European Review for Medical and Pharmacological Sciences 2020; 24: 2239-2247

MicroRNA-29c-3p inhibits osteosarcoma cell proliferation through targeting PIK3R3

H.-Z. MA, J. WANG, J. SHI, W. ZHANG, D.-S. ZHOU

Department of Orthopedic Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China

Abstract. – OBJECTIVE: MicroRNAs (miRNAs) are endogenous, non-coding small RNAs involving in pathological regulation. Previous studies have shown that microRNA-29c-3p is a tumor-suppressor gene. However, the role of microRNA-29c-3p in osteosarcoma (OS) has not been reported. This study aims to investigate the potential influence of microRNA-29c-3p on the progression of OS.

PATIENTS AND METHODS: Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was applied to examine microRNA-29c-3p levels in 40 matched pairs of OS tumor tissues and adjacent ones. The correlation between microRNA-29c-3p expression and clinical indicators in OS patient was analyzed. At the same time, qRT-PCR was used to detect microRNA-29c-3p level in OS cell lines. In addition, microRNA-29c-3p knockdown and the overexpression models were constructed in OS cell lines. The effects of microRNA-29c-3p on the biological functions of OS cells were analyzed via cell counting kit-8 (CCK-8) and transwell assays. Finally, the potential mechanism underlying microRNA-29c-3p in OS was explored by Western Blot and cell recovery experiment.

RESULTS: QRT-PCR results revealed that microRNA-29c-3p level in OS tumor tissues was conspicuously lower than that in adjacent tissues. Compared with OS patients with the high expression of microRNA-29c-3p, those with low expression of microRNA-29c-3p had a higher incidence of distant metastasis and worse overall survival. Cell proliferative capacity and invasiveness in OS were enhanced after knockdown of microRNA-29c-3p; while the opposite results were observed after the overexpression of microRNA-29c-3p. QRT-PCR results revealed that microRNA-29c-3p negatively regulated PIK3R3 expression in OS cells. Moreover, microRNA-29c-3p and PIK3R3 levels were confirmed to be negatively correlated in OS tissues. In addition, cell reverse experiment demonstrated that PIK3R3 was responsible for the malignant progression of OS regulated by microRNA-29c-3p.

CONCLUSIONS: MicroRNA-29c-3p expression was reduced in OS, and conspicuously associated with distant metastasis and poor prognosis. MicroRNA-29c-3p might inhibit the malignant progression of OS by modulating PIK3R3 expression.

Key Words:
MicroRNA-29c-3p, PIK3R3, OS, Malignant progression.

Introduction

Osteosarcoma (OS) is a highly malignant tumor that mainly affects children and adolescents. It occurs in the mesenchymal tissues, and osteogenesis is its main characteristic[1-3]. Due to the strong metastasis in the early stage, OS develops rapidly and thus leads to an extremely poor prognosis. Most OS patients experience lung metastasis in the early phase, and the 5-year survival rate is lower than 20%. Amputation used to be the main procedure for OS treatment. Later, with the gradual development of chemotherapy drugs, surgical adjuvant chemotherapy has emerged. The 5-year survival rate of OS patients can be as high as 70%, but the 5-year survival rate of patients with metastasis has not improved[4-6]. OS is a differentiation defect, which is mainly caused by the genomic changes in the osteogenic differentiation pathway. The inactivation of tumor-suppressor genes, abnormal expressions of oncogenes, chromosomal abnormalities, or signal transduction pathway abnormalities are potential risk factors for OS[7,8]. However, so far, we have not fully clarified the molecular mechanism of the occurrence and metastasis of OS, and there are still many aspects requiring further studies[9,10].

MicroRNA (miRNA) is a kind of endogenous, multifunctional, small molecular RNA discovered in recent years. MiRNAs have numerous functions in affecting growth, metabolism, en-

Corresponding Author: Huanzhi Ma, MD; e-mail: mahuanzhi2006@163.com

docrine system, hormone secretion, and embryonic stem cells, so as to regulate environmental adaption[11-13]. Though it cannot encode proteins, miRNA can degrade the mRNA or direct block protein translation by base-pairing with the 3'-untranslated region of the downstream target. MiRNAs are found in almost all eukaryotes, which are conserved, time-specific, and tissue-specific[14-16]. It is believed that miRNAs may play an important role in the occurrence, growth, and metastasis of tumors. It is likely to be involved in tumor cell proliferation, differentiation, and apoptosis process[17-19].

MicroRNA-29c-3p is one of the newest members of the miRNA family, which is generally believed to be closely related to the occurrence and development of many kinds of tumors[20,21]. However, the regulatory effect of microRNA-29c-3p on OS still remains elusive. In this study, we mainly explored the influence of microRNA-29c-3p on the proliferative ability and invasiveness of OS cells. Our findings hope to provide a new molecular biological target for the diagnosis and treatment of OS.

Patients and Methods

Patients and OS Samples

A total of 40 matched pairs of OS tissues and paracancerous ones were surgically resected and confirmed by pathology. The samples were registered, immediately placed in liquid nitrogen, and preserved at -80°C. The specimens were obtained with informed consent from patients and their families. The Hospital Ethics Committee approved this research.

Cell Lines and Reagents

OS cell lines, including HOS, U2OS, SOSP-9607, MG63, 143B, and SaOS-2 and human osteoblasts hFOB cell line, were purchased from 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; HyClone, South Logan, UT, USA) at 37°C, with 5% CO_2.

Transfection

OS cells were inoculated into 6-well plates with about 50% to 70% confluence. Subsequently, miRNA-NC, miRNA-mimics, or miRNA-inhibitor were respectively transfected into OS cells. After cell culture for 48 hours, the transfected cells were collected for quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) analysis and cell function experiments.

Cell Counting Kit-8 (CCK-8) Assay

After transfection for 48 h, the cells were collected and inoculated into 96-well plates with 2000 cells per well. The cells were cultured for 6 h, 24 h, 48 h, and 72 h respectively, and then CCK-8 (Dojindo Laboratories, Kumamoto, Japan) reagent was applied. After incubation for 2 hours, the optical density (OD) value of each well was measured in the microplate reader at 490 nm absorption wavelength.

Transwell Assay

After transfection for 48 hours, the cells were digested, centrifuged, and resuspended in FBS-free medium at the density of 5×10^5 cells/mL. 200 μL of cell suspension (1×10^5 cells) was added to the upper chamber, and 700 μL of medium containing 20% FBS was added to the bottom chamber. After 48 hours incubation, the transwell chamber was washed 3 times with 1 × phosphate-buffered saline (PBS), and fixed in methanol for 15 min. The chamber was stained in 0.2% crystal violet for 20 min, and the cells on the upper surface of the chamber were carefully wiped off with a cotton swab. Penetrating cells in 10 randomly selected fields per well were observed under a microscope.

QRT-PCR

The total RNA was extracted from OS cell lines and tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using Primescript RT Reagent (TaKaRa, Otsu, Shiga, Japan). QRT-PCR reactions were performed using SYBR® Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan), and StepOne Plus Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The data analysis was performed using ABI Step One software. The relative expression levels of mRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used in this study were as follows: PIK3R3, F: 5'-CGAGGGAACAACACCTGTACGTC-3', R: 5'-CGTACGGCGATGTCGGCAACGGCA-3'; microRNA-29c-3p, F: 5'-GCTGTCAACACACTCCTCGACTCG-3', R: 5'-ACTCTCGCCGTTGCGAGTCCG-3'; U6: F: 5'-GCTTC-

GGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blot Assay

The transfected cells were lysed using cell lysis buffer, shaken on ice for 30 minutes, and centrifuged at 4°C, 14,000 × g for 15 minutes. The total protein concentration was calculated by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). The extracted proteins were separated using a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) 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 anti-PIK3R3, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the secondary antibodies were anti-mouse and anti-rabbit ones (Cell Signaling Technology, Danvers, MA, USA).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 6 V6.01 software (La Jolla, CA, USA). The *t*-test was used for analyzing measurement data. The differences between the two groups were analyzed using the Student's *t*-test. The comparison between multiple groups was done using one-way ANOVA test followed by post-hoc test (Least Significant Difference). Independent experiments were repeated at least three times. The data were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant (*$p<0.05$, **$p<0.01$ and ***$p<0.001$).

Results

MicroRNA-29c-3p Was Lowly Expressed in OS Tissues and Cell Lines

The expression of microRNA-29c-3p in 40 matched OS tissues and paracancerous tissues was detected by qRT-PCR. The results revealed that microRNA-29c-3p was downregulated in OS tumor tissues compared with adjacent tissues (Figure 1A). Likewise, microRNA-29c-3p was lowly expressed in OS cell lines compared to the human normal osteoblasts (hFOB) (Figure 1B). It is suggested that microRNA-29c-3p may be involved in the progression of OS.

MicroRNA-29c-3p Expression Was Correlated with Distant Metastasis and Overall Survival in OS Patients

According to the average level of microRNA-29c-3p, 40 OS patients were divided into high expression group and low expression group. The Chi-square test was conducted to analyze the relationship between microRNA-29c-3p expression and age, sex, Enneking stage, and distant

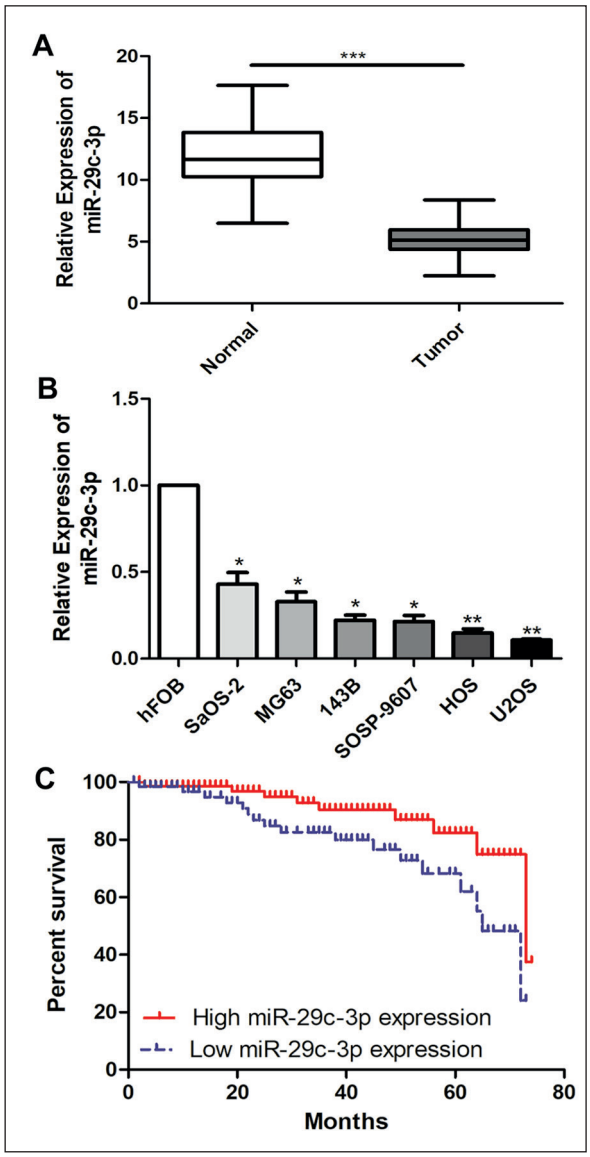

Figure 1. MiR-29c-3p was lowly expressed in osteosarcoma tissues and cell lines. **A,** qRT-PCR was used to detect the differential expression of miR-29c-3p in osteosarcoma tumor tissues and adjacent tissues. **B,** qRT-PCR was used to detect the expression level of miR-29c-3p in osteosarcoma cell lines. **C,** The Kaplan Meier survival curves of osteosarcoma patients based on miR-29c-3p expression. Data are expressed as mean ± SD, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

metastasis in OS patients. As shown in Table I, high expression of microRNA-29c-3p was positively correlated with distant metastasis, rather than the others. In addition, the Kaplan-Meier survival curves revealed that the low expression of microRNA-29c-3p was associated with poor prognosis of OS ($p<0.05$; Figure 1C). These results showed that microRNA-29c-3p expression was correlated with distant metastasis and overall survival in OS patients.

Knockdown/Over-expression of MicroRNA-29c-3p Promoted/Inhibited Cell Proliferation, Migration and Invasion

To explore the effect of microRNA-29c-3p on OS cell proliferation and metastasis, we first successfully constructed the microRNA-29c-3p overexpression and knockdown models (Figure 2A). CCK-8 assay showed that the proliferation rate in OS cells overexpressing microRNA-29c-3p markedly decreased, while it increased after transfection of microRNA-29c-3p inhibitor (Figure 2B). The transwell assay results showed that after the overexpression of microRNA-29c-3p in SaOS-2 cells, the number of transmembrane OS cells in the transwell chamber was conspicuously reduced, suggesting that migration and invasion were weakened. However, after the knockdown of microRNA-29c-3p in U2OS cells, the number of transmembrane OS cells in the transwell chamber increased (Figure 2C). These results demonstrated that microRNA-29c-3p could regulate cell proliferation, migration, and invasion in OS.

MicroRNA-29c-3p Targeted PIK3R3 in Human OS Cells

To further explore the mechanism underlying microRNA-29c-3p in alleviating the malignant progression of OS, we predicted the correlation between microRNA-29c-3p and three potential targets through bioinformatics software (Figure 3A). Among the three targets, PIK3R3 was the most differentially expressed after overexpression of microRNA-29c-3p in OS cells (Figure 3B). Subsequently, we constructed PIK3R3 overexpression/knockdown vectors. QRT-PCR results revealed that the overexpression of PIK3R3 down-regulated microRNA-29c-3p expression, while the knockdown of PIK3R3 up-regulated microRNA-29c-3p expression (Figure 3C). In addition, qRT-PCR experiments confirmed that PIK3R3 was highly expressed in OS tissues compared to that of paracancerous tissues, and the difference was statistically significant (Figure 3D). Also, as shown in Figure 3E, PIK3R3 was highly expressed in OS cells compared to that of hFOB. In addition, a negative correlation between microRNA-29c-3p and PIK3R3 expression levels was identified in OS tissues (Figure 3F). These results indicated that PIK3R3 might be the downstream target of microRNA-29c-3p in human OS cells.

Overexpression/Knockdown of PIK3R3 Promoted/Inhibited Cell Proliferation, Migration and Invasion

To further explore the effect of PIK3R3 on OS cell proliferation and metastasis, we constructed PIK3R3 overexpression/knockdown models in

Table I. Association of miR-29c-3p expression with clinicopathologic characteristics of osteosarcoma.

Parameters	Number of cases	miR-29c-3p expression		*p*-value
		High (%)	Low (%)	
Age (years)				0.197
< 21	16	6	10	
≥ 21	24	14	10	
Gender				0.648
Male	28	13	15	
Female	11	6	5	
Enneking stage				0.730
IA	5	4	1	
IIA	11	6	5	
IIB	24	13	11	
III	6	3	3	
Distance metastasis				0.010
No	24	16	8	
Yes	16	4	12	

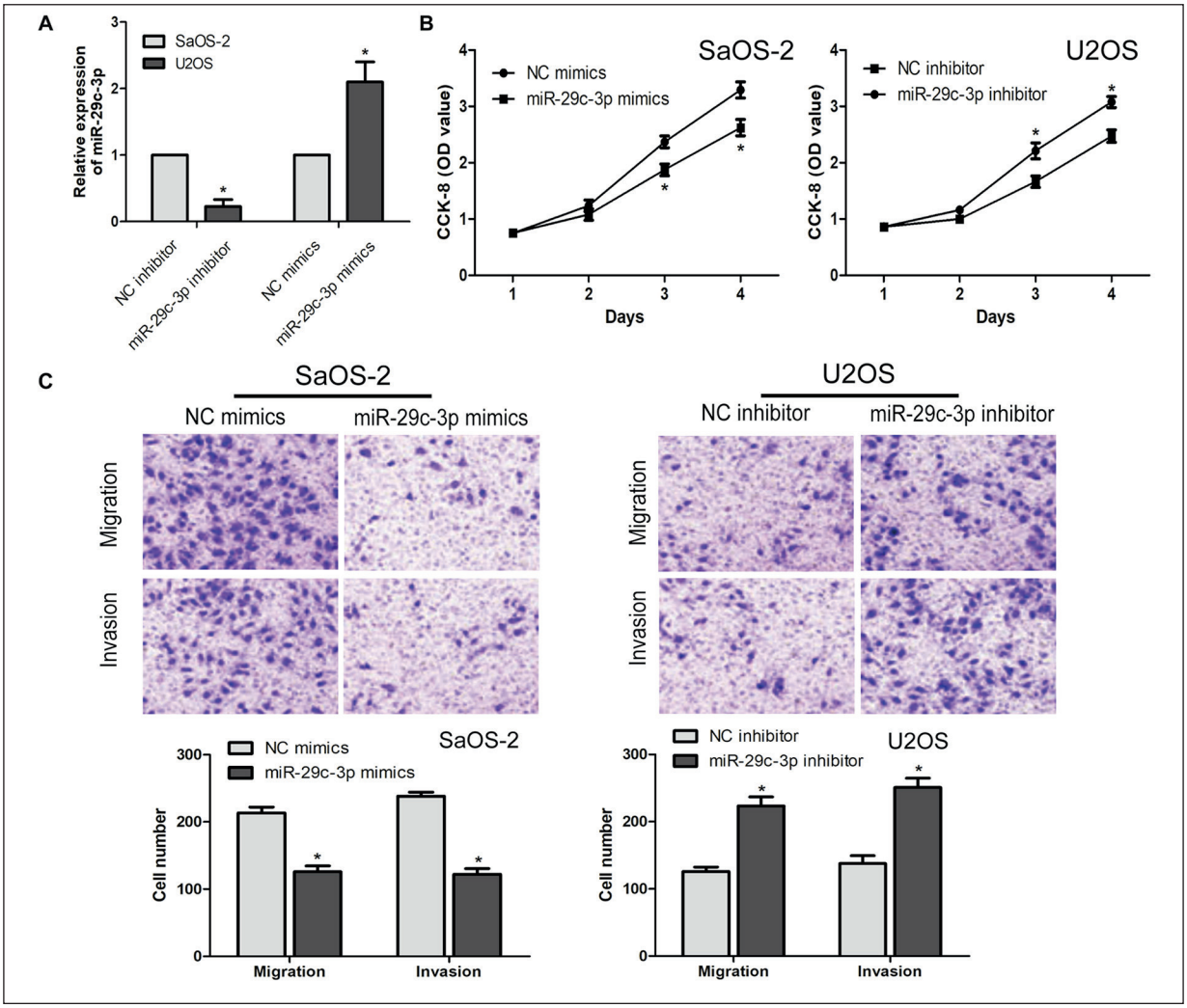

Figure 2. Overexpression/knockdown of miR-29c-3p inhibited/promoted osteosarcoma cell proliferation and metastasis. **A,** qRT-PCR verified the transfection efficiency after the overexpression/knockdown of miR-29c-3p in SaOS-2 and U2OS cell lines. **B,** CCK-8 assay detected the proliferation in SaOS-2 and U2OS cells after transfection of miR-29c-3p mimics or inhibitor. **C,** The transwell assay detected the invasion and migration in SaOS-2 and U2OS cells after transfection of miR-29c-3p mimics or inhibitor (magnification: 40×). Data are expressed as mean ± SD, *$p<0.05$.

SaOS-2 and U2OS cells, respectively (Figure 4A). Subsequently, CCK-8 was used to detect cell proliferation. As shown in Figure 4B, the proliferation rate markedly increased after the overexpression of PIK3R3, while the knockdown of PIK3R3 achieved the opposite result. In addition, the transwell assay showed that the overexpression of PIK3R3 accelerated the migration and invasion capacities in OS (Figure 4C).

PIK3R3 Was Responsible for OS Progression Regulated by MicroRNA-29c-3p

To explore the interaction between microRNA-29c-3p and PIK3R3 in OS cells, rescue experiments were conducted. PIK3R3 levels in the co-transfected OS cells were examined by qRT-PCR and Western Blot (Figures 5A, 5B). In addition, the transwell assay results detected that PIK3R3 reversed the effect of microRNA-29c-3p on invasion and migration in OS cells (Figure 5C), confirming that microRNA-29c-3p regulated OS progression by interacting with PIK3R3.

Discussion

During the pathological progression of OS, a large number of tumor-related genes are abnormally expressed, which could, in turn, influence

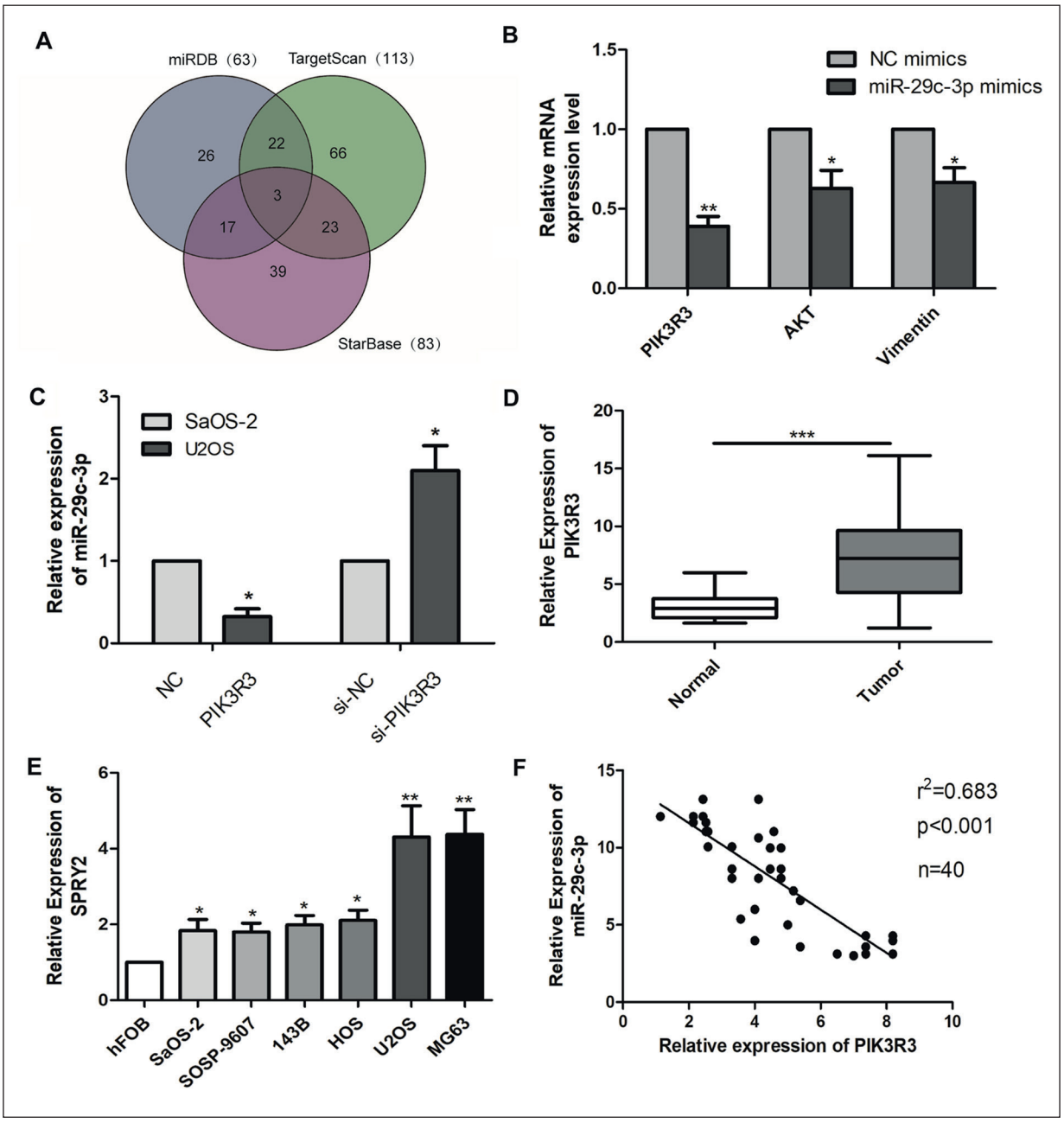

Figure 3. MiR-29c-3p regulated PIK3R3. **A,** Potential binding mRNAs of miR-29c-3p predicted in TargetScan, miRbase, and MiRcode. **B,** Potential expressions of three mRNAs after overexpression of miR-29c-3p, of which PIK3R3 was the most pronounced. **C,** MiR-29c-3p expression after overexpression/knockdown of PIK3R3. **D,** qRT-PCR was used to detect the differential expression of PIK3R3 in osteosarcoma tumor tissues and adjacent tissues. **E,** qRT-PCR was used to detect the expression level of PIK3R3 in osteosarcoma cell lines. **F,** A negative correlation between miR-29c-3p and PIK3R3 expressions in osteosarcoma. Data are expressed as mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

malignant phenotypes of tumor cells[1-4]. These certain genes are able to intervene in tumor-related pathways and networks in OS, and are required to be well explored[5-7]. These complex cellular biological regulatory mechanisms include signal transduction, regulatory responses to growth factors, regulation of gene transcription patterns, intercellular interactions, intercellular adhesion, angiogenesis, and regulation of cell cycle, etc.[8]. The occurrence and development of OS are ex-

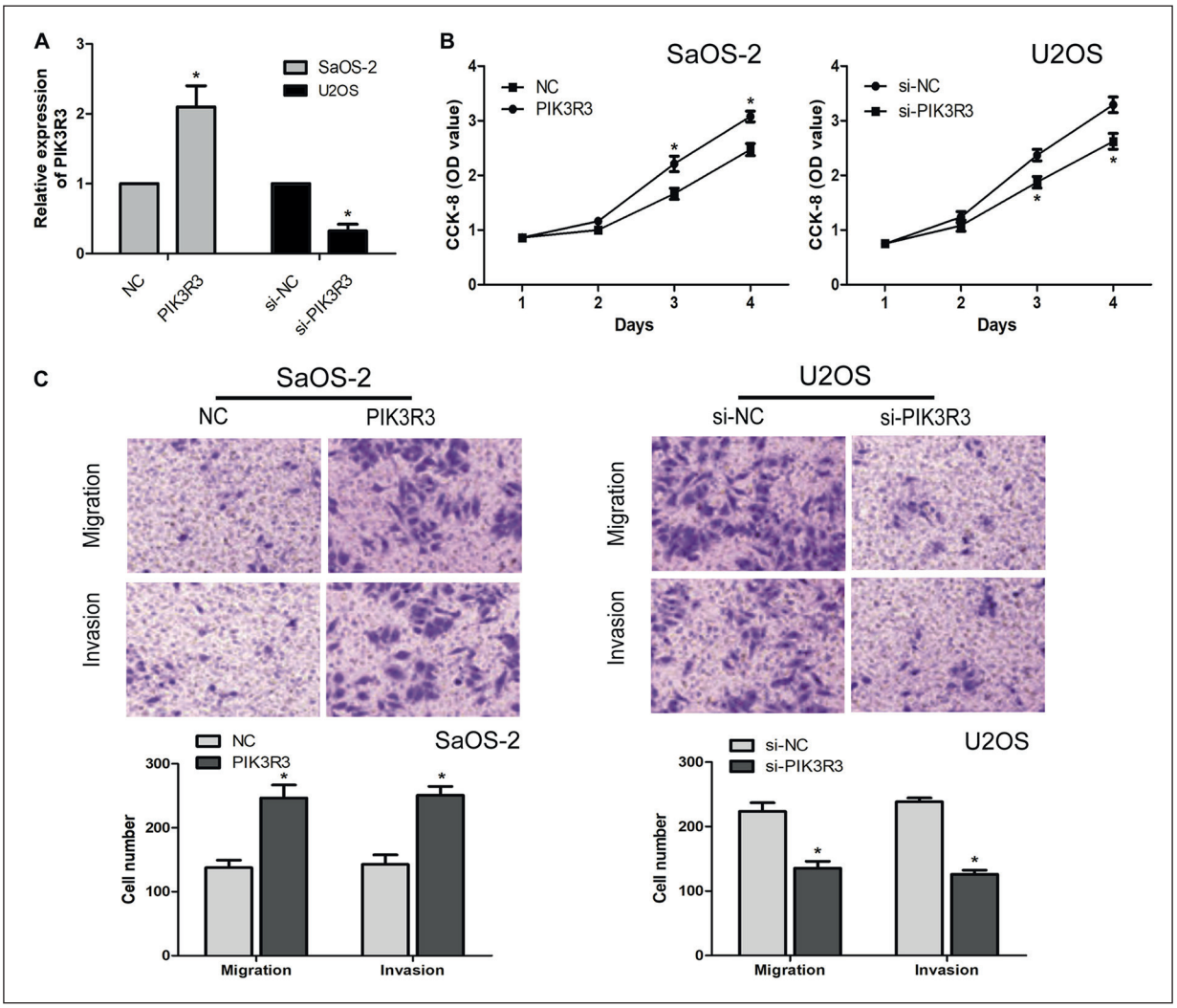

Figure 4. Overexpression/knockdown of PIK3R3 promoted/inhibited osteosarcoma cell proliferation and metastasis. **A,** qRT-PCR verified the transfection efficiency after overexpression/knockdown of PIK3R3 in SaOS-2 and U2OS cell lines. **B,** The CCK-8 assay detected proliferation in SaOS-2 and U2OS cells influenced by PIK3R3. **C,** The transwell assay detected migration and invasion in SaOS-2 and U2OS cells influenced by PIK3R3 (magnification: 40×). Data are expressed as mean ± SD, *$p<0.05$.

tremely complex processes involving multiple genes and signaling pathways[9]. The tumor-related genes and pathways vary a lot in different types of tumors[9,10].

MiRNAs are small, non-coding RNAs composed of about 22 nucleotides, which have been shown to play an important regulatory role in the growth of tissues and cells[11,12]. MiRNAs are involved in the occurrence and metastasis of human tumors by directly acting on oncogenes and tumor-suppressor genes[13,14]. MiRNAs have become important biomarkers for cancer diagnosis and prognosis[15]. MicroRNA-29c-3p is closely related to the malignant progression of tumor cells[13,18]. MicroRNA-29c-3p is capable of affecting multiple phenotypes of OS cells[20,21]. Our results uncovered that microRNA-29c-3p was downregulated in OS. Its level was correlated with the distant metastasis and poor prognosis of OS, suggesting that microRNA-29c-3p may exert an anti-cancer role in OS. To further explore the influence of microRNA-29c-3p on the biological functions of OS, the overexpression/knockdown expression models of microRNA-29c-3p were constructed. The overexpression of microRNA-29c-3p markedly attenuated proliferation, migration, and invasion in OS.

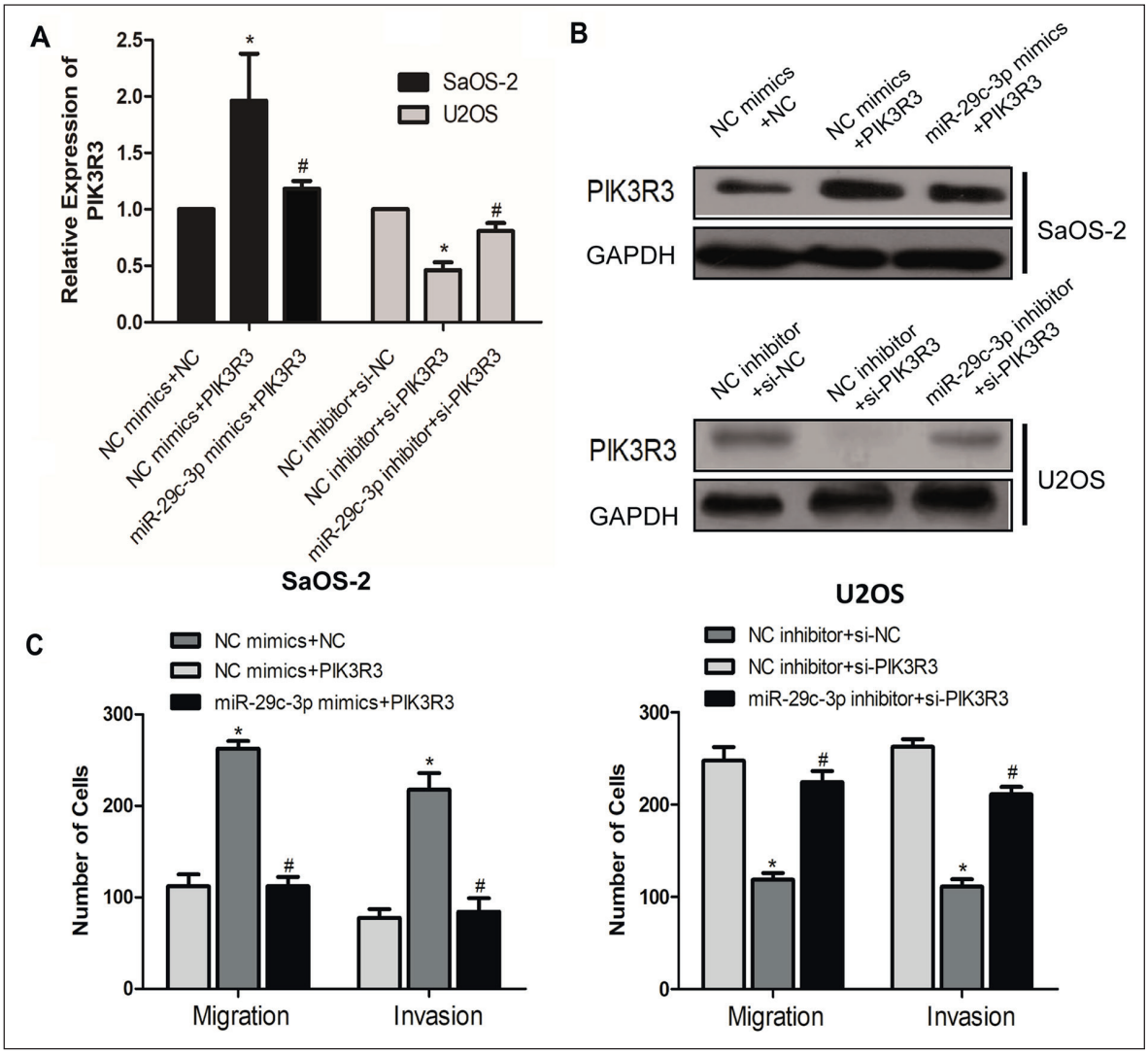

Figure 5. MiR-29c-3p regulated the expression of PIK3R3 in osteosarcoma cell lines. **A,** PIK3R3 expression levels in miR-29c-3p and PIK3R3 co-transfected cell lines were detected by qRT-PCR. **B,** The expression level of PIK3R3 expression levels in co-transfected cells detected by Western blot. **C,** The transwell assay detected the abilities of miR-29c-3p and PIK3R3 in regulating invasion and migration in SaOS-2 and U2OS cells. Data are expressed as mean ± SD, $*^{\#}p<0.05$.

MicroRNAs exert biological effect by regulating target genes. Some miRNAs are involved in the malignant transformation of OS. MiRNA-protein interaction has been well investigated because of its regulatory effect in the progression of tumor diseases[15-19]. In this study, a negative correlation was identified between the expression levels of microRNA-29c-3p and PIK3R3 in OS tissues. Furthermore, the rescue experiments confirmed the involvement of PIK3R3 in OS progression regulated by microRNA-29c-3p. These results reflected that microRNA-29c-3p was able to inhibit the malignant progression of OS by targeting PIK3R3.

Conclusions

We first detected that microRNA-29c-3p expression was conspicuously reduced in OS cells and tissues, which was correlated with distant metastasis and poor prognosis of OS patients. Besides, microRNA-29c-3p may inhibit the malignant progression of OS *via* regulating PIK3R3.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Irac SE, Oksa A, Jackson K, Herndon A, Allavena R, Palmieri C. Cytokine expression in canine lymphoma, osteosarcoma, mammary gland tumour and melanoma: comparative aspects. Vet Sci 2019; 6. pii: E37.
2) Chao LM, Sun W, Chen H, Liu BY, Li PF, Zhao DW. MicroRNA-31 inhibits osteosarcoma cell proliferation, migration and invasion by targeting PIK3C2A. Eur Rev Med Pharmacol Sci 2018; 22: 7205-7213.
3) Camuzard O, Santucci-Darmanin S, Carle GF, Pierrefite-Carle V. Role of autophagy in osteosarcoma. J Bone Oncol 2019; 16: 100235.
4) Zhu J, He T, Wei Z, Wang Y. Retrospective analysis of the effect of treatment of osteosarcoma complicated by pathological fracture by neoadjuvant chemotherapy combined with limb salvage surgery. J BUON 2018; 23: 1809-1815.
5) Han X, Wang W, He J, Jiang L, Li X. Osteopontin as a biomarker for osteosarcoma therapy and prognosis. Oncol Lett 2019; 17: 2592-2598.
6) Tang QX, Wang LC, Wang Y, Gao HD, Hou ZL. Efficacy of methotrexate, doxorubicin, and cisplatin for osteosarcoma: study protocol for a systematic review of randomized controlled trial. Medicine (Baltimore) 2019; 98: e14442.
7) Chen Y, Wang S, Geng B, Yi Z. Pelargonidin induces antitumor effects in human osteosarcoma cells via autophagy induction, loss of mitochondrial membrane potential, G2/M cell cycle arrest and downregulation of PI3K/AKT signalling pathway. J BUON 2018; 23: 735-740.
8) Li YS, Liu Q, Tian J, He HB, Luo W. Angiogenesis process in osteosarcoma: an updated perspective of pathophysiology and therapeutics. Am J Med Sci 2019; 357: 280-288.
9) Chen R, Wang G, Zheng Y, Hua Y, Cai Z. Drug resistance-related microRNAs in osteosarcoma: translating basic evidence into therapeutic strategies. J Cell Mol Med 2019; 23: 2280-2292.
10) Zamborsky R, Kokavec M, Harsanyi S, Danisovic L. Identification of prognostic and predictive osteosarcoma biomarkers. Med Sci (Basel) 2019; 7. pii: 28.
11) Shcherbata HR. MiRNA functions in stem cells and their niches: lessons from the Drosophila ovary. Curr Opin Insect Sci 2019; 31: 29-36.
12) Lu S, Liao QS, Tang L. MiR-155 affects osteosarcoma cell proliferation and invasion through regulating NF-kappaB signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 7633-7639.
13) Shirjang S, Mansoori B, Asghari S, Duijf P, Mohammadi A, Gjerstorff M, Baradaran B. MicroRNAs in cancer cell death pathways: apoptosis and necroptosis. Free Radic Biol Med 2019; 139: 1-15.
14) Subramaniam S, Jeet V, Clements JA, Gunter JH, Batra J. Emergence of microRNAs as key players in cancer cell metabolism. Clin Chem 2019. pii: clinchem.2018.299651. doi: 10.1373/clinchem.2018.299651. [Epub ahead of print].
15) Tang XJ, Wang W, Hann SS. Interactions among lncRNAs, miRNAs and mRNA in colorectal cancer. Biochimie 2019; 163: 58-72.
16) Takahashi RU, Prieto-Vila M, Kohama I, Ochiya T. Development of miRNA-based therapeutic approaches for cancer patients. Cancer Sci 2019; 110: 1140-1147.
17) Tassinari V, Cesarini V, Silvestris DA, Gallo A. The adaptive potential of RNA editing-mediated miRNA-retargeting in cancer. Biochim Biophys Acta Gene Regul Mech 2019; 1862: 291-300.
18) Ors-Kumoglu G, Gulce-Iz S, Biray-Avci C. Therapeutic microRNAs in human cancer. Cytotechnology 2019; 71: 411-425.
19) Biswas S. MicroRNAs as therapeutic agents: the future of the battle against cancer. Curr Top Med Chem 2018; 18: 2544-2554.
20) Zhang S, Jin J, Tian X, Wu L. Hsa-miR-29c-3p regulates biological function of colorectal cancer by targeting SPARC. Oncotarget 2017; 8: 104508-104524.
21) Chen G, Zhou T, Li Y, Yu Z, Sun L. P53 target miR-29c-3p suppresses colon cancer cell invasion and migration through inhibition of PHLDB2. Biochem Biophys Res Commun 2017; 487: 90-95.