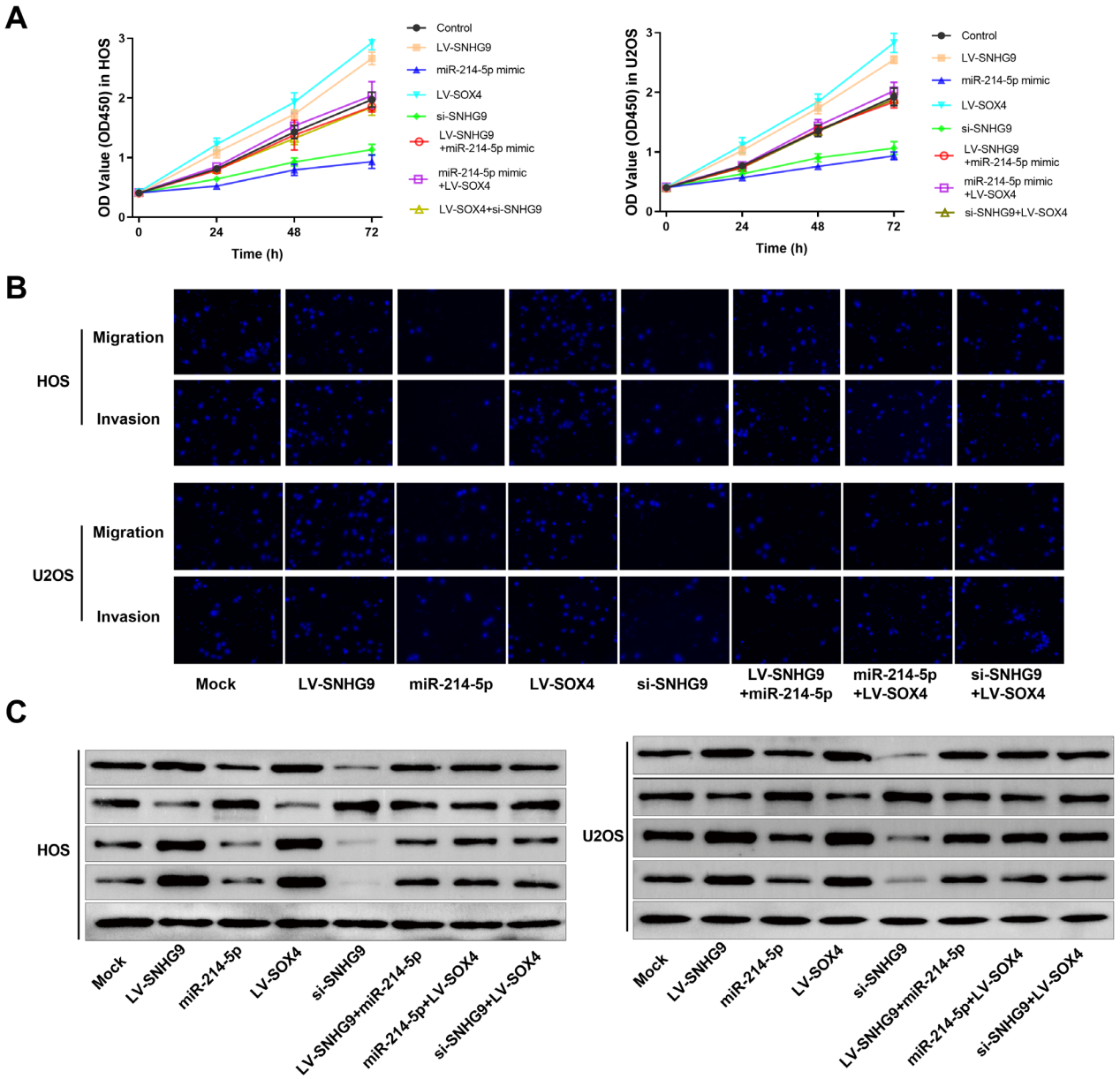

Figure 7. The interaction of SNHG9, miR-214-5p, and SOX4 regulates the proliferation, migration, and invasion of OS cells. A) Growth curve derived from the OD values. B) Transwell assay images of abilities of migratory and invasion. C) The expression of EMT-related protein.

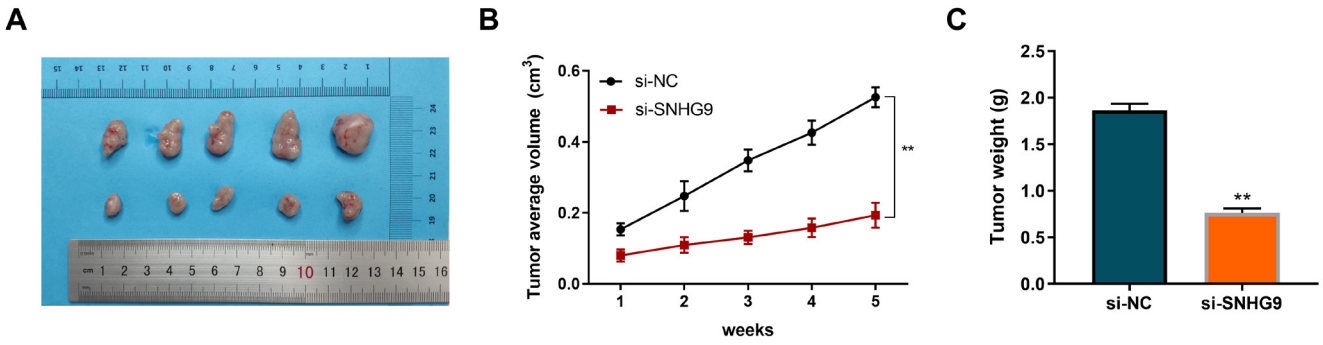

Figure 8. The knockdown of SNHG9 inhibits OS tumor growth *in vivo*. A) The representative images of tumors from mice injected with si-SNHG9 and si-NC cells. B) The volume of tumors from mice injected with si-SNHG9 and si-NC cells. C) The weight of tumors from mice injected with si-SNHG9 and si-NC cells. *p<0.05; **p<0.01

migration, and invasion *in vitro* and *in vivo*. LncRNAs are relatively abundant in the whole cell, being located in various subcellular compartments, including the nucleus, cytoplasm, and/or multiple organelles [22, 23]. LncRNAs located in the nucleus are mainly involved in chromatin modification or transcription regulation; whereas lncRNAs located in the cytoplasm are mainly related to mRNA stability and regulation of protein expression [24]. We demonstrated that SNHG9 is located in the cytoplasm of OS cells and inhibits miR-214-5p expression as a molecular sponge.

In the present study, bioinformatics analyses and dual-luciferase reporter assays showed that miR-214-5p is a direct target of SNHG9 in OS cells. Previous studies showed that miR-214 plays an important role in tumor suppression. The expression of miR-214 promotes apoptosis and reduces cell growth, proliferation, migration, and invasion in several cancer cells, such as gastric cancer [25], cervical cancer [26], and nasopharyngeal carcinoma [27]. We found that miR-214-5p had a low expression level in OS tissues and cells; in addition, OS cells with upregulated miR-214-5p expression had reduced proliferation, migration, and invasion abilities. Furthermore, the transcription factor SOX4 was verified to be the target gene of miR-214-5p and was shown to positively regulate SNHG9. SOX4 was overexpressed in OS tissues and cells and increased the proliferation, migration, and invasion abilities of cells. SOX4 is highly expressed in several malignant tumors and promotes the malignant phenotype [28]. As a transcription factor, the function of SOX4 in cancer most likely involves direct regulation of transcriptional target genes. We found that SNHG9 expression was regulated by SOX4 in OS cells.

The interaction of SNHG9, miR-214-5p, and SOX4 affected the expression levels of each other. Importantly, our results showed that miR-214-5p overexpression with upregulation of SNHG9 or SOX4 did not affect the expression of SOX4 or SNHG9, and attenuated the effects of overexpression of SNHG9 or SOX4 in OS cells. At the same time, the expression of miR-214-5p and cell abilities (proliferation, migration, and invasion) did not change in the OS cells transfected with si-SNHG9 and LV-SOX4 vectors compared to the control cells. Hence, we suggest that the SNHG9/miR-214-5p/SOX4 feedback loop contributes to the malignant phenotype of OS.

In conclusion, SNHG9 was overexpressed in OS cells and tissues, and the expression of SNHG9 was positively correlated with carcinogenesis and tumor growth in OS. We found that the SNHG9/miR-214-5p/SOX4 feedback loop plays an important role in OS progression, and might be used as a new potential therapeutic target for the treatment of OS.

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