

# Long non-coding RNA HOTAIR promotes the progression of synovial sarcoma through microRNA-126/stromal cell-derived factor-1 regulation

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**Abstract.** The long non-coding RNA (lncRNA) HOTAIR is an oncogene, that has been reported to be aberrantly expressed in multiple types of malignant tumor tissues. However, its expression and association with synovial sarcoma (SS) remains unclear. The present study aimed to elucidate the expression level of HOTAIR in SS tissues and also identify its role. Reverse transcription-quantitative PCR was used to detect the expression level of HOTAIR and microRNA (miR)-126 in 54 tissue samples from patients with SS, in 10 tissue samples from synovium tissues of normal patients, and in SW982 cells. The protein expression level was measured using western blot analysis and cellular immunofluorescence. Cellular proliferation, invasion and migration were assessed using MTT, Transwell and wound healing assays, respectively. HOTAIR was expressed at high levels in SS tissues. In contrast, miR-126 was expressed at low levels in SS tissues, and was negatively correlated with HOTAIR expression. HOTAIR knockdown in SW982 cells inhibited cellular proliferation *in vitro*, but also significantly increased the ratio of cells in the G<sub>1</sub>/G<sub>0</sub> phase of the cell cycle, and decreased the ratio of cells in the G<sub>2</sub>/S phase. In addition, HOTAIR knockdown inhibited the invasion and migration of the SW982 cells, as observed in the Transwell and wound healing assays. Furthermore, HOTAIR knockdown increased miR-126 expression level and decreased the expression level of stromal cell-derived factor-1 (SDF-1) at the protein level. On the other hand, while miR-126-mimic decreased the protein expression level of SDF-1, miR-126-inhibitor increased its expression level in SW982 cells. Notably, HOTAIR knockdown or SDF-1 knockout significantly decreased the protein expression levels of CDK1, CDK2, cyclin D1, MMP-9,

vimentin and N-cadherin, and significantly increased the protein expression levels of p21, p53 and E-cadherin in SW982 cells. HOTAIR was highly expressed in SS tissues, wherein it could promote the proliferation, invasion and migration of SS cells by increasing the expression of SDF-1 via miR-126 inhibition.

## Introduction

Synovial sarcoma (SS) is a malignant mesenchymal tumor that is characterized by partial epithelial differentiation, and it constitutes 8-10% of soft tissue sarcoma (1,2). Despite its low incidence, SS is not easy to diagnose, progresses rapidly, and has a low 5-year survival rate that ranges between 21-40% (1,2). While sensitive to a variety of chemotherapeutic drugs, distant metastasis is another malignant manifestation of SS. Patients with SS often exhibit distant metastasis at early stages, and the majority of metastatic sites are concentrated in the lymph nodes, lungs and the liver (3,4). In fact, the main cause of death in patients with SS is tumor recurrence and metastasis, wherein ~40% of patients with SS develop lung, liver and regional lymph node metastasis within 2 years (5,6). Therefore, elucidating the mechanisms underlying SS recurrence and metastasis would greatly improve the survival rate of patients.

Long non-coding RNAs (lncRNAs) are a type of RNA, that are >200 bp in length, which generally have no open reading frame and do not encode a protein. In addition, they are usually transcribed by RNA polymerase II and are composed of multiple spliced exons (7,8). Recently, various lncRNAs have been associated with the development of different types of cancer (7,9), and multiple studies have shown that lncRNA HOTAIR can promote the progression of neuroglioma (10,11). In this regard, the lncRNA HOTAIR has been shown to act as an oncogene, which is aberrantly expressed in multiple malignant tumor tissues, including colorectal cancer (12), pancreatic cancer (13), hepatocellular carcinoma (14), rhinitis (15) and cervical cancer (16). In addition, HOTAIR has been demonstrated to promote cancer metastasis (17). It has been shown that HOTAIR can regulate the biological characteristics of gastric cancer cells and glioma cells by binding to and inhibiting microRNA (miR)-126 expression (11,18). On the

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other hand, stromal cell-derived factor-1 (SDF-1), a target of miR-126 (19), has been shown to be highly expressed in SS tissues and to be associated with poor prognosis in patients with SS (20).

However, the expression of HOTAIR in SS tissues and its association with miR-126 remains unclear. In this regard, the hypothesis used in the present study was that the association between HOTAIR expression and the prognosis of patients with SS is associated with the regulation of SDF-1 via miR-126 inhibition.

## Materials and methods

**Synovial sarcoma tissues and cell lines.** As a prospective study, a total of 54 synovial sarcoma tissues from the limbs were collected from patients at the Fourth Hospital of Hebei Medical University (Shijiazhuang, China) between January 2011 and December 2017. The inclusion criteria were as follows: i) Synovial sarcoma identified by pathological diagnosis; ii) complete clinical and histological features, including sex, age, tumor size, histological grade, distant metastasis and the 8th Edition of American Joint Committee on Cancer (AJCC) (21) staging; iii) no radiotherapy, chemotherapy, immunotherapy or molecular targeting treatment prior to surgery; iv) regular follow-up or review following surgery and a clear cause or time of death; v) signed informed consent. The exclusion criteria were: i) Combined with other malignant tumors; ii) postoperative death due to non-tumor related causes; iii) combined with other severe diseases, such as chronic infection, organ disorders and cardiovascular and cerebrovascular diseases; iv) poor physical fitness, unable to tolerate surgery or other related examinations, poor postoperative mental state, and the prognosis affected by the basic conditions; v) postoperative follow-up is lost or the cause of death is unclear. In addition, 10 normal synovium tissues from the limbs were collected at the same hospital, over the same time period, from patients who underwent limb amputation with no history of joint disease. The tissues were snap-frozen in liquid nitrogen until required. Informed consent was obtained from all patients participating in the study or from their family members. The study was approved and monitored by the Ethics Committee of The Fourth Hospital of Hebei Medical University.

Wild-type (WT) SW982 (HTB-93; American Type Culture Collection) cell lines were cultured at 37°C and 5% CO<sub>2</sub> in DMEM (Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Thermo Fisher Scientific, Inc.). SDF-1 knockout (KO) SW982 cell lines were obtained from Synbio Technologies LLC., and were cultured under the same conditions as the WT-SW982. WT cells were used as the untreated control group.

**Cell proliferation assay.** A MTT Cell Proliferation and Cytotoxicity Assay kit (Beyotime Institute of Biotechnology) was used to assess cell proliferation according to the manufacturer's instructions. A total of 2x10<sup>3</sup> SW982 cells/well were initially seeded in a 96-well plate for the MTT assay. Following 24-h culture, the medium was removed, the cells were washed twice with PBS, and MTT reagent was added to incubate for ≥2 h at 37°C. The MTT solvent included in the assay kit was used to dissolve the purple formazan, and the absorbance was measured at 570 nm.

**Cell transfection.** Small interfering (si)-negative control (NC), si-HOTAIR-1, si-HOTAIR-2, miR-126-NC, miR-126-mimic and miR-126-inhibitor were designed and synthesized by Shenggong Bioengineering Co., Ltd. For siRNA or miRNA mimic/inhibitor transfection, 50 nmol/l siRNA, miRNA mimic or inhibitor was transfected into 2.5x10<sup>6</sup> cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 6 h at 37°C according to the manufacturer's protocol. At 48 h post-transfection, the follow-up experiments were conducted. The sequences were as follows: si-NC: 5'-GAA ACAUUAUGAAAAUCAAUU-3'; si-HOTAIR-1: 5'-AGA CUAAGACGGAUAAACGCGU-3'; si-HOTAIR-2: 5'-ACA AUAUCUACUUUGGAUCAC-3'; mimic-NC: 5'-AUCUCA ACUUCGCGGCA-3'; miR-126-mimic: 5'-UCGUACCGU GAGUAAU-3'; inhibitor-NC: 5'-AUUGCUGGCUAUAA CG-3'; miR-126-inhibitor: 5'-AGCAUGGCACUCAUUA-3'.

**Dual-luciferase reporter assay.** First, WT-SDF-1 and mutant (MUT)-SDF-1 were integrated into pmirGLO plasmids (Promega Corporation) and co-transfected into cells (1x10<sup>6</sup> cells/well) with miR-126-NC, miR-126-mimic and miR-126-inhibitor (50 nmol/l) using Lipofectamine® 2000 for 6 h at 37°C. At 48 h post-transfection, the Dual-Lucy Assay kit (Beijing Solarbio Science & Technology Co., Ltd.) was used to detect the activity of luciferase according to the manufacturer's instructions. Briefly, cells were collected at 48 h post-transfection and lysed for 5 min on ice before centrifugation (12,000 x g for 1 min at room temperature) to collect the cell supernatant. Subsequently, 5X firefly or *Renilla* luciferase reaction solution were added to the cell lysate, and the enzyme activity was detected using a multifunctional microplate reader (Thermo Fisher Scientific, Inc.), and the enzyme activity was standardized to that of *Renilla* luciferase.

**Reverse transcription-quantitative PCR (RT-qPCR).** The mRNA expression levels of HOTAIR and miR-126 were detected using the RT-qPCR Fluorescence quantitative-polymerase chain reaction kit (Hangzhou Bori Technology Co., Ltd.), as previously described (22). Total RNA was extracted using RNAiso Plus (Takara Bio, Inc.) and reverse transcribed into cDNA using the PrimeScript RT reagent kit with gDNA Eraser (Takara Bio, Inc.) at 37°C for 15 min and 85°C for 5 sec. U6 was used as a reference gene for miRNA (miR-126 and miR-429) measurements, whereas GAPDH was used as a reference gene for lncRNA HOTAIR. The following PCR primers were used: HOTAIR forward, 5'-GGCAAATGT CAGAGGGTT-3' and reverse, 5'-GTGTAACAGGCAGGT GGA-3'; GAPDH forward, 5'-GGCTGTATTCCCCTCCAT CG-3' and reverse, 5'-CCAGTTGGTAACAATGCCATGT-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3'; and reverse, 5'-AACGCTTCACGAATTTGCGT-3'; and miR-126 forward, 5'-ACACTCCAGCTGGGTCTGACCGTG-3' and reverse, 5'-TGGTGTCTGTTGAGTTCG-3'. The 2<sup>-ΔΔCq</sup> method was used to calculate quantitative gene expression (23).

**Western blot analysis.** Protein expression in SW982 cells was measured using western blot analysis as previously described (22), and GAPDH was used as the a loading control. Proteins were separated using 10% SDS-PAGE at 90 V for 1.5 h, and transferred on to PVDF membranes

(EMD Millipore) at 300 mA for 1 h. The membranes were then blocked in 5% skimmed milk at room temperature for 1 h, then incubated with the primary antibody for 2 h at room temperature, followed by the secondary antibody for 1 h at room temperature. The antibodies used were as follows: Anti-CDK1 [EPR165] (1:1,000; cat. no. ab133327; Abcam), anti-Cdk2 [E304] (1:1,000; cat. no. ab32147; Abcam), anti-cyclin D1 (1:2,000; cat. no. ab226977, Abcam), anti-p21 [HUGO291] (1:1,500; cat. no. ab107099; Abcam), anti-p53 (DO-1) (1:500; cat. no. sc-126; Santa Cruz Biotechnology, Inc.), anti-MMP9 (1:1,000; cat. no. ab38898; Abcam), anti-vimentin [VI-10] (1:2,000; cat. no. ab20346; Abcam), anti-E-cadherin [4A2] (1:500; cat. no. ab231303; Abcam), anti-N-cadherin [EPR1791-4] (1:1,000; cat. no. ab76011; Abcam), SDF1/CXCL12 (D32F9) Rabbit mAb (1:1,500; cat. no. 3350; Cell Signaling Technology, Inc.), HRP Conjugate Goat Anti-Rabbit IgG (H+L) (1:3,000; cat. no. HS101-01) and HRP Conjugate Goat Anti-Mouse IgG (H+L) (1:3,000; cat. no. HS201-01) (both TransGen Biotech Co., Ltd.). The proteins were visualized with ECL solution (Beijing Xingjike Biotechnologies Co., Ltd), followed by densitometry analysis using ImageJ v1.8.0 (National Institutes of Health). GAPDH was loaded as control.

**Cell invasion assay.** A 24 mm Transwell® with 3.0  $\mu$ m Pore Polycarbonate Membrane Insert (Corning Inc.) was used to assess the invasive capacity of SW982 cells, as previously described (24). In brief, 25  $\mu$ l Matrigel (diluted with 3X serum-free medium; Sigma-Aldrich; Merck KGaA) was added to the upper chamber of the Transwell plate (Corning, Inc.) for 30 min at 37°C. A total of  $0.5 \times 10^6$  cells/ml were added into the Transwell upper chamber in serum-free DMEM, and media containing 20% FBS (Gibco; Thermo Fisher Scientific, Inc.) was added into the lower chamber. The plate was incubated at 37°C for 24 h. Subsequently, the Transwell insert was removed and washed twice with PBS, and 100% methanol was used for fixation for 30 min at room temperature, followed by drying. The membrane was stained with crystal violet for 20 min at room temperature, and the relative migration was determined by measuring the absorbance at 595 nm.

**Wound healing assay.** A total of  $5 \times 10^5$  cells/well were seeded with 2 ml medium in a 6-well plate. A scratch perpendicular to the back of the horizontal line was created using a vertically positioned (not tilted) pipette tip against a ruler, and images were captured to determine the distance the wound at the start of the assay using a light microscope (Olympus Corporation) (x40 magnification). Serum-free DMEM medium was added after washing three times with PBS to remove the scratched cells. Images were captured after incubation for 24 h at 37°C in a humidified incubator with 5% CO<sub>2</sub>. ImageJ v1.8.0 was used for quantification.

**Cell cycle and apoptosis assay.** The cell cycle phases were detected using the Cell Cycle and Apoptosis Analysis kit (cat. no. 40301ES50; Shanghai Yeasen Biotechnology Co., Ltd.) using the MACSQuant® Analyzer 10 Flow cytometer (Miltenyi Biotec GmbH), according to the manufacturer's instructions. Data were analyzed by FlowJo X 10.0.7 software (Beckman Coulter, Inc.).

**Immunofluorescence assay.** A total of  $3 \times 10^4$  cells/well were seeded onto a Lab-Tek Chambered cover glass (cat. no. LOT1228622; Thermo Fisher Scientific, Inc.). After incubation for 24 h, the medium was removed, and the cells were washed twice with PBS. The cells were incubated with 0.3% Triton X-100 in PBS-Tween-20 (PBS-T) for 30 min at room temperature, washed twice with PBS-T and incubated with the SDF-1 antibody (1:50; cat. no. ab155090; Abcam) for 30 min at room temperature. After washing three times with PBS-T, the cells were incubated with the Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) secondary antibody (1:500; cat. no. ab150077; Abcam) for 1 h at room temperature. The nuclei were finally stained with 5  $\mu$ g/ml DAPI for 5 min at room temperature. A Leica TCS SP5 microscope with the LAS AF Lite v4.0 image browser software (DMI3000; Leica Microsystems GmbH) was used for fluorescence detection and analysis.

**Statistical analysis.** The data was analyzed using SPSS v20.0 software (IBM, Corp.). Pairwise comparisons between groups were performed using either the unpaired Student's t-test, the  $\chi^2$  test or Fisher's exact test, while multi-group statistical analysis was performed using one-way ANOVA followed by the Tukey's post hoc test. Pearson's coefficient was used for correlation analysis.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**HOTAIR is highly expressed in SS tissues.** As shown in Fig. 1A, HOTAIR was highly expressed in the 54 SS tissues compared with that in the 10 normal synovium tissues. By contrast, miR-126 was expressed at low levels in the SS tissues (Fig. 1B) compared with that in the normal synovium tissues, and was negatively correlated with HOTAIR expression level in the SS tissues (Fig. 1C). In addition, patients with SS were divided into groups based on the median HOTAIR or miR-126 expression. Analysis using either  $\chi^2$  or Fisher's exact tests found that both HOTAIR and miR-126 expression levels were significantly associated with histological grade, distant metastasis and AJCC staging in the patients with SS (Table I).

**HOTAIR knockdown inhibits cellular proliferation and blocks the cells in the G<sub>1</sub> phase.** A MTT assay was used to measure the proliferation of the SW982 cells *in vitro* following knockdown of HOTAIR, using si-HOTAIR-1 and si-HOTAIR-2 (Fig. 2A). The results showed that HOTAIR knockdown significantly reduced the cell proliferation of the SW982 cells (Fig. 2B). In addition, HOTAIR knockdown significantly increased the ratio of the SW982 cells in the G<sub>1</sub>/G<sub>0</sub> phase of the cell cycle (Fig. 2C). In addition, HOTAIR knockdown decreased the protein expression level of CDK1, CDK2 and cyclin D1, while it increased the expression level of p21 and p53 (Fig. 2D).

**HOTAIR knockdown inhibits invasion and migration of the SS cells.** The Transwell and wound healing assays were used to evaluate the invasive and migration abilities of the SS cells following knockdown of HOTAIR, respectively. As shown in Fig. 3A and B, the number of invasive SS cells was significantly reduced after HOTAIR knockdown. In addition,

Table 1. Clinicopathological variables and HOTAIR/miR-126 expression level.

Variable	Number	HOTAIR			miR-126		
		Low, n	High, n	P-value	Low, n	High, n	P-value
Sex				0.846			0.409
Female	23	12	11		13	10	
Male	31	17	14		14	17	
Age, years				0.816			0.413
$\geq 30$	25	13	12		14	11	
$< 30$	29	16	13		13	16	
Tumor size, cm				0.918			0.580
$< 5$	32	17	15		15	17	
$\geq 5$	22	12	10		12	10	
Histological grade				0.033			0.021
I	12	3	9		10	2	
II	20	8	12		9	11	
III	22	15	7		8	14	
Distant metastasis				0.001			0.001
No	34	24	10		11	23	
Yes	20	5	15		16	4	
AJCC staging				0.015			0.003
I/II	25	9	16		7	18	
III/IV	29	20	9		20	9	

miR, microRNA; AJCC, American Joint Committee on Cancer (8th edition).

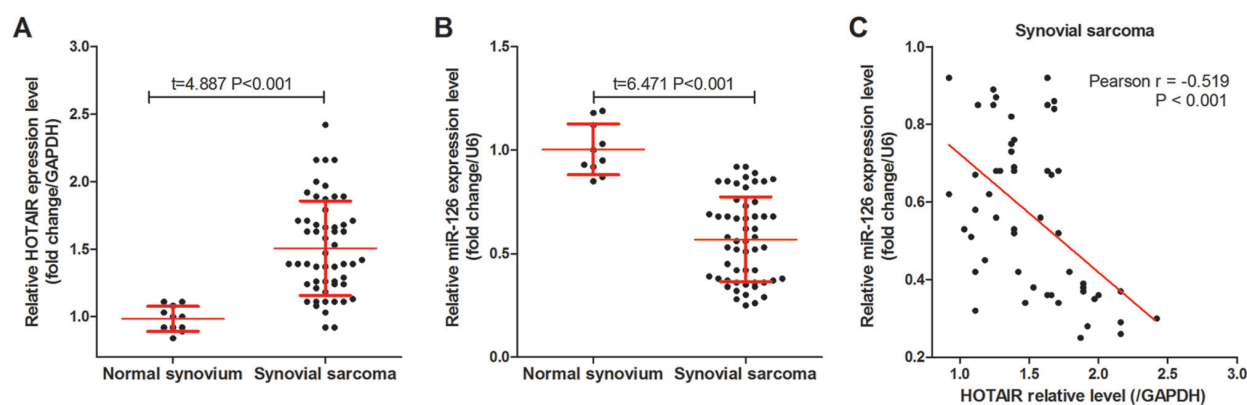


Figure 1. HOTAIR and miR-126 expression level in SS and normal synovium tissues. Reverse transcription-quantitative PCR was used to measure (A) HOTAIR and (B) miR-126 expression level in SS (n=54) and normal synovium tissue (n=10). (C) Correlation between HOTAIR and miR-126 expression level in SS tissues. SS, synovial sarcoma; miR, microRNA.

the migration distance by the SS cells was also significantly reduced. Similarly, the expression level of the proteins involved in cellular invasion and migration were measured. While HOTAIR knockdown significantly decreased the protein expression level of MMP-9, vimentin and N-cadherin, it increased the expression level of E-cadherin in the SW982 cells compared with that in the control cells (Fig. 3C).

*HOTAIR promotes SDF-1 expression level by inhibiting miR-126.* HOTAIR knockdown significantly increased miR-126 expression

level in the SW982 cells (Fig. 4A). Notably, sequence analysis showed that HOTAIR and SDF-1 contained complementary sequences to miR-126 (Fig. 4B). In accordance with the sequence analysis, a luciferase gene reporter assay indicated that the miR-126-mimics significantly reduced, and the miR-126-inhibitor significantly increased the luciferase activity in SW982 cells transfected with WT-SDF-1, whereas the miR-126-mimics and miR-126-inhibitor did not affect the luciferase activity in SW982 cells transfected with MUT-SDF-1 (Fig. 4C and D). Both HOTAIR knockdown and miR-126-mimics significantly



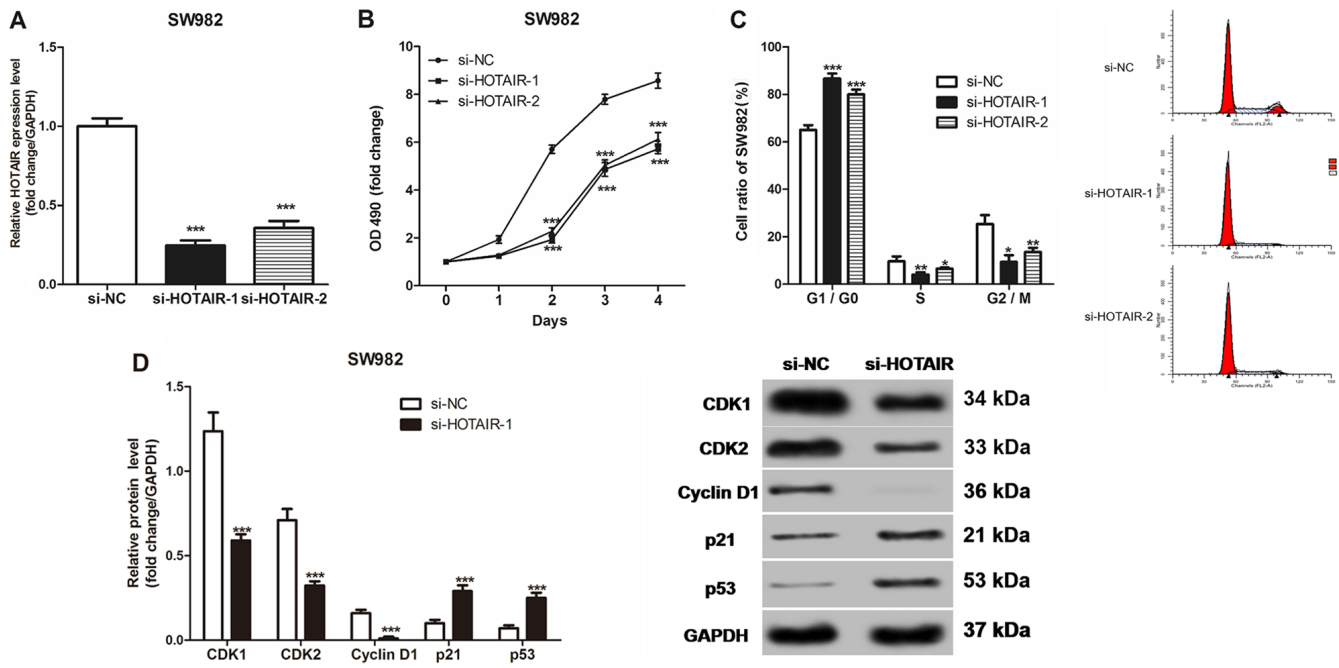


Figure 2. Effects of HOTAIR expression on the proliferation, cell cycle and cell cycle-related protein expression of the SS cells *in vitro*. (A) HOTAIR expression level in the SW982 cells transfected with si-NC or 2 siRNAs. (B) The proliferation of the SW982 cells was measured using MTT assays. (C) The cell cycle of SW982 was detected using flow cytometry. (D) Western blot analysis was used to measure CDK1, CDK2, cyclin D1, p21, and p53 protein expression levels in the SW982 cells. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 vs. si-NC. si, short inhibiting; NC, negative control; OD, optical density.

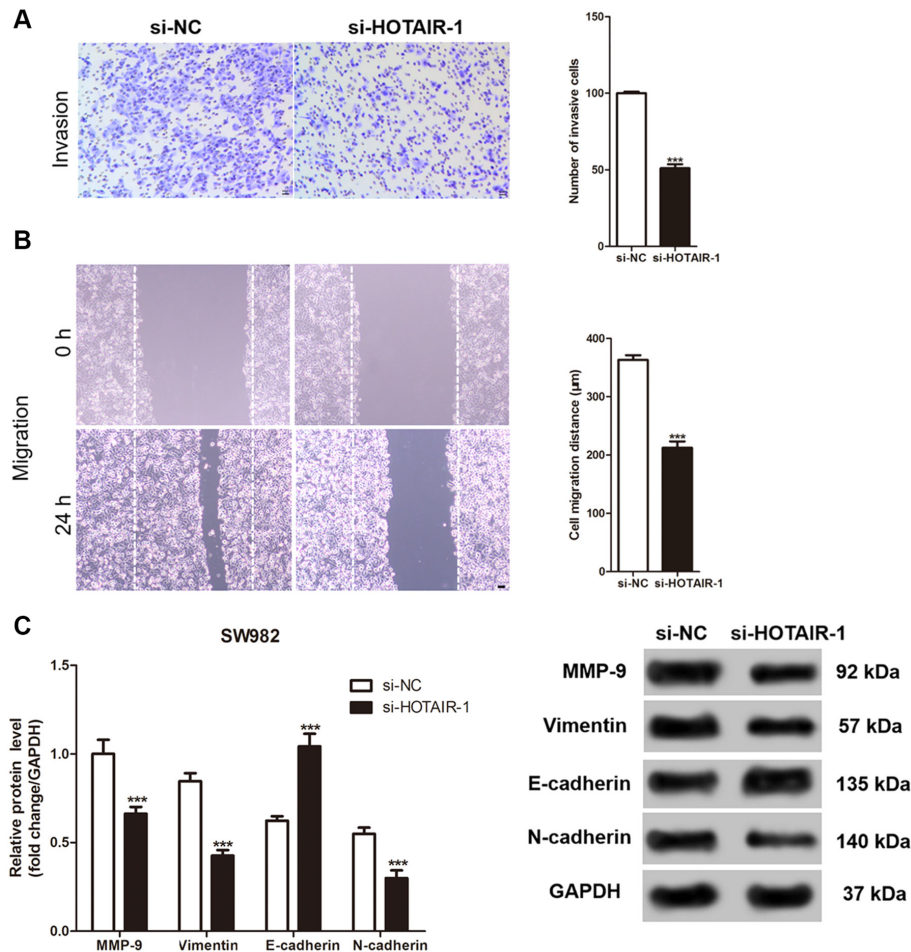


Figure 3. Effect of HOTAIR expression on migration and invasion of the SS cells *in vitro*. (A) Matrigel and (B) cell-scratch assays were used to evaluate the invasion and migration of the SW982 cells, respectively. (C) MMP-9, vimentin, E-cadherin and N-cadherin protein expression level in the SW982 cells transfected with si-NC and si-HOTAIR-1. Scale bar, 50  $\mu$ m; \*\*\*P<0.001 vs. si-NC. si, short inhibiting; NC, negative control.

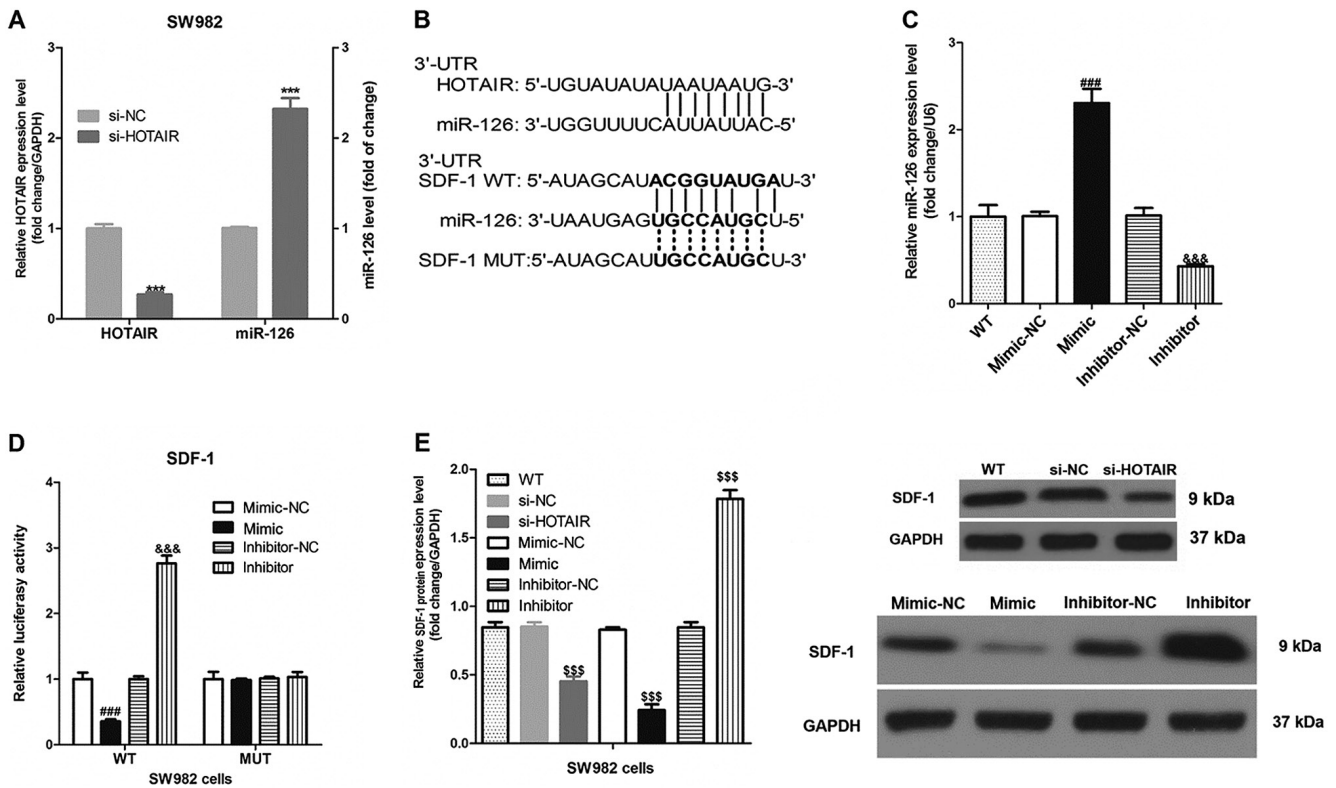


Figure 4. HOTAIR regulates SDF-1 expression level via miR-126 in the SS cells *in vitro*. (A) Effect of HOTAIR expression level on miR-126 expression level in the SW982 cells *in vitro*. \*\*\* $P < 0.001$  vs. si-NC group. (B) The complementary sequences in HOTAIR and miR-126 with SDF-1 were identified, then a WT-SDF-1 3'-UTR or MUT-SDF-1 3'-UTR luciferase reporter vector with the miR-126 binding sites was constructed. (C) miR-126 expression level in the SW982 cells. \*\*\* $P < 0.001$  vs. mimic-NC; &&& $P < 0.001$  vs. inhibitor-NC. (D) Luciferase activity was detected in the SW982 cells with transfected with miR-126-NC, -mimics and -inhibitor. \*\*\* $P < 0.001$  vs. mimic-NC (WT); &&& $P < 0.001$  vs. inhibitor-NC (WT). (E) SDF-1 protein expression level in the SW982 cells with different treatment. \$\$\$ $P < 0.001$  vs. inhibitor-NC group. WT, wild-type; MUT, mutant; miR, microRNA; UTR, untranslated region; NC, negative control.

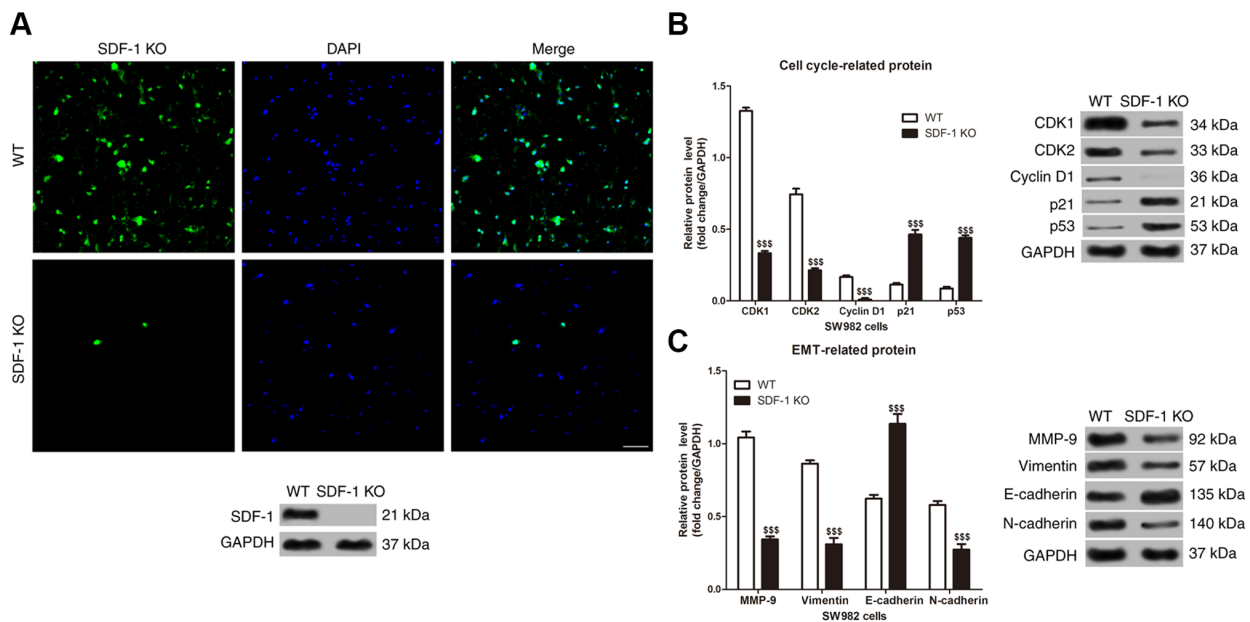


Figure 5. Effects of SDF-1 knockout on cell cycle- and EMT-related protein expression level in synovial sarcoma cells. (A) Measurement of SDF-1 protein expression level using cellular immunofluorescence and western blot in SW982 cells. Scale bar, 100  $\mu$ m. Western blot analysis was used to detect the expression level of (B) cell cycle- and (C) EMT-related proteins in the SW982 cells. \$\$\$ $P < 0.001$ . KO, knockout; WT, wild-type; EMT, epithelial-mesenchymal transition.

decreased SDF-1 protein expression level, whereas treatment with miR-126 inhibitor significantly increased the protein expression level in the SW982 cells (Fig. 4E). Furthermore,

immunofluorescence and western blot analysis showed that the expression of SDF-1 protein in SDF-1 KO SW982 cells was significantly lower than that of wild-type SW982 cells (Fig. 5A).

In addition, SDF-1 knockdown suppressed the protein expression level of CDK1, CDK2, cyclin D1, MMP-9, vimentin and N-cadherin, while it induced the expression level of p21, p53 and E-cadherin in the SW982 cells (Fig. 5B and C).

## Discussion

It has been shown in our previous study that SDF-1 was highly expressed in SS tissues, which was also associated with poor prognosis in patients with SS (20). Notably, SDF-1 was a target gene of miR-126. Indeed, van Solingen *et al* (25) showed that miR-126 increased the number of endothelial cells by inhibiting SDF-1 expression in the endothelium. Similarly, Zhang *et al* (19) demonstrated that miR-126 could inhibit the recruitment of mesenchymal stem cells and inflammatory monocytes into the tumor tissues by inhibiting SDF-1 expression level, thereby inhibiting the metastasis of breast cancer cells. Notably, HOTAIR was shown to promote the metastasis of cancer cells by inhibiting miR-126 expression level in different malignant tumor cells, such as breast, gastric and liver cancer cells (17). In the present study, it was shown that HOTAIR mRNA was highly expressed and was negatively correlated with miR-126 expression level in SS tissues, but was also significantly associated with histological grade, distant metastasis and AJCC staging in patients with SS. These results suggested that HOTAIR was associated with the development of SS.

The knockdown of HOTAIR in the SS cells inhibited proliferation, and the invasion and migration abilities of the SS cells *in vitro*, which is consistent with previous studies reporting HOTAIR as an oncogene in various malignancies. For example, Gupta *et al* (17) showed that HOTAIR could promote cancer metastasis through epigenetic reprogramming of the cellular chromatin. As a non-coding RNA, HOTAIR may only act indirectly to regulate cellular functions through miRNA and mRNA regulation. In this regard, the results from the present study showed that HOTAIR knockdown could increase miR-126 expression level, which is consistent with previous studies reporting miR-126 to be a target of HOTAIR (11,18).

miRNAs are non-coding, single-stranded RNAs, that are 20-25 nucleotides in length, which can regulate cellular proliferation, differentiation, apoptosis, metabolism, invasion and migration through the post-transcriptional regulation of target genes (26,27). Multiple miRNAs have been recognized as biomarkers for the diagnosis of multiple diseases and have been demonstrated to serve important roles in the development of cancer, acute myocardial infarction and rheumatoid arthritis (28). Previous studies have shown (29,30) that the development of breast cancer was associated with the abnormal expression of miR-1271 and miR-192-5p. miR-126, a tumor suppressor, was shown to be abnormally expressed in numerous tumors, where it regulated multiple biological processes in tumors, such as proliferation, apoptosis, invasion, and migration (31). Feng *et al* (32) demonstrated that miR-126 could inhibit the proliferation, invasion and migration of gastric cancer cells by inducing cell-cycle arrest at the G<sub>1</sub>/G<sub>0</sub> phase. Hamada *et al* (33), on the other hand, showed that miR-126 could play a tumor suppressor role in pancreatic tumor cells through the regulation of ADAM9. Taken together,

these findings indicated that HOTAIR could regulate the proliferation, invasion and migration of SS cells via miR-126 regulation.

There have been two major interaction mechanisms reported between lncRNA and miRNA. The first mechanism suggested that lncRNA could act as 'molecular sponges' to block the post-transcriptional inhibitory effects of miRNAs on downstream target genes. These molecular sponge lncRNAs are called competitive endogenous RNA (34). The other mechanism suggested that lncRNAs and miRNAs could increase the stability of target genes by competing with miRNAs to bind target genes (34). In the present study, it was shown that HOTAIR knockdown increased miR-126 expression level, and that miR-126-mimics inhibited the protein expression level of SDF-1 in the SS cells. This suggested that the high expression level of HOTAIR may act as an oncogene in the SS cells by promoting SDF-1 expression level by regulating miR-126. Furthermore, it was also shown that SDF-1 knockout and HOTAIR knockdown resulted in similar molecular signatures in the SW982 cells, whereby they significantly reduced CDK1, CDK2, cyclin D1, MMP-9, vimentin and N-cadherin protein expression levels, while inducing p21, p53 and E-cadherin expression level.

Cyclin network systems, including CDKs and CDKIs, play important roles in the regulation of the G<sub>1</sub>/S phase of the cell cycle (35,36). p21 is a negative regulator of the cell cycle and is a member of the CDKI family. p21 can induce cell cycle arrest and block cell proliferation by binding to cyclins, CDKs or cyclin-CDKs complexes (37,38). In addition, the p21 gene has a specific binding site to the p53 protein and is considered one of the most important target genes of p53; when the cell is subjected to various stressors (such as chemical drugs or radiation), the WT p53 protein acts on the p21 gene and rapidly induces its expression (39). The p21 protein, encoded by the p21 gene, is a cell cycle inhibitor that is currently considered to have the broadest kinase inhibitor activity (39). Indeed, p21 can bind to and inhibit a wide range of cyclin-CDK complexes, such as cyclinD1-CDK4, cyclinE-CDK2, and cyclinA-CDK2, but also has a weak inhibitory activity against cyclinB-related complexes. p21 inhibits the activity of cyclinD1-CDK4 and cyclinE-CDK2, and hence, can block Rb protein phosphorylation and the release of the E2F protein, thereby inducing cell cycle arrest in the G<sub>1</sub> phase (40). On the other hand, MMP-9, vimentin, E-cadherin and N-cadherin are key regulators of epithelial-mesenchymal transition, and hence, can regulate the metastatic ability of cancer cells (41,42).

In summary, the results from the present study showed that HOTAIR could be highly expressed in SS tissues, where it can promote the proliferation, invasion and migration of the SS cells by promoting SDF-1 protein expression through the regulation of miR-126 expression. Despite these important results, the number of SS tissues, as well as their freezing time and conditions may affect the expression level of HOTAIR and miR-126. Therefore, future studies, using larger sample sizes and more cell lines are required to further support the findings.

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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

QF conceived and designed the current study and contributed to writing the manuscript. QF and DW performed the experiments. QF and DW confirm the authenticity of all the raw data. DW, PG, ZZ and JF analyzed and interpreted the data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was approved and monitored by the Ethics Committee of The Fourth Hospital of Hebei Medical University (Hebei, China). Informed consent was provided by all the patients participating in the study or from their family members.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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