

Lower miR-630 expression predicts poor prognosis of osteosarcoma and promotes cell proliferation, migration and invasion by targeting PSMC2

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Abstract. – OBJECTIVE: miR-630 has been reported as a tumor suppressor or tumor promoter in various types of cancer. However, the effect of miR-630 in osteosarcoma (OS) has not been investigated. The purpose of this study was to investigate the expression patterns, clinical value, and functional role of miR-630 in OS.

PATIENTS AND METHODS: miR-630 levels in 147 paired OS tissues and corresponding normal bone tissues were investigated by RT-PCR. The clinical data were interpreted by chi-square test, Kaplan-Meier analysis, univariate analysis, and multivariate analysis. The functional role of miR-630 was verified using cell experiments. The regulation of Proteasome 26S subunit ATPase 2 (PSMC2) by miR-630 was detected by Western blotting, dual luciferase reporter assays and rescue experiments.

RESULTS: We found that miR-630 expression was decreased in OS tissues and cell lines. A low level of miR-630 was associated with advanced clinical stage and distant metastasis. Clinical assay indicated that downregulation of miR-630 strongly correlated with poor prognosis and was an independent prognostic indicator for overall survival of OS patients. Functional investigation showed that miR-630 overexpression inhibited cell growth, colony formation, migration, invasion and EMT pathway, and promoted apoptosis in OS. Mechanistically, miR-630 was identified as direct targets of miR-630 and its overexpression significantly suppressed the levels of PSMC2. In addition, overexpression of PSMC2 recuperated the effects of miR-630 overexpression.

CONCLUSIONS: Our data indicated that miR-630 targets PSMC2 in OS and inhibited OS cell proliferation, which may offer a new mechanism underlying the development and progression of OS.

Key Words:

miR-630, PSMC2, Osteosarcoma, Proliferation, Metastasis, Prognosis.

Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor, which is prevalent in teenagers and young adults¹. The incidence of OS was the highest in people aged between 10 and 20 years old². The major treatment for these OS adolescents is combined limb-salvage surgery and neo-adjuvant chemotherapy, which results in that the 5-year survival for OS patients has increased to 60-70%^{3,4}. Unfortunately, the prognosis of some OS patients is extremely poor due to its metastatic disseminations, especially the lung^{5,6}. To date, the mechanisms underlying OS carcinogenesis and progression remain poorly understood. Therefore, the molecular pathogenesis of OS needs to be explored; such understanding may help explore novel biomarkers for developing target therapy. MiRNAs are a class of small non-coding RNAs of 20-22 nucleotides that silence gene expression usually by interfering with mRNA stability or protein translation⁷. Growing studies show that miRNAs can be key players in diverse physiological and pathological processes, such as embryonic development, tumorigenesis, metastasis, metabolism and apoptosis^{8,9}. Especially, accumulating evidence has shown that miRNAs can act either as oncogenes or as tumor suppressors in tumors, including OS^{10,11}. Recently, there is also increasing evidence that miRNA expression profiles may be indicative of disease risk and burden^{12,13}. Thus, dysregulation of miRNAs has great potential to be used as novel clinical biomarkers for OS patients. Although more and more miRNAs have been identified, there are a large number of miRNAs which remain to be elucidated¹⁴. MiR-630, a newly identified miR-

NA and a tumor-related regulator, has been reported to be dysregulated in several tumors and play an important role in regulating tumor cells proliferation and metastasis¹⁵⁻¹⁷. However, to our best knowledge, the expression pattern, biological function and potential mechanism of miR-630 in OS progression have not been investigated. In this study, we discovered that miR-630 expression was significantly down-regulated in OS and associated with prognosis of OS patients. Then, it was observed that miR-630 functioned as a novel suppressor in tumor proliferation, metastasis and the EMT process in OS by targeting PSMC2, which has been confirmed to be a tumor promoter in several tumors, including OS.

Patients and Methods

Patients and Tissue Samples

Surgically resected OS tissue samples and paired corresponding non-cancerous bone tissues were collected from 147 patients with primary OS at the First Affiliated Hospital of Jinzhou Medical University from February 2010 to March 2012. All participants enrolled in this study signed informed consents, which were reviewed and approved by the Medical Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University. The patients' clinical features were listed in Table II. The patients did not receive any perioperative radiotherapy or chemotherapy before surgery. All clinical samples were immediately frozen in liquid nitrogen and stored at -80°C for further experiments.

Cell Lines and Cell Culture

Human OS cell lines (Saos-2, U2OS, MG63 and HOS) were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Xuhui, Shanghai, China). The osteoblast hFOB1.19 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). RPMI-1640 medium (BasalMedia Technologies, Pudong, Shanghai, China) was used to culture the cells. Besides, 10% fetal bovine serum (FBS, Life Technologies, Carlsbad, CA, USA) as well as antibiotics (100 microg/mL streptomycin and 100 U/mL penicillin) were added into the cell culture medium and the cells were at 37°C with 5% CO₂.

Cell Transfection

Cell transfection was conducted using Lipofectamine 3000 reagent (Invitrogen Co., Carls-

bad, CA, USA) according to the manufacturer's instructions. Briefly, the cells (1×10^5 cells per well) were seeded into 12-well plates (Excell Bio, Taicang, Jiangsu, China) and maintained until the cell confluence reached 70%. Then, appropriate concentration of miRNA mimic or plasmid was mixed with Lipofectamine 3000 reagent in the Opti-MEM (Invitrogen Co., Carlsbad, CA, USA). After 24 h incubation, the medium was changed and the cells were used for further experiments. The miRNA mimics (NC mimic or miR-630 mimic) were purchased from Generay Biotech Co., Ltd. (Songjiang, Shanghai, China). The PSMC2 overexpression plasmid, pcDNA3.1-PSMC2 was constructed by Vigene Biosciences Co., Ltd. (Jinan, Shandong, China).

Reverse Transcription-Quantitative Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from cultured cells or clinical samples using Total RNA Fast Extraction Kit (BioTeke, Haidian, Beijing, China) according to the manufacturer's protocols, and the concentration was determined by a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA). The qRT-PCR assays were performed using an ABI7500 real-time PCR instrument (ABI Co., Oyster Bay, NY, USA). The mRNA detection was conducted by the use of FastKing One Step RT-PCR Master Mix Kit, which was purchased from TIANGEN Biotechnology Co., Ltd. (Haidian, Beijing, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal reference for mRNA. The miRNA was isolated using miRNA Purification Kit (Qiagen, Hilden, Germany) and detected by TransScript Green miRNA Two-Step qRT-PCR SuperMix Kit which was purchased from TRANSGEN Biotechnology Co., Ltd. (Haidian, Beijing, China). The relative expression was analyzed using $2^{-\Delta\Delta C_t}$ method. All primer sequences were listed in Table I.

Western Blot Analysis

Cells were collected and lysed using radio immunoprecipitation assay (RIPA) buffer (Beyotime, Pudong, Shanghai, China) containing protease inhibitor cocktail (TRANSGEN Biotech, Haidian, Beijing, China). A Bicinchoninic Acid (BCA) Protein Assay Kit (BioTeke, Haidian, Beijing, China) were then utilized to assess the protein concentration. Subsequently, equal amount of proteins was loaded onto a 10% SDS-polyacrylamide gel and transferred onto polyvinylidene

difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) using a BioRad Bis-Tris Gel system (Bio-Rad, Richmond, CA, USA) at 300 mA. After the membranes were blocked by 5% non-fat milk, they were incubated overnight with the primary antibodies against vimentin, N-Cadherin, PSMC2 and GAPDH, at 4°C, and subsequent incubation with secondary antibodies at room temperature for 1 h. Chemiluminescence Western blotting reagents (Cell Signaling Technology, Danvers, MA, USA) and ECL System (GE Healthcare, Madison, WI, USA) were applied to observe the protein bands. The optical density of the protein bands was assessed by ImageJ software (version 1.8.0; NIH, Bethesda, MD, USA).

Cell Viability Assays

Cells at a density of 3000 cells per well were seeded into 96-well plates (Excell Bio, Taicang, Jiangsu, China). At indicated time points at 37°C, Cell Count Kit-8 (CCK-8) assays were conducted by adding 10 µl CCK-8 reagent (Solarbio, Tongzhou, Beijing, China) into each well. After culturing for additional 1-2 h, the absorbance at a wavelength of 450 nm was determined by a microplate reader (BioTek Instruments, Bio-Tek, Winooski, VT, USA).

Colony Formation Assay

MG63 or U2OS cells (500 cells per well) transfected with indicated miRNA mimics or plasmids were seeded in 6-well plates at 37°C with 5% CO₂. After culturing for two weeks, the cell colonies were fixed with ethanol and stained with 0.1% crystal violet (Sigma Aldrich, Pudong, Shanghai, China). Colonies with over 50 cells were recorded using an inverted microscope (Carl Zeiss, Oberkochen, Germany).

Apoptosis Assay

Cell apoptosis were evaluated by flow cytometry. In short, the collected MG63 or U2OS cells were re-suspended in 400 µl binding buffer

and subsequently incubated with 5 µl Annexin V-FITC, and 5 µl propidium iodide (PI) using Annexin V-FITC Apoptosis Detection Kit which was purchased from KeyGEN Biotechnology Co., Ltd. (Nanjing, Jiangsu, China). FACS ArialII system (BD Biosciences, San Jose, CA, USA) was then employed to analyze the cell apoptosis.

Wound Healing Assay

The cell migration was assessed by wound healing assays. Briefly, cell suspensions (70 µl, 2×10⁵ cells/ml) were seeded into each chamber of the culture insert in a 35 mm high culture µ-dish (Ibidi, Martinsried, Germany). After the cell attachment for 24 h, the culture inserts were gently removed using sterile tweezers. Then, the photographs of wounded areas were taken by an inverted microscope (Carl Zeiss, Oberkochen, Germany) at 0 h and 24 h.

Transwell Invasion Assay

To measure cell invasion ability, the transwell chambers (8 µm pore size; BD Biosciences, Franklin Lakes, NJ, USA) were firstly pre-coated with 50 µl of Matrigel (200 mg/ml; BD Biosciences, Franklin Lakes, NJ, USA). Then, cells were collected by trypsin digestion and re-suspended in serum-free medium. Afterwards, the cell suspensions (200 µl; 2.5 × 10⁵ cells per ml) were added into the top side of the chambers, while the lower chamber was supplemented with 500 µl medium containing 10% FBS. After culturing for 24 h, the cells on the bottom surface of the membrane were fixed with 4% formaldehyde (Meilun Biotechnology, Dalian, Liaoning, China), stained with 0.5% crystal violet (Sangong, Pudong, Shanghai, China) and counted using an inverted microscope (Carl Zeiss, Oberkochen, Germany).

Dual Luciferase Reporter Assays

Sequences corresponding to the 3'-untranslated region (UTR) of PSMC2 mRNA and containing the wild-type (PSMC2 WT) or mutat-

Table 1. Primer sets used in the present study.

Gene names	Primer sequences (5'-3')
miR-630: Forward	GTCAGCGCAGTATTCTGTAC
miR-630: Reverse	GTGCAGGGTCCGAGGT
PSMC2: Forward	GAGCACTTACTCTAGGCAGATCA
PSMC2: Reverse	GTACACCTGGCAACCTGTAAAG
GAPDH: Forward	ACAACCTTGGTATCGTGGAAGG
GAPDH: Reverse	GCCATCACGCCACAGTTTC

Table II. Correlation between miR-630 expression and different clinicopathological features in 147 osteosarcoma patients.

Clinicopathological features	Number of cases	miR-630 expression		<i>p</i> -value
		Low	High	
Age				NS
< 20 years	71	31	40	
≥ 20 years	76	41	35	
Gender				NS
Female	53	21	32	
Male	94	51	43	
Tumor size				NS
< 8 cm	96	42	54	
≥ 8 cm	51	30	21	
Anatomic location				NS
Tibia/femur	78	37	41	
Elsewhere	69	35	34	
Clinical stage				0.010
I/A	93	38	55	
I/B/III	54	34	20	
Distant metastasis				0.006
Absent	94	42	52	
Present	43	30	13	

Table III. Univariate and multivariate analysis of clinicopathological factors for overall survival in 147 patients with CRC.

Risk factors	Univariate analysis			Multivariate analysis		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Age	1.542	0.569-2.311	0.156	-	-	-
Gender	1.781	0.844-2.432	0.177	-	-	-
Tumor size	1.478	0.659-2.784	0.114	-	-	-
Anatomic location	1.548	0.894-2.327	0.138	-	-	-
Clinical stage	3.427	1.569-4.982	0.013	2.785	1.236-3.416	0.025
Distant metastasis	3.895	1.674-5.655	0.004	3.237	1.238-4.678	0.009
miR-630 expression	4.216	1.477-6.556	0.001	3.679	1.215-5.235	0.003

ed (PSMC2 MUT) miR-630 binding site were synthesized by Generay Biotechnology Co., Ltd. (Songjiang, Shanghai, China). The sequences were sub-cloned into pGL3 Basic vector (Promega, Madison, WI, USA) by IGE Biotechnology Co., Ltd. (Guangzhou, Guangdong, China). After MG63 and U2OS cells were seeded into 24-well plates for 48 h, NC mimics or miR-630 mimics were co-transfected with 10 µg PSMC2 WT plasmids or PSMC2 MUT plasmids using Lipofectamine 3000 reagent (Invitrogen Co., Carlsbad, CA, USA) according to the manufacturer's instructions. Dual luciferase reporter assays were then carried out using Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA) according to manufacturer's protocols.

Statistical Analysis

All the values in this study were shown as mean ± the standard error of the mean (SEM). Kaplan-Meier survival and log-rank test were used for survival analysis and comparison of differences in overall survival. The variables were used in multivariate analysis on the basis of the Cox proportional hazards model. Statistical analyses were performed with the SPSS 20.0 statistics software (SPSS, Inc., Chicago, IL, USA). The differences were evaluated by one-way ANOVA or Student's *t*-test. Tukey's post hoc test was used to validate the ANOVA for comparing measurement data between groups. The difference between the groups was considered significant and very significant when *p* < 0.05.

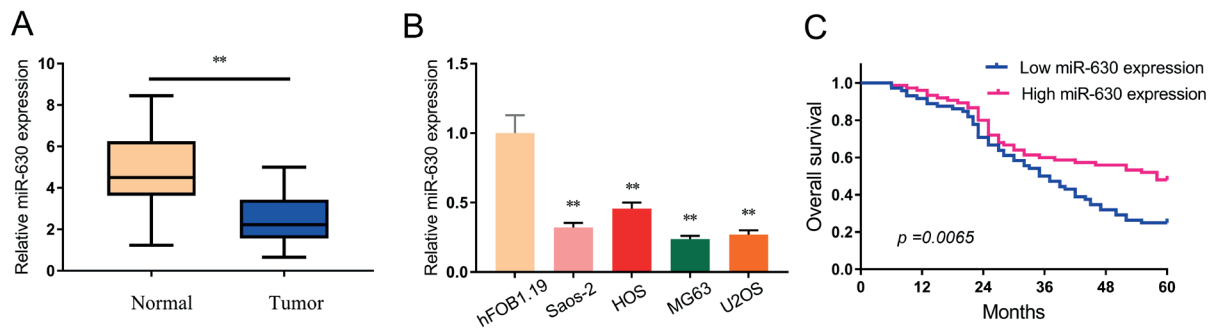


Figure 1. Expression levels of miR-630 in OS tissues and cell lines, and its prognostic significance. **(A)** Difference in expression levels of miR-630 between OS tissues and matched non-tumor bone tissues. The expression of miR-630 was normalized to GAPDH. **(B)** The expression levels of miR-630 were measured in four OS cell lines and normal osteoblast hFOB1.19. **(C)** Kaplan-Meier curve showed that OS patients with low miR-630 expression had a poor overall survival compared to patient with high miR-630 expression. * $p < 0.05$, ** $p < 0.01$.

Results

MiR-630 Expression was Downregulated in OS Patients and Associated with Poor Prognosis

In order to explore the possible role of miR-630 in OS, we firstly detected its expression in OS patients. As shown in Figure 1A, we found that the expression levels of miR-630 was significantly down-regulated in OS tissues compared to matched normal bone tissues ($p < 0.01$). Then, we also found that miR-630 expression in four OS cell lines (Saos-2, U2OS, MG63 and HOS) was reduced compared to a normal human osteoblast cell line (hFOB1.19) (Figure 1B). Our results indicated that miR-630 may play an important role in progression of OS.

Then, in order to further explore the clinical significance of miR-630 in OS patients, OS samples were classified into low miR-630 expression group ($n = 72$) and the high miR-630 expression group ($n = 75$) according to the median miR-630 expression level of all OS samples. As shown in Table II, we found that low miR-630 expression was significantly associated with clinical stage ($p = 0.010$) and distant metastasis ($p = 0.006$). However, there were no significant correlations between miR-630 expression and other clinicopathological factors of patients. Moreover, Kaplan-Meier method and log-rank test were used to evaluate differences of overall survival between low expression group and high-expression group. As shown in Figure 1C, a significant difference was found that OS patients with low miR-630 expression level had distinctly shorter overall survival than patients with high miR-630 expression level ($p = 0.065$). Of note, mul-

tivariate analysis revealed that miR-630 expression level (HR = 3.679, 95% CI: 1.215-5.235, $p = 0.003$) was independently associated with the overall survival (Table III). Taken together, our findings suggested miR-630 as a potential biomarker for OS patients.

Ectopic Expression of miR-630 Inhibited the Proliferation of OS Cells and Promoted Cell Apoptosis

Considering that miR-630 was downregulated in OS tissues and closely associated with favorable prognosis, we next aimed to investigate the biological roles of miR-630 in OS. To achieve that, miR-630 mimics were transfected into OS cell lines, MG63 and U2OS, to enhance the expression of miR-630. The results of qRT-PCR analysis demonstrated that miR-630 was effectively improved in MG63 and U2OS cells after they were transfected with miR-630 mimics (Figure 2A). Thereafter, CCK-8 assays were performed to evaluate the cell proliferation, and the data indicated that miR-630 overexpression notably inhibited the proliferative rates of MG63 and U2OS cells (Figure 2B and C). Similarly, colony formation assays also confirmed the inhibitory effect of miR-630 on MG63 and U2OS cells (Figure 2D and E). We next investigated the effects of miR-630 on the apoptosis of OS cells by the use of flow cytometry analysis. It was demonstrated that the apoptotic rates were significantly lower in miR-630 overexpression MG63 and U2OS cells than in the control cells (Figure 2F and G). Collectively, these data confirmed that overexpression of miR-630 could significantly inhibit the proliferation of OS cells and accelerate the cell apoptosis.

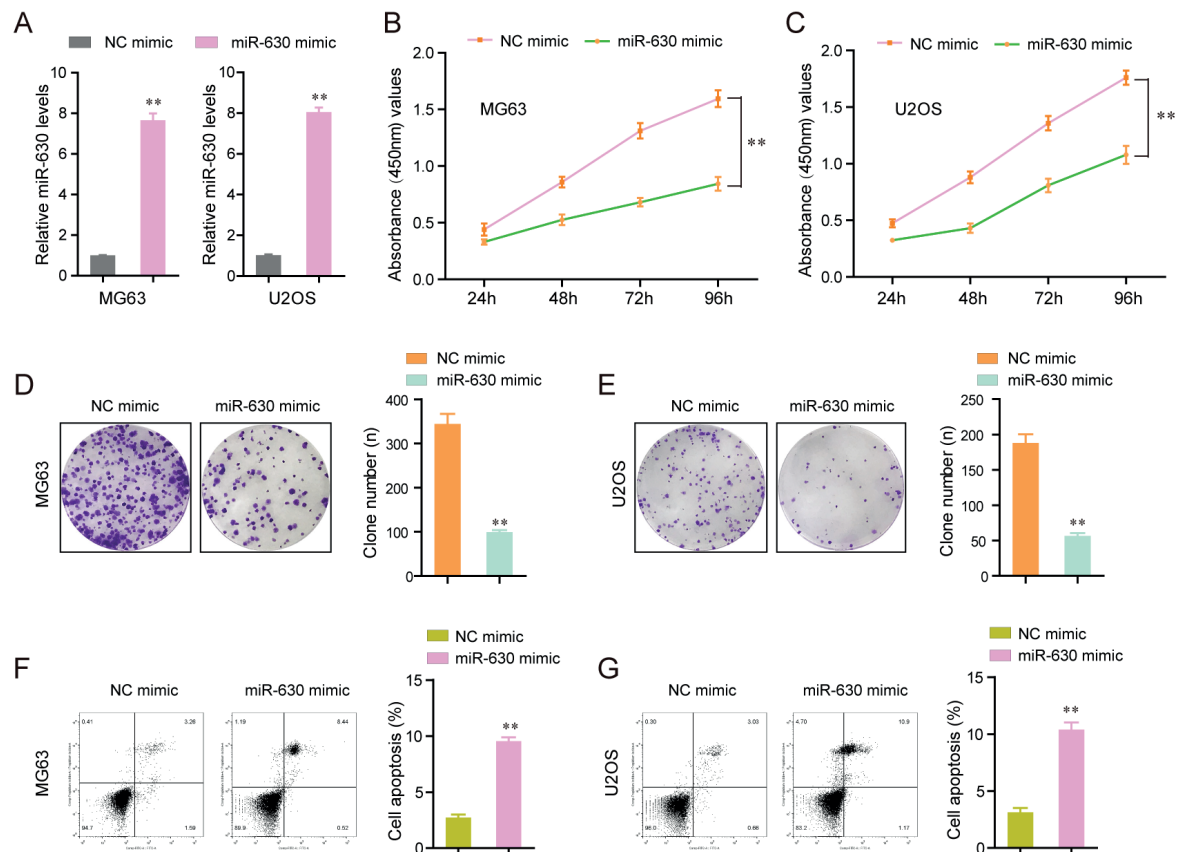


Figure 2. Ectopic expression of miR-630 inhibited OS cell proliferation and invasion. **(A)** Relative expression levels of miR-630 in MG63 and U2OS cells transfected with negative control miRNA mimic (NC mimic) or miR-630 mimic. **(B and C)** The proliferation rates of MG63 and U2OS cells assessed by CCK-8 assays. **(D and E)** Representative images of the colony formation assays and statistical analysis of MG63 and U2OS cell colony number. **(F)** Flow cytometry analysis detected the apoptotic rates of MG63 and U2OS cells transfected with NC mimic or miR-630 mimic. * $p < 0.05$, ** $p < 0.01$.

Overexpression of miR-630 Impaired the Migration and Invasion of OS Cells

To evaluate whether miR-630 contributed to the progression of OS, we next examined the effects of miR-630 on the migratory and invasive behaviors of MG63 and U2OS cells using wound healing and transwell invasion assays. The results of wound healing assays revealed that the up-regulation of miR-630 remarkably attenuated the migratory capacities of MG63 and U2OS cells (Figure 3A and B). In addition, transwell invasion assays clearly showed that transfection of miR-630 mimics dramatically impeded the invasive abilities of MG63 and U2OS cells (Figure 3C and D). Besides, the expressing levels of epithelial to mesenchymal (EMT) markers such as N-cadherin and vimentin in MG63 and U2OS cells were further examined by Western blot assays. According to the data, enhancing expression of miR-630 resulted in a marked decline of the protein levels

of N-cadherin and vimentin in MG63 and U2OS cells (Figure 3E and F). Overall, our data suggested that overexpression of miR-630 suppressed the migration and invasion of OS cells.

PSMC2 was a Novel Direct Target of miR-630 in OS Cells

To further ascertain the molecular mechanisms by which miR-630 exerted its tumor-suppressing roles in OS, we next employed bioinformatics analysis to predict the potential target of miR-630 using "miRDB" (<http://www.mirdb.org/>). The results indicated that a putative binding site for miR-630 was identified in the 3'-UTR of PSMC2 (Figure 4A). To certify whether the 3'-UTR of PSMC2 could be directly targeted by miR-630, we next performed dual luciferase reporter assays. The data revealed that when co-transfected with PSMC2 3'-UTR wild-type (PSMC2-WT) plasmid, miR-630 resulted in a remarkable de-

crease in the luciferase activities of MG63 and U2OS cells, whereas the luciferase activities were unaffected when the cells were co-transfected with PSMC2 3'-UTR mutant (PSMC2-MUT) plasmid as well as miR-630 mimics (Figure 4B). Furthermore, the qRT-PCR assays suggested that the expression levels of PSMC2 were dramatically decreased in MG63 and U2OS cells transfected miR-630 mimics (Figure 4C). Analogously, ectopic expression of miR-630 significantly reduced the protein levels of PSMC2 in MG63 and U2OS cells (Figure 4D). Taken together, our data demonstrated that PSMC2 was directly interacted with miR-630 in OS cells.

Alteration of PSMC2 Expression Abrogated the Inhibitory Effects of miR-630 on OS Cell Proliferation and Invasion

Given the low expression levels of miR-630 in OS cells, rescue experiments were carried out by

co-transfecting the miR-630 mimics with or without pcDNA3.1-PSMC2 plasmid followed by determination of the cell proliferation and invasion (Figure 5A). CCK-8 assays validated that transfection of miR-630 mimics alone notably inhibited cell growth, while reintroduction of pcDNA3.1-PSMC2 plasmid could effectively reverse the inhibitory effects of miR-630 on the proliferation of MG63 and U2OS cells (Figure 5B). Similarly, cell colony formation assays revealed that, restored PSMC2 expression markedly rescued the suppressive effects of miR-630 on the colony formation capabilities of MG63 and U2OS cells (Figure 5C and D). In addition, transwell assays suggested that the invasive cells were significantly decreased after transfecting miR-630 mimic, while co-transfection of pcDNA3.1-PSMC2 plasmid and miR-630 remarkably elevated the cell number of invaded MG63 and U2OS cells (Figure 5E). In summary, these results supported that PSMC2 was a downstream functional effector of miR-630 in OS cells.

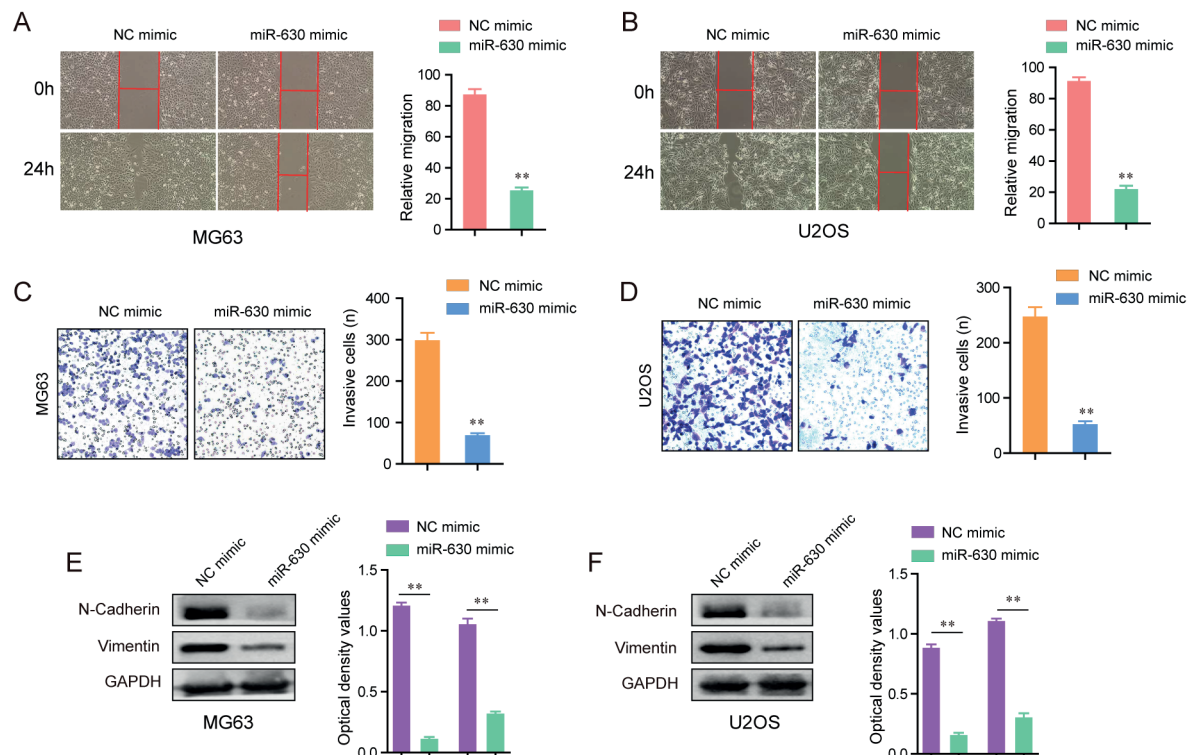


Figure 3. The effects of miR-630 on the migration and invasion of MG63 and U2OS cells. (*A* and *B*) The wound healing assays showed that transfection of miR-630 mimic reduced the migratory abilities of MG63 and U2OS cells. (*C* and *D*) The invasive cell numbers of MG63 and U2OS cells transfected with miR-630 mimic were significantly decreased using transwell invasion assays. (*E* and *F*) N-cadherin and vimentin in MG63 and U2OS were evaluated by Western blot assays. * $p < 0.05$, ** $p < 0.01$.

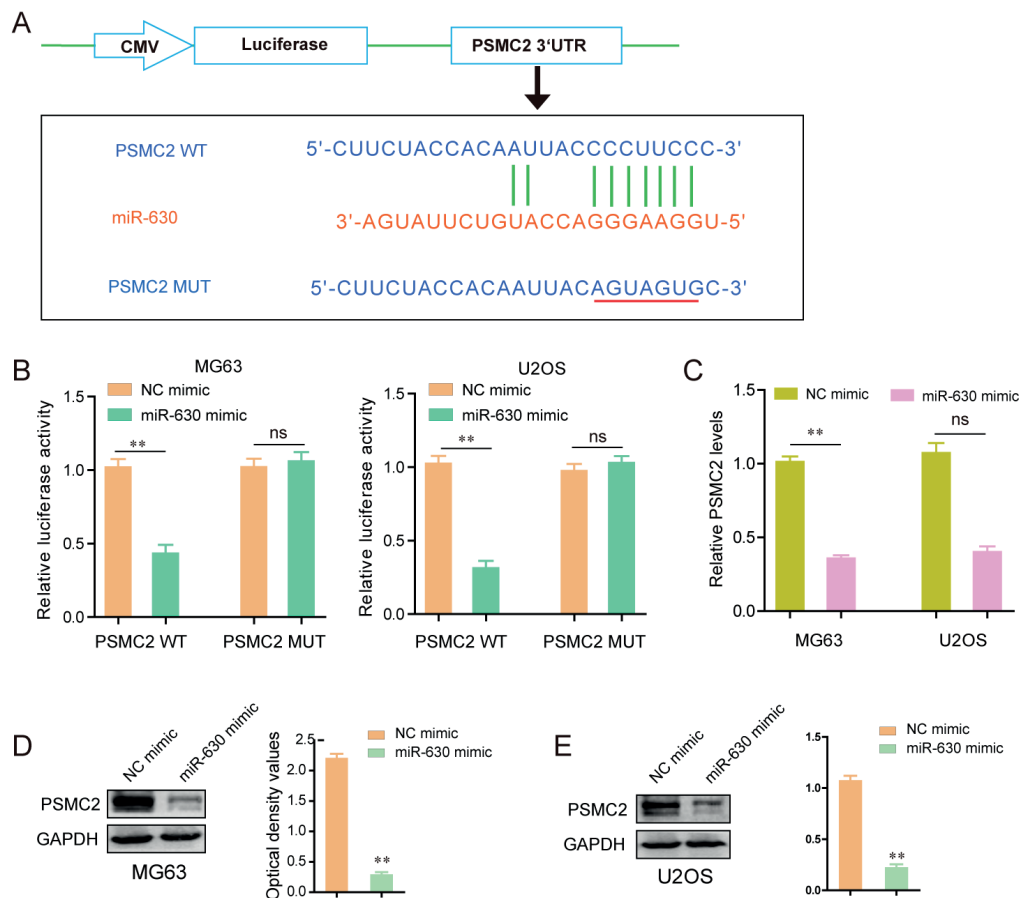


Figure 4. miR-630 directly targeted PSMC2. **(A)** Bioinformatics tool “miRBD” predicted that PSMC2 mRNA 3'-UTR harbored the miR-630 binding site. **(B)** The luciferase activities of MG63 and U2OS cells were assessed by dual-luciferase reporter assays. **(C)** qRT-PCR assays were applied to evaluate the effects of miR-630 on PSMC2 mRNA expression. **(D and E)** Western blot assays were applied to evaluate the effects of miR-630 on PSMC2 protein expression. * $p < 0.05$, ** $p < 0.01$.

Discussion

OS remains a great challenge for clinical treatments. The great majority of OS patients are represented by high-grade tumors with advanced phenotypes at the time of diagnosis^{18,19}. In clinical practice, evaluation of prognosis of OS patients is very important for decision of treatment methods²⁰. Although several clinical parameters and some genes biomarkers have been used to predict the prognosis of OS patients, these systems may not be sufficient to estimate patient prognosis^{21,22}. Recently, some functional miRNAs become ideal candidates for this problem. In this study, our attention focused on miR-630. By RT-PCR, it was observed that miR-630 expression was significantly down-regulated in both OS tissues and cell lines. Furthermore, by analyzing clinical data, we found that low miR-630 expression was asso-

ciated with advanced clinical stage and positive distant metastasis, suggesting that it might be involved in the carcinogenesis of OS. Moreover, according to multivariate analysis, miR-630 was an independent prognostic marker for OS. These findings indicated that miR-630 plays a key role in OS progression and may be used as a novel biomarker for OS prognosis. MiR-630 has been reported as a potential tumor suppressor, which was identified as one of the miRNAs downregulated in renal cell carcinoma²³, nasopharyngeal carcinoma²⁴, colorectal cancer²⁵ and lung cancer²⁶. In addition, the oncogenic role of miR-630 was also reported in several tumors. For instance, overexpression of miR-630 inhibited lung cancer proliferation by targeting CDC7²⁷. In breast cancer, it was reported that miR-630 functioned as a tumor suppressor by directly targeting BMI1²⁸. However, Zhang et al²⁹ reported that miR-630

was highly expressed in epithelial ovarian cancer and its overexpression promoted epithelial ovarian cancer cell proliferation and migration by targeting KLF6. These results revealed that miR-630 may play a different role according to the cell situation. In this work, we investigated the effects of miR-630 on cell proliferation, metastasis and invasion apoptosis. It was observed that overexpression of miR-630 significantly suppressed tumor growth, migration and invasion, and promoted apoptosis in OS. In addition, the results of Western blot indicated that forced miR-630 expression may suppress EMT path-

way. Taken together, our study, for the first time, showed that miR-630 acted as a tumor suppressor in progression of OS. Proteasome 26S subunit ATPase 2 (PSMC2), located in 7q22.1-q22.3 in the genome, is a pivotal member of the 19S regulatory subunit of the 26S proteasome³⁰. Recently, more and more evidences show that PSMC2 acted as functional gene in regulating tumor progression^{31,32}. Of note, recently Song et al³³ firstly reported that PSMC2 was highly expressed in OS and its knockdown could suppress OS cell proliferation and migration, and promoted apoptosis. However, the potential mechanism underling OS

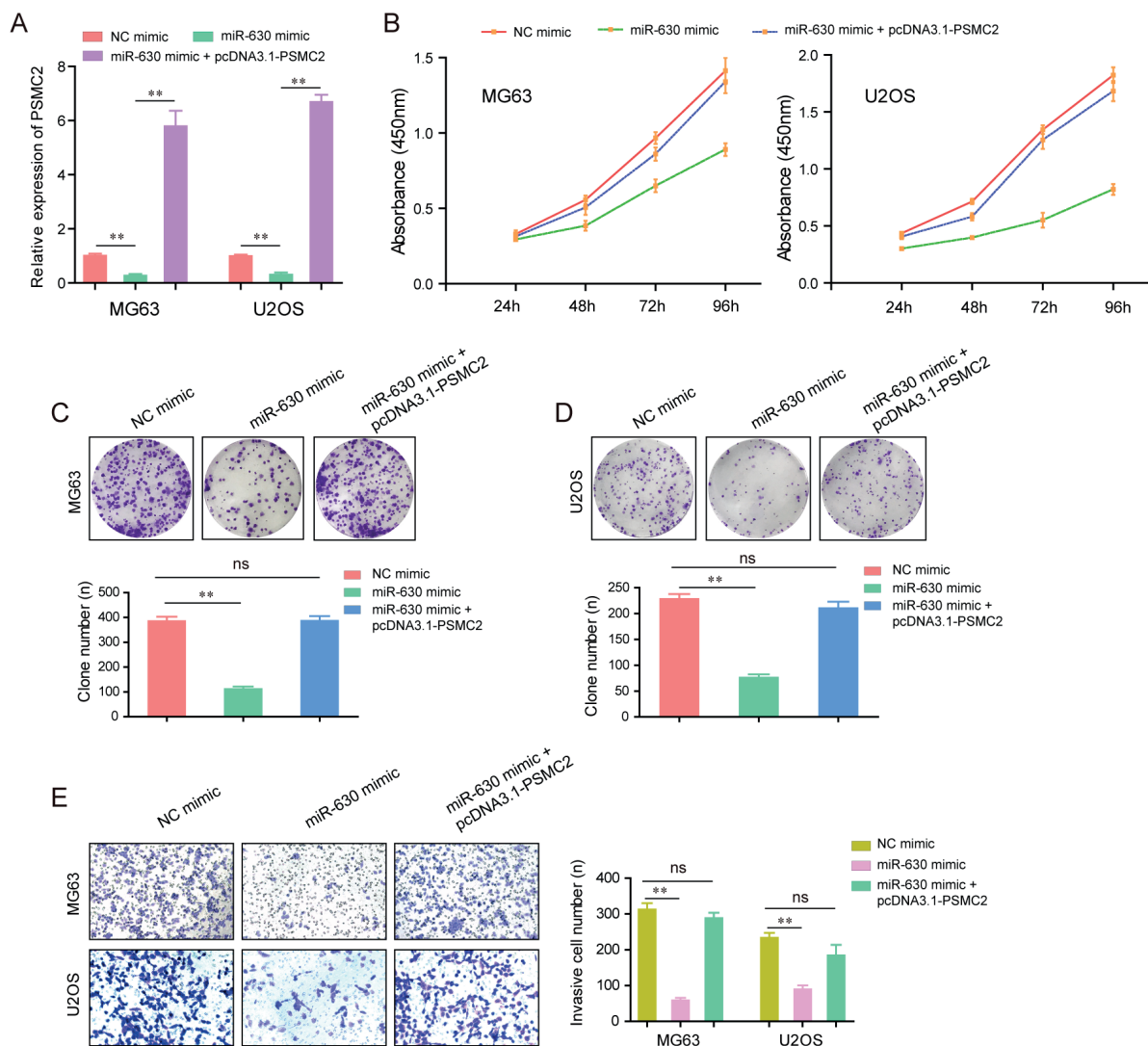


Figure 5. Overexpression of PSMC2 abrogated the tumor suppressive roles of miR-630. (A) Relative mRNA expression levels of PSMC2 in MG63 and U2OS cells transfected with NC mimic, miR-630 mimic or co-transfected with miR-630 mimic and pcDNA3.1-PSMC2 plasmid. (B) The proliferative rates of MG63 and U2OS cells determined by CCK-8 assays. (C and D) Cell colony formation assays evaluated the alteration of clonogenic abilities of MG63 and U2OS cells. (E) Representative images of the transwell invasion assays and statistical analysis of MG63 and U2OS cell invasive number in different groups. * $p < 0.05$, ** $p < 0.01$.

progression remains largely unclear. In this report, by bioinformatics analysis, we found that we identified PSMC2 as a possible target of miR-630 among the regulated genes. Further, we used series of experiments to confirm whether PSMC2 was a direct negative target gene of miR-630 in OS. By a dual-luciferase reporter assay, miR-630 was demonstrated to bind directly to the 3'-UTR of PSMC2. Furthermore, we also found that overexpression of miR-630 significantly suppress the levels of PSMC2. In addition, we observed that PSMC2 re-expression attenuates miR-630 mediated inhibition of cell proliferation, colony formation, migration and invasion in OS cells. Taken together, our findings suggested that miR-630 may suppress OS progression by targeting PSMC2. On the other hand, other target molecules might help miR-630 to inhibit OS cell migration and PSMC2 expression.

Conclusions

To our knowledge, we presented the first evidence that miR-630 expression was reduced in OS cell lines and its downregulation related to poor prognosis of OS patients. Functional assay indicated that miR-630 suppresses OS growth and metastasis, at least partially through targeting PSMC2. These findings provided novel insights into understanding the molecular pathogenesis of OS and suggests.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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