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# MicroRNA-330-5p promotes the development of osteosarcoma by regulating SPRY2

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**Abstract. – OBJECTIVE: MicroRNA is an endogenous, non-coding small RNA that has a significant role in regulating organisms and pathology. Previous studies have demonstrated that microRNA-330-5p was a cancer-promoting gene. However, the role of microRNA-330-5p in osteosarcoma (OS) has not been reported. The aim of this work was to explore the characteristics of microRNA-330-5p expression in OS, and to further study its expression in OS and its relationship with clinicopathological parameters and prognosis.**

**PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed to investigate the expression of microRNA-330-5p in 48 pairs of OS tissues and paracancer tissues, and to analyze the relationship between the expression of microRNA-330-5p and OS clinical indicators and patient prognosis. Meanwhile, qRT-PCR was performed to verify the microRNA-330-5p expression in OS cells. In addition, the microRNA-330-5p knockdown expression model was constructed using lentivirus in OS cell lines U2OS and MG63. The effects of microRNA-330-5p on the biological function of OS cells were analyzed by Cell Counting Kit-8 (CCK-8) and transwell experiments. The potential mechanism was explored by Western blot.**

**RESULTS: In this paper, qRT-PCR results showed that the expression of microRNA-330-5p in OS was higher than that in paracancer tissues, and the difference was statistically significant. Compared with microRNA-330-5p low expression group, patients with high expression of microRNA -330-5p had a higher prevalence of distant metastasis and a lower overall survival rate. *In vitro* experiment showed that the proliferation, invasion and metastasis abilities of the cells in the microRNA-330-5p silencing group were markedly decreased compared with the negative control group (NC group). Western blot results demonstrated that microRNA-330-5p inhibitor can activate SPRY2 and regulate the expression of key proteins, such as p-Smad2, p-Smad3, TGF-β1, MMP9 and Vimentin in the TGF-β1/Smad signaling pathway. It was found that there was a mutual regulation between microRNA-330-5p and SPRY2, which promoted the malignant progression of OS.**

**CONCLUSIONS: The expression of microRNA-330-5p was markedly increased in OS, which was associated with distant metastasis and poor prognosis. Furthermore, we found that microRNA-330-5p may promote the vicious progression of OS by inter-modulating SPRY2 and the TGF-β1/Smad signaling pathways.**

*Key Words:*
MicroRNA-330-5p, SPRY2, Progression, Osteosarcoma.

## Introduction

Osteosarcoma (OS) is one of the most malignant primary bone tumors with the highest incidence in the clinic. It occurs in the metaphysis of the lower end of the femur and the upper end of the humerus. It can also occur in the tibia and spine[1-3]. According to the epidemiological authority data of the US Department of Health's SEER, osteosarcoma is more common in adolescents and the elderly, with an incidence of 4.4/1 million in the 0-24 age group, second only to lymphoma[4,5]. Osteosarcoma is accompanied by high degree of malignancy, about 80%-90% of the cases can metastasize. If the disease is not discovered at early stage, it may break through the cortical and medullary cavity, and then hematogenous metastasis occurs. The most common metastasis is to the lungs while a small number can be transferred to the brain, prostate, kidney and other organs[5]. However, both primary and secondary osteosarcoma are resistant to conventional chemotherapeutic drugs, and drug resistance increases with time. Therefore, recurrence to drugs is still one of the important factors in the failure of surgical treatment and chemotherapy[6-8]. A comprehensive analysis of the etiology, cancer, proliferation, metastasis and drug resistance of osteosarcoma has important implications for the treatment of osteosarcoma and the improvement of survival prognosis[6-8]. Tumor metastasis is a complex process involving multiple factors, including not only the characteristic changes of tumor cells themselves, but also the changes in the host environ-

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ment[6-8]. The tumor microenvironment is a dynamic network composed of tumor cells, extracellular matrix and interstitial tissues. The specific tumor microenvironment can affect the proliferation of tumor cells, induce the formation of tumor angiogenesis, regulate the expression of metastasis-related genes and promote the degradation of the extracellular matrix and the occurrence of metastasis[9-12].

MicroRNA is an endogenous small RNA that is widely distributed in eukaryotes with 18-25 nt in length and has no protein-encoding and no open reading frame (ORF), therefore it does not encode proteins, but has a significant role in the organism by regulating target genes[12,13]. The matured microRNA has a phosphate group at the 5' end and a hydroxyl group at the 3' end. It is conserved in various species and variously expressed at different stages of biological development and different tissues. MicroRNA mainly inhibits the transcription of mRNA or the translation process of proteins by binding to the 3' non-translated region of the target gene, thereby regulating the life activities of the cells in a variety of ways, including cell cycle regulation, proliferation, apoptosis, differentiation, and cellular stress response. About 3% of human genes are encoded by microRNA, which regulates about 30% of human protein expression, and more than half of them are located in tumor-associated gene regions[15]. MicroRNAs are involved in the formation of RNA silencing complexes (RISC) by incomplete base pairing with the target gene mRNA, resulting in inhibition of target mRNA transcription or protein translation[16,17]. MicroRNA-330-5p is one of the significant members of the microRNA family, and Tong et al[18] have demonstrated that it exhibited high expression in a variety of malignant tumors, showing a tumor-promoting gene function.

In this work, we examined microRNA-330-5p expression in human osteosarcoma tissues and explored the expression change of microRNA-330-5p and its possible role in the development of osteosarcoma. This may provide new ideas for further studies of the etiology and treatment of osteosarcoma.

## Patients and Methods

### Patients and OS Samples

We collected 48 cases of osteosarcoma tissues that were surgically removed from our department and confirmed by pathology. The age of the patients ranged from 6 to 48 years old, with an average of 22.24±9.58 years old. The samples were numbered and registered after cutting, and then stored in liquid nitrogen at -80°C. This study was approved by the Ethics Committee of the Shanxian Central Hospital. Signed informed consents were obtained from all participants before the study.

### Cell Lines and Reagents

Osteosarcoma cell lines HOS, SOSP-9607, MG63, U2OS, 143B, SaOS-2 and human osteoblasts hFOB cells were all purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) in a 37°C, 5% $CO_2$ incubator. The medium was changed every 2 to 3 days. The passage was performed when the cell confluence reached 90%. The cells were then maintained in logarithmic growth and used for the experiments.

### Transfection

The OS cells were plated into a 6-well plate and cultured until the cell density reached 50% to 70%. Cells were transfected with microRNA mimics and inhibitors as well as their corresponding negative controls, respectively. In particular, siRNA SPRY2 or the SPRY2 NC was co-transfected with microRNA inhibitors and its negative controls. The transfected cells were continuously cultured, and cells were collected 48 hours later for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and cell function experiments.

### Cell Proliferation Assays

48 h after transfection, the cells were harvested and seeded into 96-well plates at 2000 cells per well. After 6 h, 24 h, 48 h and 72 h, the cells were added with Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) reagent. After incubation for another 2 hours, the optical density (OD) value of each well at different time point was detected in the microplate reader and then the data was analyzed.

### Cell Migration and Invasion Assay

48 h after transfection, the cells were digested, centrifuged and resuspended in serum-free medium at the density of 5 x $10^5$ cells/mL. A cell suspension of 200 uL (1 x $10^5$ cells) was added to the upper chamber, and 700 μL of a medium containing 20% FBS was added to the lower chamber. The cells were returned to the incubator for a specific period of time-based on the different

migration abilities of each cell line. The transwell chamber was clipped, washed 3 times with 1 x Phosphate-Buffered Saline (PBS; Gibco, Grand Island, NY, USA), and placed in methanol for 15 min cell fixation. After the chamber was stained in 0.2% crystal violet for 20 min, the cells on the upper surface of the chamber were carefully wiped off with water and a cotton swab. The cells were placed under a microscope, and 10 fields of view were randomly selected for counting. Then, statistical analysis was performed.

### Quantitative Real Time-Polymerase Chain Reaction

Total RNA was extracted from OS cell lines and tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and RNA was reverse transcribed into cDNA using Primescript RT Reagent (TaKaRa, Otsu, Shiga, Japan). The qRT-PCR reactions were performed using SYBR® Premix Ex TaqTM (TaKaRa, Otsu, Shiga, Japan), and StepOne Plus Real Time-PCR System (Applied Biosystems, Foster City, CA, USA). The following primers were used for qRT-PCR reaction: microRNA-330-5p: forward: 5'-CTCTCCGACCTGCCACAGA-3', reverse: 5'-AACCTAGATGGGCGCGATCT-3'; U6: forward: 5'-CGCTTCGGCAGCACATATAC-3', reverse: 5'-TTCACGAATTTGCGTGTCAT-3'; SPRY2: forward: 5'-CTCGCTrCGGCAGCACA-3', reverse: 5'-AACGCTI'CACGAATTTGCGT-3'; β-actin:forward:5'-CCTGGCACCCAGCACAAT-3', reverse: 5'-TGCCGTAGGTGTCCCTTTG-3'. Data analysis was performed using a StepOne software (Applied Biosystems, Foster City, CA, USA) and relative mRNA expressions were calculated using the $2^{-\Delta\Delta Ct}$ method.

### Western Blot

The transfected cells were lysed using cell lysis buffer, shaken on ice for 30 minutes, and centrifuged at 14,000 x g for 15 minutes at 4°C. The total protein concentration was calculated by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Waltham, MA, USA). The extracted proteins were separated using a 10% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) gel and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Western blot analysis was performed according to standard procedures. The primary antibodies were SPRY2, p-Smad2, p-Smad3, TGF-β1, N-cad, MMP9, Vimentin, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the secondary antibodies were anti-mouse and anti-rabbit, which were all purchased from Cell Signaling Technology (Danvers, MA, USA).

### Statistical Analysis

Statistical analysis was performed using GraphPad Prism 6 V6.01 software (La Jolla, CA, USA). Statistical differences between the two groups were analyzed using Student's *t*-test. The comparison between groups was made using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). Independent experiments were repeated at least three times for each experiment and the data were expressed as mean ± standard deviation ($\bar{x}\pm s$). There were three levels of $p<0.05$, $p<0.01$ and $p<0.001$ at the significance level, and $p<0.05$ was considered statistically significant.

## Results

### MicroRNA-330-5p Was Highly Expressed in OS Cell Lines and Tissues

We examined the microRNA-330-5p expression in 48 pairs of OS tissues and their paracancerous tissues and OS cell lines. The results illustrated that microRNA-330-5p in OS tissues was markedly higher than that in paracancerous tissues (Figure 1A, 1B). At the same time, *in vitro* analysis showed that compared with human normal osteoblasts (hFOB), microRNA-330-5p was significantly expressed in OS cells with a statistically significant difference (Figure 1C), suggesting that microRNA-330-5p was highly expressed in OS cell lines and tissues.

### MicroRNA-330-5p Expression Was Correlated With Distance Metastasis and Overall Survival in OS Patients

Based on the qRT-PCR results of 48 pairs of microRNA-330-5p expression in OS tissues and paracancerous tissues, we divided these tissues into high expression group and low expression group, and counted the number of each group. Chi-square test was performed to analyze the relationship between microRNA-330-5p expression and age, sex, clinical stage and distant metastasis of OS patients. As shown in Table I, the high expression of microRNA-330-5p was positively correlated with distant metastasis, but not with age, gender, and Enneking stage. In addition, to explore the relationship between the expression of microRNA-330-5p and the prognosis of OS pa-

**Table I.** Association of miR-330-5p expression with clinicopathologic characteristics of osteosarcoma.

| Parameters | Number of cases | miR-330-5p expression | | *p*-value |
|---|---|---|---|---|
| | | Low (%) | High (%) | |
| Age (years) | | | | 0.242 |
| <21 | 20 | 8 | 12 | |
| ≥21 | 28 | 16 | 12 | |
| Gender | | | | 0.680 |
| Male | 32 | 15 | 17 | |
| Female | 15 | 8 | 7 | |
| Enneking stage | | | | 0.835 |
| IA | 7 | 5 | 2 | |
| IIA | 13 | 7 | 6 | |
| IIB | 26 | 14 | 12 | |
| III | 8 | 4 | 4 | |
| Distance metastasis | | | | 0.019 |
| No | 28 | 18 | 10 | |
| Yes | 20 | 6 | 14 | |

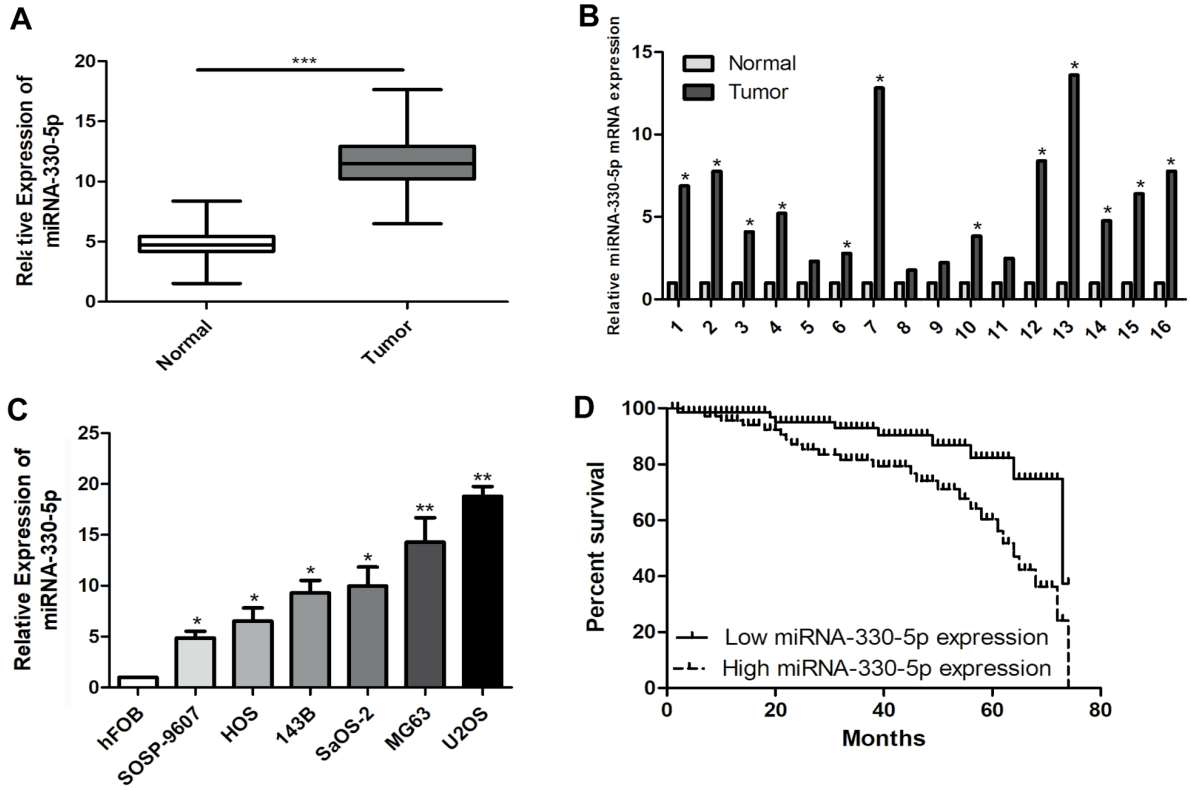


**Figure 1.** MicroRNA-330-5p was highly expressed in osteosarcoma tissues and cell lines. **A-B,** qRT-PCR detection of differential expression of microRNA-330-5p in osteosarcoma tumor tissues and paracancerous tissues. **C,** qRT-PCR detection of microRNA-330-5p expression levels in osteosarcoma cell lines. **D,** Kaplan-Meier survival curve of osteosarcoma patients based on microRNA-330-5p expression; the prognosis of patients with high expression was significantly worse than that of the bottom expression group. Data are mean ± SD, *$p$<0.05, **$p$<0.01, ***$p$<0.001.

tients, we collected relevant follow-up data. Kaplan-Meier survival curves illustrated that high expression microRNA-330-5p was significantly associated with poor OS prognosis. The higher the microRNA-330-5p expression, the worse the prognosis ($p$<0.05; Figure 1D). These data suggested that microRNA-330-5p may be a new biological indicator for predicting OS prognosis.

### *Knockdown of MicroRNA-330-5p Inhibited Cell Migration, Proliferation and Invasion*

To explore the effect of microRNA-330-5p on OS cell proliferation, we first successfully constructed a microRNA-330-5p interference expression model (Figure 2A). We used the CCK-8 assay to detect proliferation in the NC group and the microRNA-330-5p interference groups. As shown in Figure 2B, the cell proliferation rate of the microRNA-330-5p inhibitor group was markedly down-regulated compared to that of the NC group, suggesting that the knockdown of microRNA-330-5p inhibited cell proliferation.

Next, we used the transwell migration assay to explore the effect of microRNA-330-5p on OS cell invasion and migration. In the MG63 and U2OS cell lines, the experimental results (Figure 2C) illustrated that, compared with the NC group, the number of OS cells transfected through the transwell chamber after microRNA-330-5p knockdown was reduced, suggesting that the invasion and migration ability of cells was inhibited.

### *SPRY2 Modulated MicroRNA-330-5p Expression in Human Osteosarcoma Cells*

To further explore the pathways in which microRNA-330-5p promoted the malignant progression of osteosarcoma, we found a possible relationship between SPRY2 and microRNA-330-5p through bioinformatics analysis. Using qRT-PCR and Western blot experiment, we

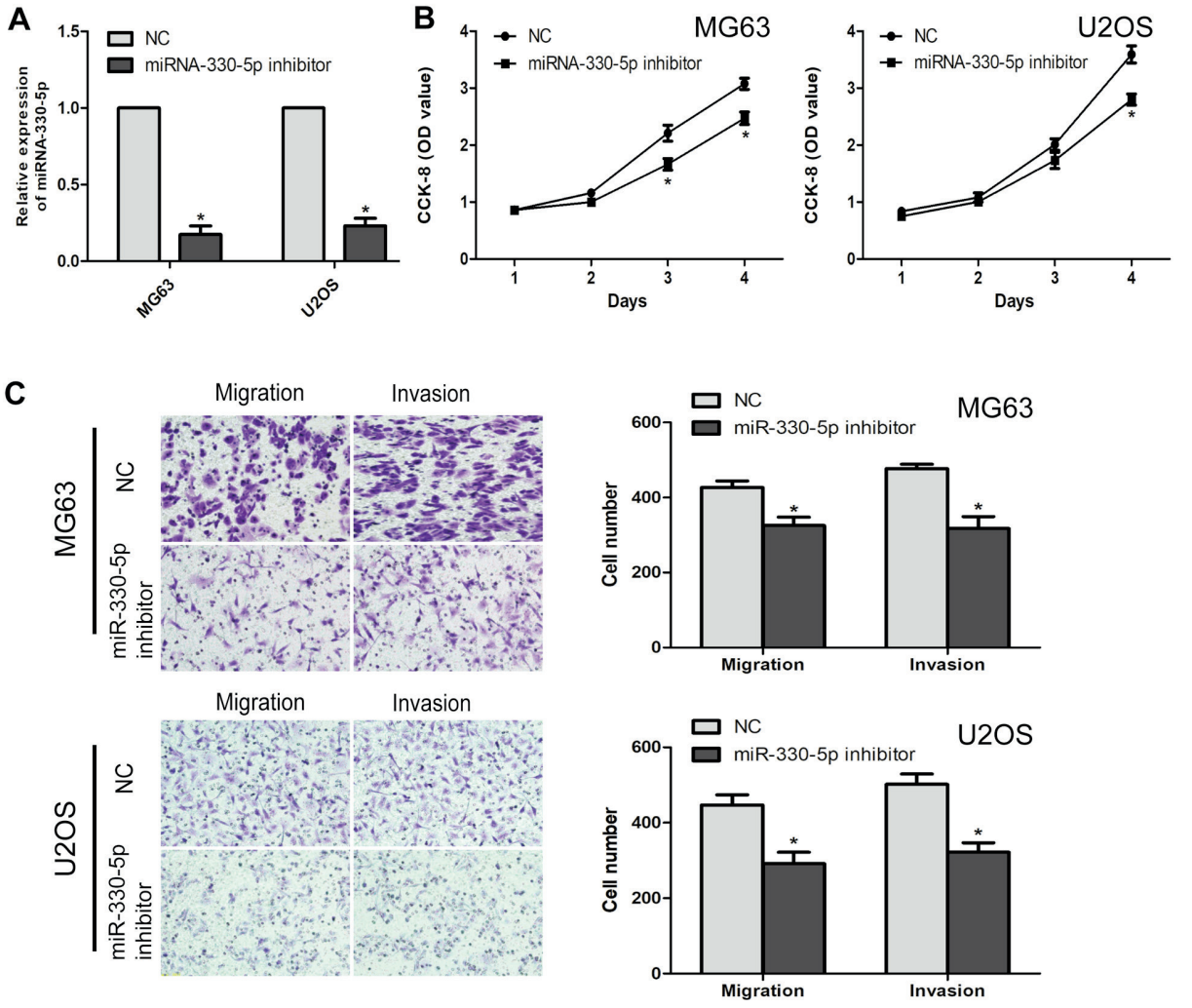


**Figure 2.** Inhibition of proliferation and metastasis of osteosarcoma cells after silencing microRNA-330-5p. **A,** qRT-PCR verified the interference efficiency after transfection of microRNA-330-5p inhibitor in U2OS and MG63 cell lines. **B,** CCK-8 assay detects the effect of knocking down microRNA-330-5p expression on proliferation of osteosarcoma cells in U2OS and MG63 cell lines. **C,** Transwell assay was used to detect the migration and invasion of osteosarcoma cells after interference with microRNA-330-5p in U2OS and MG63 cell lines (magnification: 40×). Data are mean ± SD, *$p<0.05$.

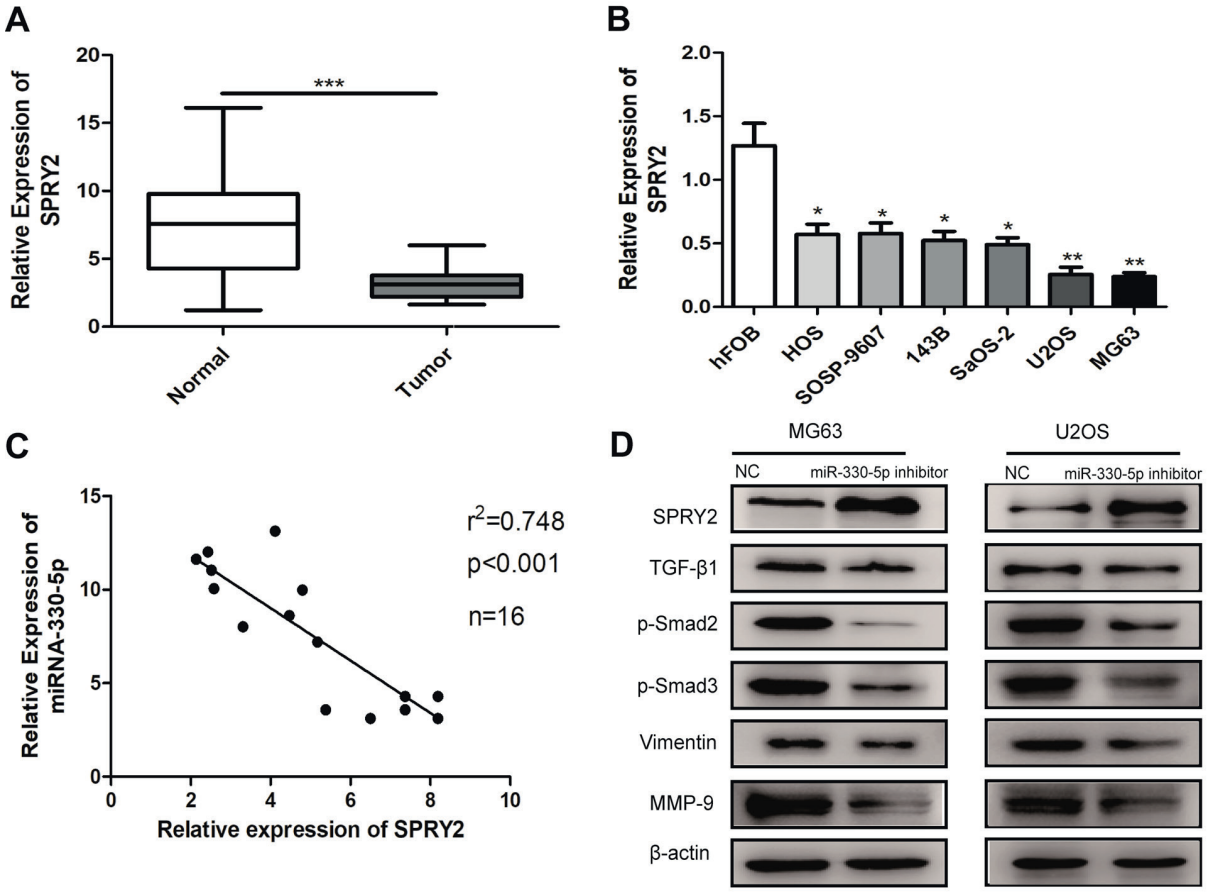


**Figure 3.** MicroRNA-330-5p regulated the expression of SPRY2 in osteosarcoma tissues and cell lines. **A,** qRT-PCR detection of SPRY2 expression in osteosarcoma tumor tissues and paracancerous tissues. **B,** qRT-PCR detection of SPRY2 expression in osteosarcoma cell lines. **C,** There was a significant negative correlation with the expression of SPRY2 and the expression of microRNA-330-5p in osteosarcoma tissues. **D,** Silencing microRNA-330-5p expression significantly decreased the expression of the TGF-β1/Smad signaling pathways genes, including TGF-β1, p-Smad2, p-Smad3, N- Cad, Vimentin and MMP9. Data are mean ± SD, *$p$<0.05, **$p$<0.01, ***$p$<0.001.

explored the expression of SPRY2 in 48 pairs of OS tissues and their corresponding paracancerous tissues as well as OS cell lines. The results illustrated that the expression of SPRY2 protein in OS tissues was lower than that in paracancerous tissues, and the difference was statistically significant (Figure 3A). Furthermore, as shown in Figure 3B, we found that SPRY2 was markedly less expressed in OS cells than hFOB, and the difference was statistically significant. In addition, we selected 16 pairs of samples from 48 pairs of OS tissues, and detected the expression of microRNA-330-5p and SPRY2 by qRT-PCR. The results demonstrated that microRNA-330-5p and SPRY2 were all expressed in osteosarcoma, but a negative correlation was presented (Figure 3C).

Subsequently, to analyze the potential mechanism of microRNA-330-5p to promote cell proliferation, invasion and migration, we examined the key protein TGF-β1, p-Smad2 in the TGF-β1/Smad pathway after knockdown of microRNA-330-5p by Western blot. The expression of p-Smad3, N-cad, Vimentin and MMP9 was down-regulated (Figure 3D), which demonstrated that microRNA-330-5p may function through the TGF-β1/Smad pathway.

### *SPRY2 Was Expressed Highly in OS Cell Lines and Tissues*

To further explore the interaction between microRNA-330-5p and SPRY2 in OS cells, we constructed a SPRY2 overexpression model at the cellular level and qRT-PCR was used to examine

the transfection efficiency of SPRY2 (Figure 4A). Subsequently, the proliferation was detected by CCK-8 assay in the NC and SPRY2 overexpression groups. As shown in Figure 4B, the cell proliferation rate of the SPRY2 overexpression group was remarkably decreased compared to that of the NC group. In addition, we used transwell migration and invasion experiments to explore the effects of SPRY2 on OS cell invasion and migration. In the MG63 and U2OS cell lines, the experimental results (Figure 4C) illustrated that the number of transmembrane OS cells in the transwell chamber of the SPRY2 overexpressing group was decreased compared with the NC group, suggesting that migration and invasion ability of the cells was inhibited.

### *MicroRNA-330-5p Interacted With SPRY2 and Regulated its Expression in Human Osteosarcoma Cells*

To further investigate the ways in which microRNA-330-5p promoted the malignant progression of OS, we found a possible relationship between microRNA-330-5p and the TGF-β1/Smad signaling pathways by qRT-PCR and Western blot. In addition, to explore the interaction between NR027113 and SPGF2 in OSF cells in the TGF-β1/Smad signaling pathway, we knocked down SPRY2 in the cell line after silencing microRNA-330-5p in OS cells, and explored their roles of the OS. Subsequently, the expression of SPRY2 in the microRNA-330-5p inhibitor+siNC and microRNA-330-5p inhibitor+si-SPRY2 groups were examined by

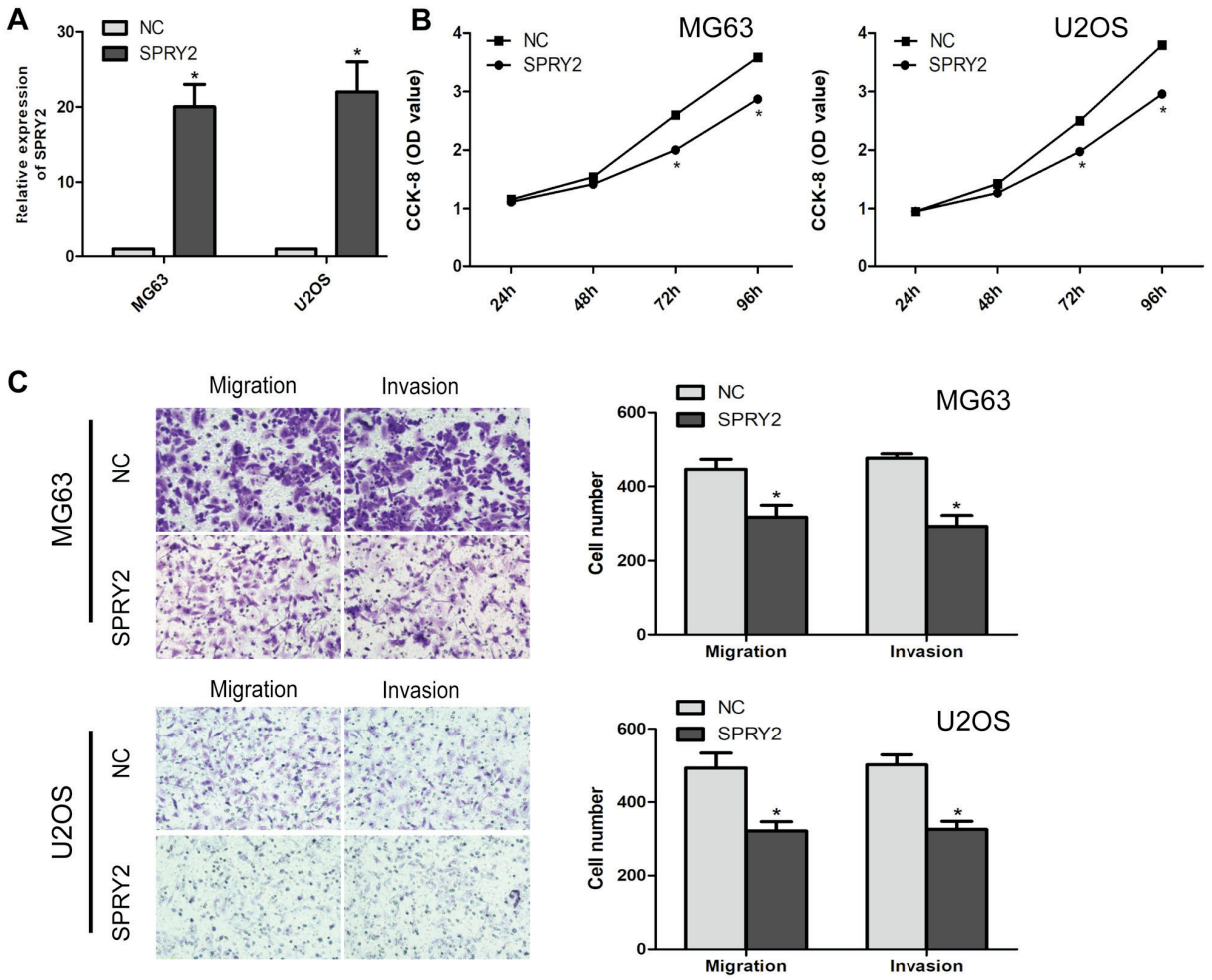


**Figure 4.** Inhibition of osteosarcoma cell migration and invasion after overexpression of SPRY2. **A,** qRT-PCR validated the interference efficiency after transfection of SPRY2 in U2OS and MG63 cell lines. **B,** CCK-8 assay detected the effect of overexpression of SPRY2 on osteosarcoma cell proliferation in U2OS and MG63 cell lines. **C,** Transwell assay was used to examine the effects of the overexpression of SPRY2 on osteosarcoma cell migration and invasion in U2OS and MG63 cell lines (magnification: 40×). Data are mean ± SD, *$p<0.05$.

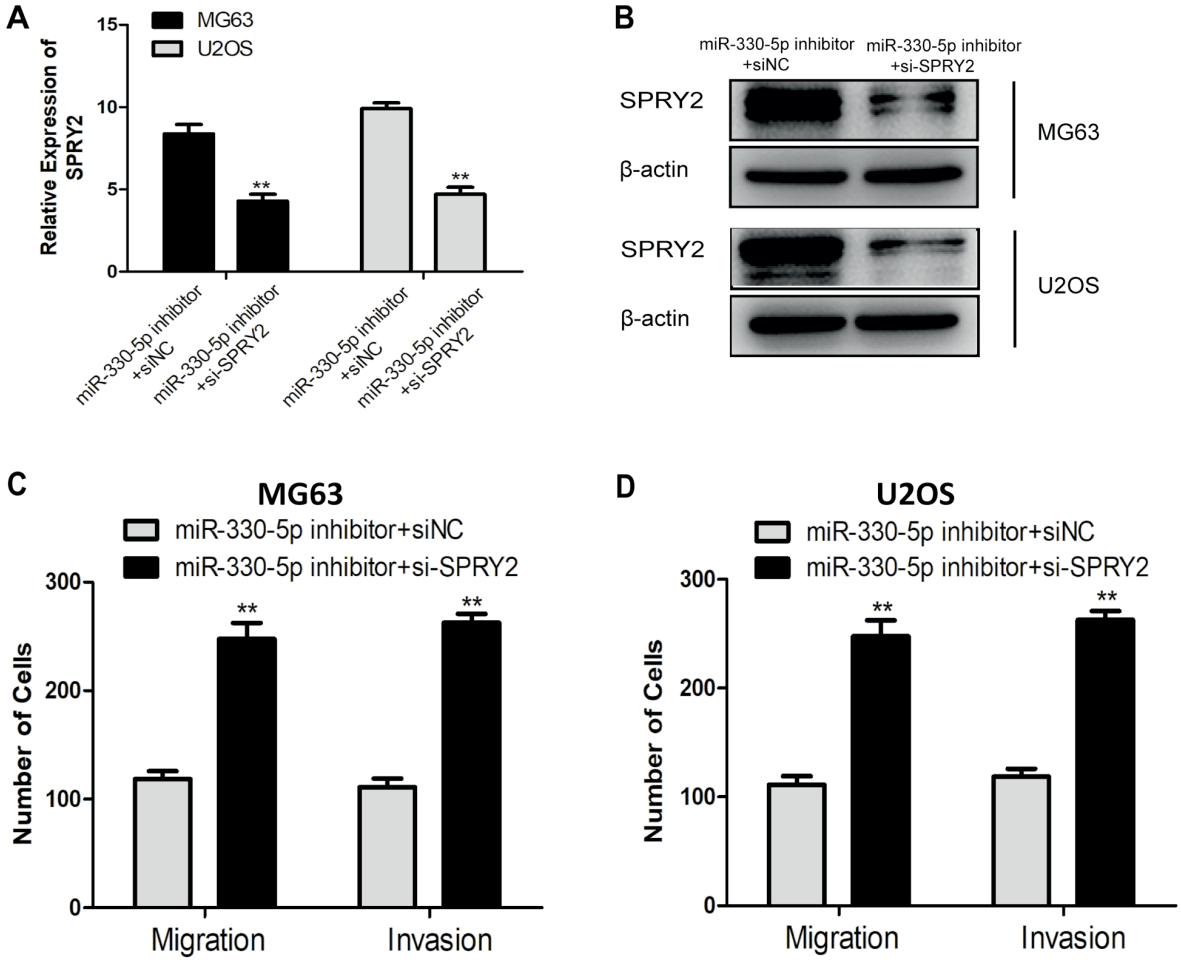


**Figure 5.** MicroRNA-330-5p regulated the expression of SPRY2 in osteosarcoma tissues and cell lines. **A,** Detection of SPRY2 expression in microRNA-330-5p and SPRY2 co-transfected cell lines by qRT-PCR.* Detection of SPRY2 expression in microRNA-330-5p and SPRY2 co-transfected cell lines by Western blot. **C,** The transwell assay was used to detect the migration and invasion of osteosarcoma cell U2OS after co-transfection of microRNA-330-5p and SPRY2. **D,** The transwell assay detected changes in migration and invasion of sarcoma cell MG63 after co-transfection of microRNA-330-5p and SPRY2. Data are average ± SD, *#$p$<0.05.

qRT-PCR and Western blot, respectively (Figure 5A, 5B). We performed the transwell assay and found that SPRY2 reversed the effect of microRNA-330-5p on invasion and migration of OS cells (Figure 5C, 5D). MicroRNA-330-5p interacted with SPRY2 and regulated its expression in human osteosarcoma cells.

## Discussion

Metastasis refers to the process in which tumor cells are detached from the primary tumor lesions and continue to grow to form new secondary tumor foci after direct spread, lymphatic metastasis, hematogenous metastasis and implantation. Tumor metastasis is one of the problems in current cancer treatment and research work. According to statistics, more than 90% of cancer patients die from tumor metastasis[6,8,14]. Tumor metastasis is a complex process with multiple factors involved[6]. From the perspective of cell biology, tumor metastasis is the result of the interaction between tumor cells and the host microenvironment. Tumor cells need to pass through the host extravascular matrix and vessel wall into the blood vessels, survive in the blood circulation and successfully escape the surveillance of the immune system to reach the appropriate target organ. After that, the tumor cells cross over the blood vessel wall and survive and grow to form a

new metastatic tumor lesion. From a genetic point of view, tumor metastasis is the result of multiple genes involved in coordinated control. Tumor-promoting genes can promote tumor metastasis, while metastasis suppressor genes act as inhibitors[3,6,12]. In the process of tumor metastasis, the dysfunction of the tumor-promoting genes and tumor-inhibiting genes is the molecular basis of tumor metastasis[4,6].

MicroRNAs are a class of endogenous small molecules that do not encode proteins and regulate the expression of protein-encoding genes by binding to the 3' UTR of the target genes[15,17]. At present, the microRNA function is usually identified by classical gene function research. The loss-of-function phenotype can be observed by down-regulating or inhibiting the expression of microRNA, and the gain-of-function phenotype can be observed by up-regulating the expression of microRNA. The up-regulation of phenotypic changes can identify target genes regulated by this microRNA and its signaling pathways[19]. Transient transfection of microRNA antisense or mature sequences can down-regulate or up-regulate the expression of a microRNA[13,19]. Early studies[13,18] have reported that, under the pathological conditions, the expression disorder of microRNA-330-5p was closely related to the malignant progression of tumor cells, exhibiting cell cycle regulation, tumor invasion to hematopoietic differentiation, immune regulation and other biological functions. The effects of microRNA-330-5p on osteosarcoma cells and the specific mechanism have not been reported, and most of the studies on microRNA-330-5p focus on the effects of tumor cell proliferation and apoptosis. The relationship between cell invasion and metastasis has rarely been reported in the literature[18]. The ability of osteosarcoma to invade and metastasize is very strong, which is the main reason for its poor prognosis. In this work, in addition to investigating the effects of microRNA-330-5p on osteosarcoma cell proliferation, the effect of microRNA-330-5p on osteosarcoma cell invasion and metastasis was also investigated. The results of this study demonstrated that the expression of microRNA-330-5p was verified in 48 pairs of OS tissues and adjacent tissues, and the up-regulation of microRNA-330-5p expression was positively correlated with OS stage, distant metastasis, lymph node metastasis and poor prognosis. We believe that microRNA-330-5p may play a role in promoting cancer in OS. To further explore the effect of microRNA-330-5p on the biological function of OS, we constructed a microRNA-330-5p knockdown expression model using lentivirus. The results of the CCK-8 assay, cell cloning experiments, invasion and migration experiments all indicated that microRNA-330-5p can promote the development of OS and have a regulatory role in OS, but its specific molecular mechanism remained still unclear.

By biological information prediction we found that microRNA-330-5p may hadve an interaction with SPRY2, thus we studied the relationship between SPRY2 and tumor. We found that SPRY2 had a dual effect on tumors, which can inhibit the proliferation of tumor cells in the early stage of tumorigenesis and could promote the metastasis of tumor cells in the advanced stage of tumorigenesis[20,21]. In this work, we found that there was a mutual regulation between microRNA-330-5p and SPRY2 through cell recovery experiments. As the research deepened, further understanding of the biological functions of SPRY2 gene and its role in the development of tumors will be more conducive to the diagnosis, treatment and prognosis of tumors[22,23]. To demonstrate whether microRNA-330-5p could promote the development of OS by influencing SPRY2, we examined the expression of SPRY2 after knockdown of microRNA-330-5p, and found that microRNA-330-5p promoted cell invasion and metastasis of OS by regulating SPRY2. In addition, our results also illustrated that the expression of SPRY2 was markedly changed after microRNA-330-5p knockdown, suggesting that microRNA-330-5p may promote the malignant progression of OS by regulating SPRY2.

## Conclusions

The expression of microRNA-330-5p in OS is markedly increased, which is significantly associated with OS stage, distant metastasis and poor prognosis. Besides, microRNA-330-5p may be capable of promoting the malignant progression of OS by regulating SPRY2.

### Conflicts of interest

The authors declare no conflicts of interest.

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