MicroRNA-208a-3p promotes osteosarcoma progression via targeting PTEN

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Abstract. Osteosarcoma (OS) is a malignant bone tumor with a poor prognosis. Accumulated evidence has suggested that microRNAs (miRNAs/miRs) may function as either oncogenes or tumor suppressors, which are associated with tumorigenesis and the progression of different types of cancer. In the present study, the role of miR-208a-3p in OS was investigated. The expression levels of miR-208a-3p in OS tissues and cell lines were determined via reverse transcription-quantitative PCR (RT-qPCR). MTT and colony formation assays were performed to verify the proliferation rate of OS cells. In addition, the effects of miR-208a-3p on the migration and invasion of OS cells were revealed using wound-healing and Transwell assays, respectively. Furthermore, the association between miR-208a-3p and phosphatase and tensin homolog (PTEN) 3'-untranslated region was determined via luciferase reporter assays, western blot and RT-qPCR analysis. The results indicated that miR-208a-3p was upregulated in OS tissues and cell lines compared with adjacent normal tissues and human osteoblastic cells, respectively. miR-208a-3p overexpression promoted and miR-208a-3p knockdown inhibited OS cells proliferation and metastatic potential. Additionally, PTEN was validated as a direct target of miR-208a-3p and its expression was negatively associate with that of miR-208a-3p in OS cells. Taken together, these results may suggest that miR-208a-3p promoted OS cells proliferation and metastatic potential via targeting PTEN. Therefore, miR-208a-3p may be considered as a diagnostic biomarker for OS.

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Introduction

Osteosarcoma (OS) is the most common malignant tumor in orthopedics and has been indicated to occur predominantly in adolescents between 1-20 years of age (1,2). OS is characterized by high malignancy, invasion and early lung metastasis (3,4). Therefore, the prognosis of patients with OS remains poor, and the 5-year survival rate following amputation has been reported to be only 5-10% (5,6). At present, the treatment of OS primarily includes amputation, radiotherapy and chemotherapy, which have been indicated to improve the survival rate of patients with OS (7,8). Although considerable progress has been achieved in the treatment and prognosis of OS, novel diagnostic biomarkers and treatments are required.

MicroRNAs (miRNAs/miRs) are a class of small endogenous non-coding RNA molecules with 15-25 nucleotides in length (9,10). The first miRNA has been discovered in nematodes (11). miRNAs bind to the 3'-untranslated region (3'-UTR) of target mRNAs, and subsequently inhibit or induce the degradation of mRNAs to achieve their biological function (12,13). Accumulating evidence has demonstrated that miRNAs may act as tumor suppressors or oncogenes, thus serving an important role in the occurrence and development of various types of cancers (14-16). miR-384 has been reported to inhibit the proliferation and invasion of OS via regulating insulin-like growth factor binding protein 3 (17). Moreover, Liu *et al* (18) reported that miR-200a induced immunosuppression via activating phosphatase and tensin homolog (PTEN).

miR-208a has been indicated to be associated with the development of numerous types of cancer. Cui *et al* (19) reported that compared with miR-negative control (NC), miR-208a enhanced cell proliferation and invasion in gastric cancer via targeting secreted frizzled-related protein 1 (SFRP1) and negatively regulating maternally expressed gene 3. Moreover, Tang *et al* (20) revealed that the level of miR-208a increased in the serum of lung cancer patients following radiotherapy, and indicated that miR-208a increased the proliferation and radioresistance of human lung cancer cells via targeting p21. In another study, Zou *et al* (21) indicated that circRAD18 interacted with miR-208a and miR-3164 to promote triple-negative

breast cancer progression via regulating the expression of insulin-like growth factor 1 and fibroblast growth factor 2. However, to the best of our knowledge, the effect of miR-208a-3p in OS has not been investigated. Therefore, the present study aimed to investigate whether miR-208a-3p may affect the progression of OS.

Materials and methods

Clinical samples. OS tissue specimens (n=10) and adjacent normal tissues were obtained from patients with OS, including 4 females (aged between 19 and 23 years), 5 males (aged between 18 and 25 years) and a child (aged 12 years), who did not undergo chemotherapy or radiotherapy prior to operation at The First Affiliated Hospital of Harbin Medical University, Harbin, China, between September 2017 and December 2018. All tissues were kept in liquid nitrogen immediately after resection operation. All patients or their legal guardians provided written informed consent and the study was approved by the Medical Ethics Committee of The First Affiliated Hospital of Harbin Medical University.

Cell culture and transfection. The human OS cell lines SaOS-2, U2OS and MG-63 were purchased from Shanghai Zhongqiao Xinzhou Biotechnology Co., Ltd. Cells were maintained in DMEM (Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Biological Industries) and 1% penicillin/streptomycin (Sigma-Aldrich; Merck KGaA) at 37˚C in a humidified atmosphere with 5% CO_2. The human osteoblastic cell line (hFOB 1.19) was purchased from Shanghai Yubo Biotechnology Co., Ltd. The cells were maintained in DMEM medium, supplemented with 10% FBS, 1% penicillin/streptomycin and 0.3 mg/ml neomycin G418 (Stemcell Technologies, Inc.) at 34˚C in a humidified atmosphere with 5% CO_2. 293T cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37˚C in a humidified atmosphere with 5% CO_2. miR-208a-3p mimics, NC mimics and miR-208a-3p and NC inhibitors were synthesized by Suzhou GenePharma Co., Ltd. The sequences used were as follows: miR-208a-3p mimics, 5'-AUAAGACGAGCAAAAAGCUUGU-3' and 5'-AGCUUUUUGCUCGUCUUAUUU-3'; miR-208a-3p inhibitor, 5'-ACAAGCUUUUUGCUCGUCUUAU-3'; NC mimics, 5'-UUCUCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGUUCGGAGAATT-3' and NC inhibitor, 5'-CAGUACUUUUGUGUAGUACAA-3'. When SaOS-2, U2OS and MG-63 cells reached 70-80% confluence, they were transfected with miR-208a-3p or mimics NC (50 nM each) and miR-208a-3p or inhibitor NC (100 nM each) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. After 24 h, the transfected cells were used for subsequent experimentations.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA from OS tissues, adjacent normal tissues and cell lines (SaOS-2, U2OS and MG-63 cells) was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Subsequently, complementary DNA (cDNA) was synthesized using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The thermocycling conditions were as follows: 42˚C for 15 min, 95˚C for 5 min and 4˚C for 10 min. PCR amplification was performed on an ABI 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR Green Master kit (Roche Diagnostics). Relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (22), U6 and GAPDH served as an internal reference. The sequences of the primers used in the present study were as follows: miR-208a-3p sense, 5'-CGGGGCATAAGACGAGCAAAAA-3' and antisense, 5'-ATCCAGTGCAGGGTCCGAGG-3'; PTEN sense, 5'-CCAGAGACAAAAAGGGAGTAACTA-3' and antisense, 5'-ACCTTTAGCTGGCAGACC-3'; U6 sense, 5'-CTCGCTTCGGCAGCACA-3' and antisense, 5'-AACGCTTCACGAATTTGCGT-3'; and GAPDH sense, 5'-CATCACTGCCACCCAGAAGAC-3' and antisense, 5'-CCAGTGAGCTTCCCGTTCAG-3'. The following thermocycling conditions were used for the qPCR: 95˚C for 30 sec, followed by 40 cycles of amplification at 95˚C for 5 sec, 59˚C for 30 sec and 72˚C for 30 sec.

Cell proliferation assay. Cell proliferation was assessed using an MTT assay. SaOS-2, U2OS and MG-63 cells transfected with miR-208a-3p mimics, inhibitor or respective NC were seeded into 96-well plates at a density of $5x10^3$ cells/well. Following cell culture for 12, 24, 36 and 48 h, the medium was removed and 100 μl MTT solution (500 μg/ml; Biosharp Life Sciences) was added into each well. Subsequently, the 96-well plates were incubated for 4 h at 37˚C, and the purple formazan was dissolved in 150 μl DMSO. The absorbance of each well was measured at a wavelength of 490 nm using a microplate reader (Tecan Group, Ltd.). The optical density value of each well indicated the OS cells proliferation rate.

Colony formation assay. SaOS-2, U2OS and MG-63 cells transfected with miR-208a-3p and NC mimics and inhibitors were seeded into 6-well plates at a concentration of $2x10^3$ cells/well. The cells were cultured at 37˚C for 2 weeks and the medium was replaced every three days. The formed colonies were subsequently fixed with 4% polyformaldehyde for 20 min at room temperature and stained with 0.5% crystal violet (Sigma-Aldrich; Merck KGaA) for 10 min at room temperature. Images of the newly formed colonies were captured using a light microscope (magnification, x1). The experiments were repeated at least three times.

Wound healing assay. SaOS-2, U2OS and MG-63 cells were cultured in 6-well plates ($5x10^5$ cells/well). Once the cells grew to ~90% confluence, wounds were created by scraping the cellular monolayer with a 200-μl pipette tip. The cells were subsequently washed with PBS to remove the suspended cells, and the culture medium was replaced with serum-free DMEM medium. Images were captured at 0, 12, 24, 36 and 48 h following wound formation using an optical microscope (magnification, x40) (Eclipse TS100; Nikon Corporation). The wound widths were analyzed using ImageJ v1.8.0 software (National Institutes of Health).

Invasion assay. The invasion assay was performed in a Transwell chamber coated with Matrigel (pore size, 8 μm;

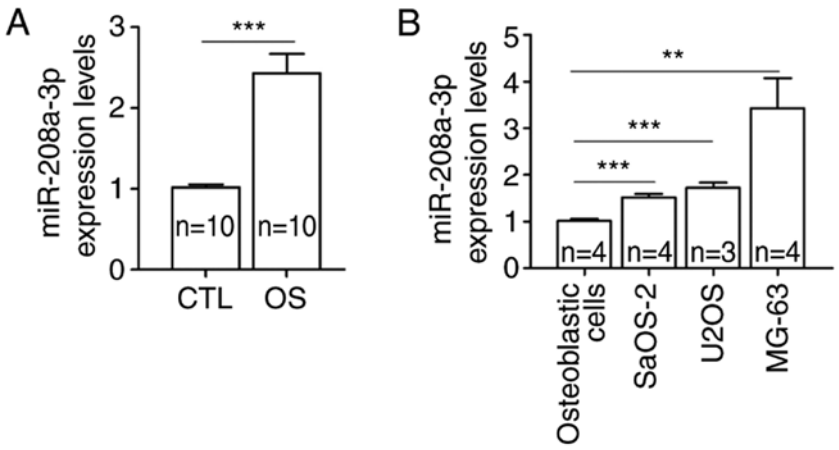

Figure 1. Expression of miR-208a-3p in OS tissues and cell lines. (A) RT-qPCR analysis of miR-208a-3p expression levels in 10 pairs of OS tissues and adjacent normal tissues. (B) RT-qPCR was performed to detect the expression levels of miR-208a-3p in osteoblast cells (hFOB 1.19) and OS cell lines SaOS-2, U2OS and MG-63. **P<0.01 and ***P<0.001. RT-qPCR, reverse transcription-quantitative PCR; miR, microRNA; CTL, control; OS, osteosarcoma.

BD Biosciences). Briefly, Matrigel was diluted in serum-free DMEM medium at a 1:5 ratio and added to the upper chamber of the Transwell inserts and maintained at 37˚C for 5 h. A total of 5x10^4 cells were suspended in 200 μl serum-free medium and seeded into the upper chamber of 24-well inserts, while the lower chamber was filled with 500 μl 10% FBS-containing medium. Following cell culture for 24 h, the invaded cells were stained with 0.1% crystal violet solution (Sigma-Aldrich; Merck KGaA) at room temperature for 20 min. The number of cells invaded through the pore was examined using an optical microscope (magnification, x100).

Western blot analysis. Total protein extracts were isolated from MG-63 cells using ice-cold RIPA lysis buffer (Beyotime Institute of Biotechnology) supplemented with protease inhibitors. Total protein concentration was determined using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. Equal amounts (40 μg/lane) of protein from each experimental group were denatured, separated using 10% SDS-PAGE and then transferred onto a nitrocellulose membrane (EMD Millipore). Following blocking with 5% skimmed milk for 2 h at room temperature, the membranes were incubated overnight at 4˚C with the following primary antibodies: Anti-PTEN (1:1,000; cat. no. ab32199; Abcam) and anti-GAPDH (1:1,000; cat. no. ab9482; Abcam). After rinsing, the membranes were incubated with an anti-mouse secondary immunoglobulin G (IgG; 1:5,000; cat. no. sc-516102; Santa Cruz Biotechnology, Inc) or anti-rabbit secondary IgG (1:5,000; cat. no. sc-2357; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Finally, immunoreactive bands were visualized using an Odyssey® CLx Imager and quantified with LI-COR Image Studio v5.2.5 Software (LI-COR Biosciences). GAPDH served as the internal control.

Dual-luciferase reporter assay. The 3'-UTR region of PTEN encompassing the putative miR-208a-3p binding site was amplified using PCR and was subsequently sub-cloned into the psiCHECK-2 dual luciferase vector (Promega Corporation). 293T cells at a density of 2x10^4 cells/well were cultured in 24-well plates and co-transfected with the luciferase vector encompassing the 3'-UTR of PTEN and miR-208a-3p or NC mimics using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Following 48 h, luciferase activity was determined using a Dual-Luciferase® Reporter assay kit (Promega Corporation) according to the manufacturer's instructions. Firefly luciferase activity was normalized to the corresponding *Renilla* luciferase activity.

Bioinformatic analysis. The potential targets of miR-208a-3p were predicted via TargetScan software v7.2 (http://www.targetscan.org/vert_72/) using the default settings.

Statistical analysis. All quantitative experimental data are presented as mean ± standard error of the mean. Statistical analyses were performed using GraphPad Prism v7.0 (GraphPad Software, Inc.). The statistical differences among experimental groups were evaluated using unpaired Student's t-test or one-way ANOVA followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference. Each experiment was repeated at least three times.

Results

miR-208a-3p is upregulated in OS tissues and cell lines. It has been reported that miR-208a-3p was dysregulated and promoted the development of several types of cancer, including gastric and colon cancer (23,24). The present study aimed to investigate the role of miR-208a-3p in OS. Therefore, RT-qPCR was performed to determine the expression levels of miR-208a-3p in 10 pairs of tissues from patients with OS and adjacent normal tissues. The results showed that the expression levels of miR-208a-3p were significantly higher in OS tissues compared with adjacent normal tissues (Fig. 1A). Similarly, miR-208a-3p was significantly upregulated in OS cell lines, namely SaOS-2, U2OS and MG-63, compared with human osteoblastic cells (Fig. 1B).

Overexpression of miR-208a-3p promotes the proliferation of OS cell lines. To verify the role of miR-208a-3p in

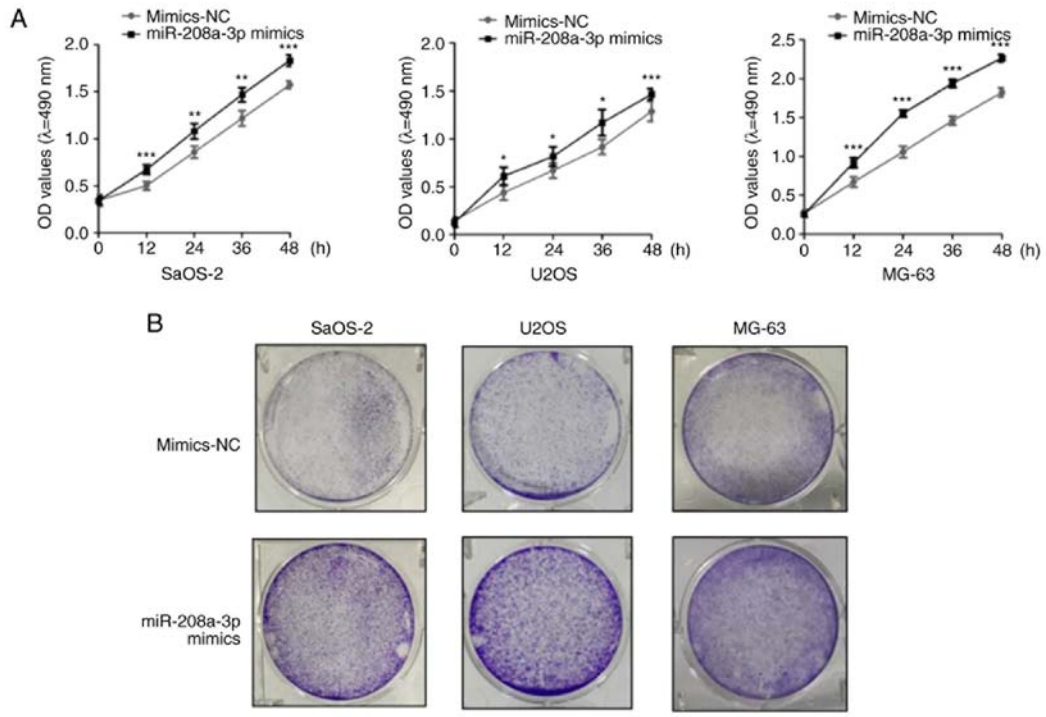

Figure 2. Overexpression of miR-208a-3p promotes proliferation in osteosarcoma cells. (A) The proliferation of SaOS-2, U2OS and MG-63 cells following overexpression of miR-208a-3p was determined via MTT assays at different time points. (B) Colony formation assays were performed to detect the proliferation of SaOS-2, U2OS and MG-63 cells following overexpression of miR-208a-3p. *P<0.05, **P<0.01 and ***P<0.001 vs. mimic NC. NC, negative control; miR, microRNA; OD, optical density.

OS cells, SaOS-2, U2OS and MG-63 cells were transfected with miR-208a-3p mimics to artificially alter the endogenous levels of miR-208a-3p (Fig. S1). Following transfection, the proliferation rate of OS cells was determined using MTT and colony formation assays. More specifically, the MTT assays demonstrated that compared with the mimics NC group, overexpression of miR-208a-3p significantly promoted the proliferation of SaOS-2, U2OS and MG-63 cells (Fig. 2A). Moreover, the results of the colony formation assays were consistent with the MTT assay findings, as miR-208a-3p overexpression was indicated to increase colony formation by SaOS-2, U2OS and MG-63 cells compared with the mimics NC group (Fig. 2B). Overall, these data suggested that overexpression of miR-208a-3p increased the proliferation rate of OS cells.

Overexpression of miR-208a-3p promotes the invasion and migration of OS cells. To determine the association between miR-208a-3p with the invasion of OS cells, Transwell assays were performed. The results revealed that the number of invading OS cells was higher in the miR-208a-3p mimics group compared with the mimics NC group (Fig. 3A). Furthermore, wound healing assays demonstrated that miR-208a-3p overexpression improved the migratory ability of SaOS-2, U2OS and MG-63 cells at 12, 24, 36 and 48 h following wound formation (Fig. 3B). These findings indicated that overexpression of miR-208a-3p positively regulated the migratory and invasive abilities of SaOS-2, U2OS and MG-63 cells.

Knockdown of miR-208a-3p inhibits the proliferation of OS cells. The aforementioned results indicated that the overexpression of miR-208a-3p promoted the proliferation, invasion and migration of OS cells; however, whether miR-208a-3p knockdown can induce an opposite phenotype in OS cells remains unclear. Therefore, additional studies were performed. OS cells were initially transfected with a miR-208a-3p inhibitor to silence miR-208a-3p expression (Fig. S1). Following miR-208a-3p knockdown, MTT assays revealed that compared with the inhibitor NC group, the proliferative ability of SaOS-2, U2OS and MG-63 cells significantly decreased in the miR-208a-3p inhibitor group (Fig. 4A). Similarly, colony formation assays demonstrated that the colony formation ability of OS cell lines in the miR-208a-3p inhibitor group was reduced compared with the inhibitor NC group (Fig. 4B). These results indicated that miR-208a-3p knockdown decreased the proliferative ability of SaOS-2, U2OS and MG-63 cells.

Knockdown of miR-208a-3p suppresses the invasion and migration of OS cells. The effect of miR-208a-3p knockdown in the migratory and invasive ability of OS cells was investigated using wound healing and Transwell assays, respectively. The results of the invasion assays indicated that miR-208a-3p knockdown decreased the number of SaOS-2, U2OS and MG-63 cells that invaded through Matrigel compared with the inhibitor NC group (Fig. 5A). This finding suggested that miR-208a-3p knockdown reduced the invasive ability of SaOS-2, U2OS and MG-63 cells. Additionally, the results of the wound healing assays indicated that miR-208a-3p knockdown inhibited the migratory ability of OS cells at 12, 24, 36 and 48 h following wound formation (Fig. 5B).

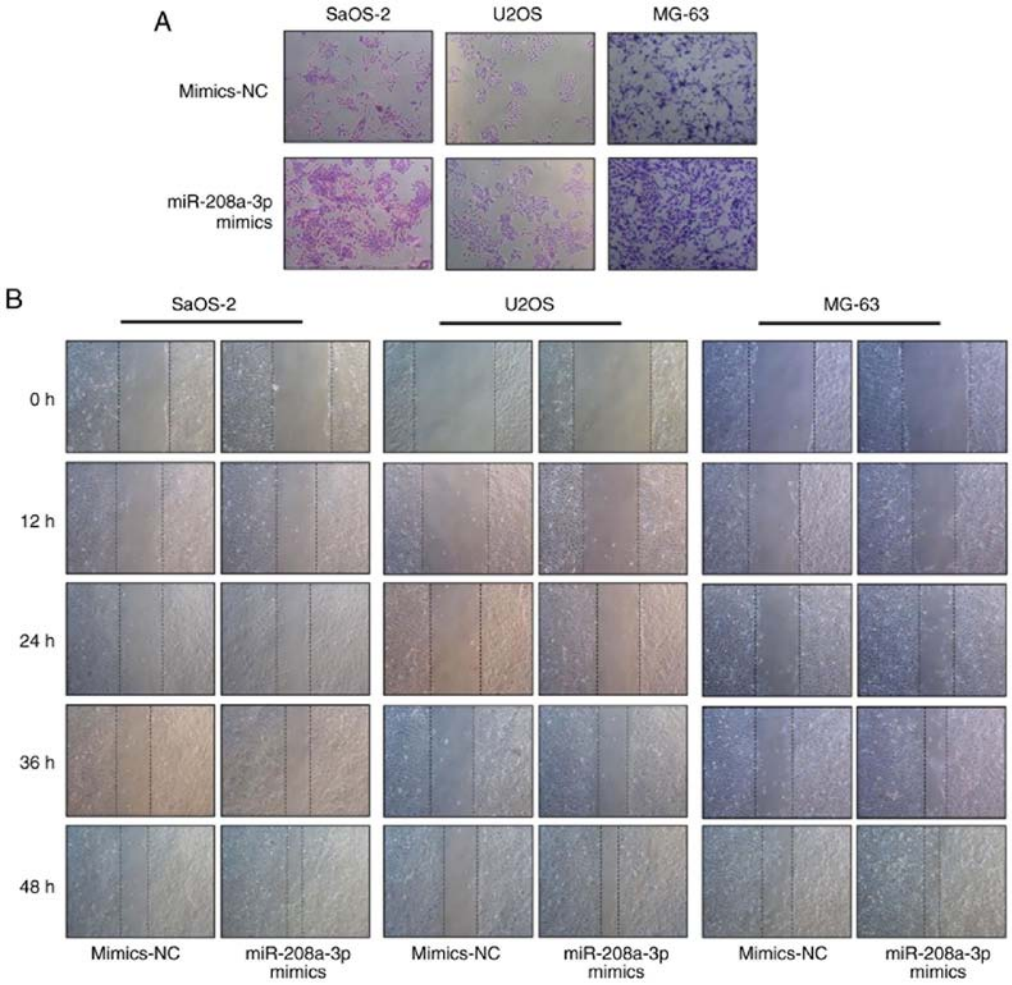

Figure 3. Overexpression of miR-208a-3p facilitates osteosarcoma cell invasion and migration. (A) The invasion of SaOS-2, U2OS and MG-63 cells following overexpression of miR-208a-3p was detected via Transwell assays (magnification, x100). (B) The migration of SaOS-2, U2OS and MG-63 cells following overexpression of miR-208a-3p was examined via wound-healing assays (magnification, x40). NC, negative control; miR, microRNA.

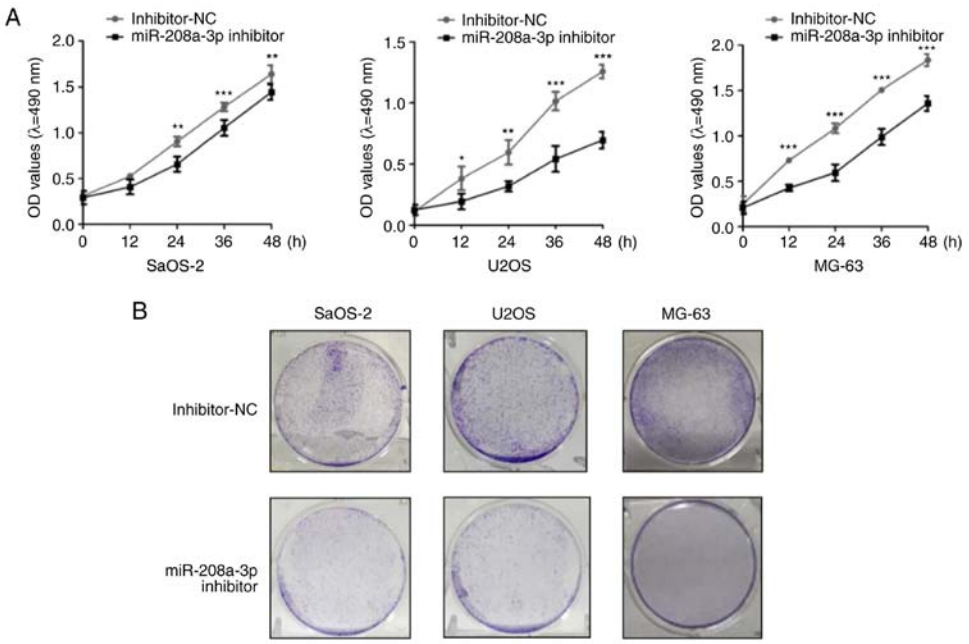

Figure 4. Knockdown of miR-208a-3p inhibits osteosarcoma cell proliferation. (A) MTT assays were performed to detect the proliferation of SaOS-2, U2OS and MG-63 cells following knockdown of miR-208a-3p at different time points. (B) The proliferation of SaOS-2, U2OS and MG-63 cells following knockdown of miR-208a-3p was examined via colony formation assays. *P<0.05, **P<0.01 and ***P<0.001 vs. inhibitor NC. NC, negative control; miR, microRNA; OD, optical density.

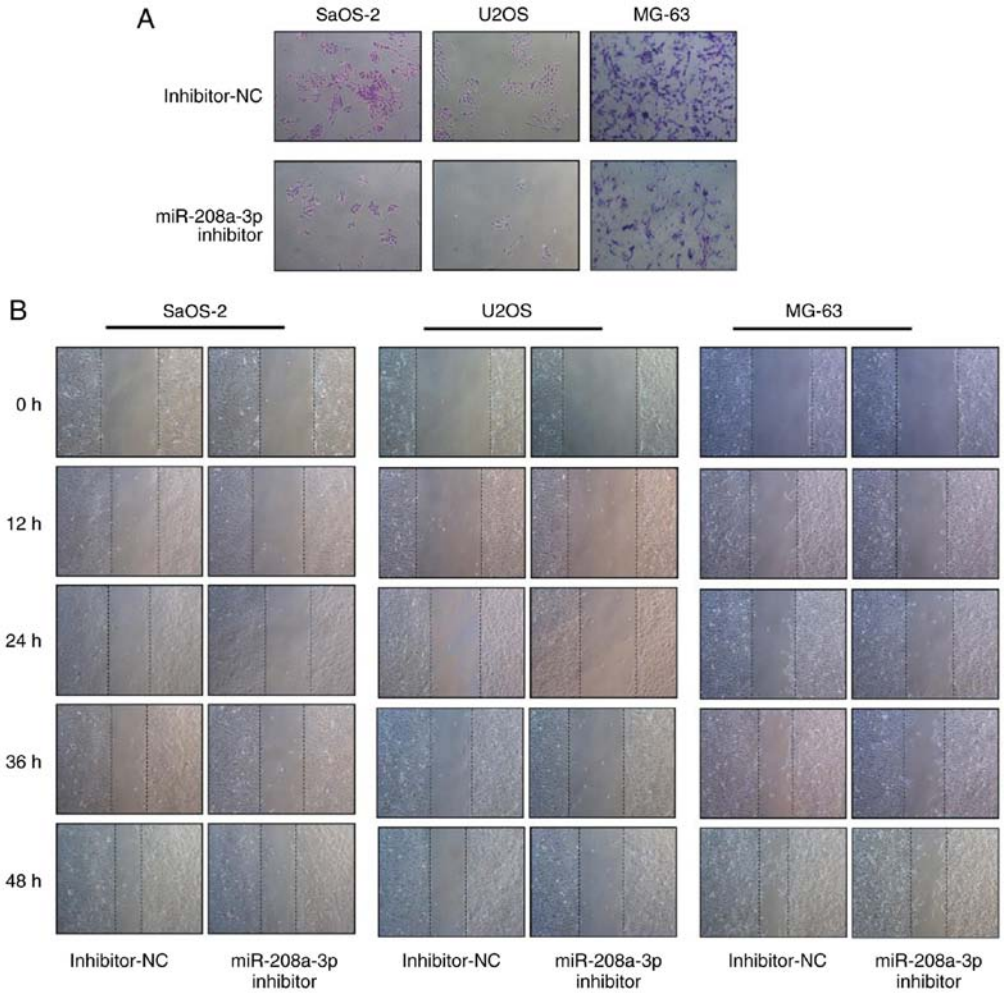

Figure 5. Knockdown of miR-208a-3p suppresses the invasion and migration of OS cells. (A) Transwell assays were performed to detect the invasion of OS cells following knockdown of miR-208a-3p (magnification, x100). (B) Wound healing assays were performed to detect the migration of OS cells following knockdown of miR-208a-3p (magnification, x40). NC, negative control; miR, microRNA; OS, osteosarcoma.

Direct targets of miR-208a-3p. To better characterize the underlying molecular mechanisms associated with miR-208a-3p-mediated progression of OS, bioinformatics analysis was performed. Therefore, TargetScan software was used to predict the potential targets of miR-208a-3p. The analysis revealed that the PTEN 3'-UTR encompassed a complementary binding site for miR-208a-3p (Fig. 6A). Furthermore, a luciferase reporter assay was employed to examine whether the 3'-UTR of PTEN was a direct target of miR-208a-3p. The results demonstrated that miR-208a-3p mimics significantly suppressed the luciferase activity of the wild-type plasmid, but not that of the mutant plasmid (Fig. 6B). In addition, RT-qPCR analysis indicated that compared with their respective NC groups, miR-208a-3p overexpression significantly suppressed the mRNA expression of PTEN, and miR-208a-3p knockdown exhibited the opposite effect (Fig. 6C). Similarly, western blot analysis revealed that compared with their respective NC groups, overexpression of miR-208a-3p significantly downregulated the protein expression of PTEN, while miR-208a-3p knockdown significantly increased PTEN protein levels (Fig. 6D). Taken together, these results suggested that miR-208a-3p may regulate the progression of OS via directly targeting PTEN.

Discussion

OS is a malignant bone tumor with a high incidence in adolescents, while its prognosis is poor due to pulmonary metastasis (25). Accumulating evidence has indicated that miRNAs act as tumor suppressor genes or oncogenes associated with the progression of OS (26,27). For example, miR-138 has been reported to inhibit cell proliferation and invasion, and promote cell apoptosis of human OS cells via targeting differentiated embryonic chondrocyte gene 2 (28). In addition, miR-33a has been revealed to be upregulated in chemoresistant OS and promote OS cell resistance to cisplatin via downregulating Twist-related protein 1 (29). Furthermore, it has been demonstrated that exosomal miR-1228 from cancer-associated fibroblasts promoted OS cell migration and invasion via directly targeting suppressor of cancer cell invasion (30). The present study aimed to investigate the role of miR-208a-3p in the regulation of OS progression.

It has been identified that miR-208a-3p is a highly conserved miRNA that serves a novel important regulator in heart diseases by affecting autophagy and other processes (31-33). miR-208a-3p has also been associated with the progression of several types of cancer. For example,

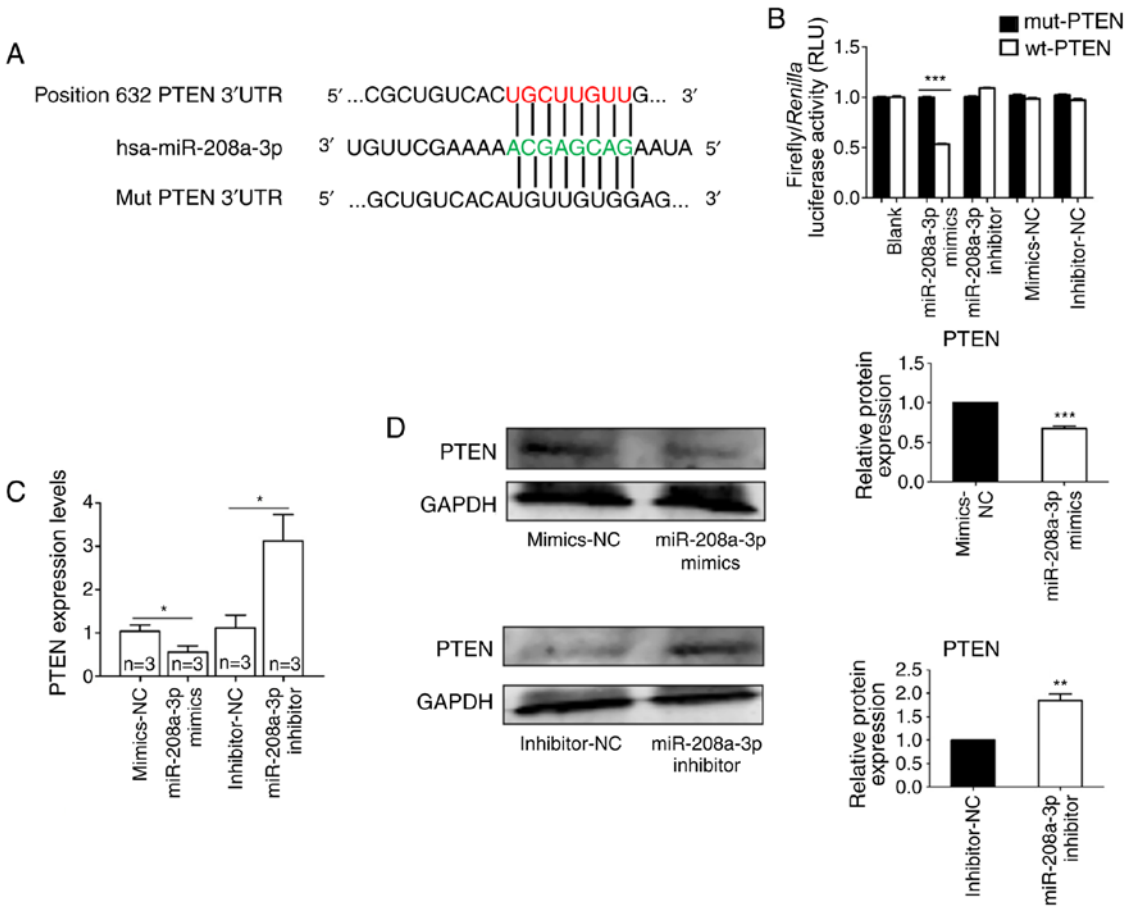

Figure 6. PTEN is a direct target of miR-208a-3p. (A) Potential binding site between miR-208a-3p and the 3'-UTR of PTEN. (B) A luciferase reporter assay was conducted to verify that PTEN was directly targeted by miR-208a-3p. ***P<0.001. (C) The mRNA expression levels of PTEN in OS cells was detected via reverse transcription-quantitative PCR. *P<0.05. (D) Western blotting was performed to measure the protein expression levels of PTEN in OS cells. **P<0.01 and ***P<0.001 vs. NC groups. UTR, untranslated region; NC, negative control; miR, microRNA; OS, osteosarcoma; wt, wild-type; mut, mutant.

miR-208a-3p, as an oncogenic miRNA, has been indicated to suppress apoptosis in gastric cancer cells (23), and promote cell proliferation, invasion and migration in colon cancer via targeting programmed cell death protein 4 (24). However, to the best of our knowledge, the role of miR-208a-3p in OS has not yet been reported. The results of the present study demonstrated that miR-208a-3p was significantly increased in OS tissues (compared with adjacent normal tissues) and three OS cell lines (compared with osteoblastic cells). Furthermore, miR-208a-3p overexpression promoted cell growth and proliferation and facilitated the invasion and migration of SaOS-2, U2OS and MG-63 cells. By contrast, knockdown of miR-208a-3p exhibited the opposite the effects compared with miR-208a-3p mimics. To the best of our knowledge, the present study was the first to demonstrate that miR-208a-3p may serve as an oncogene via regulating cell growth, proliferation and metastasis of OS cells, which was consistent with a previous report indicating that miR-208a-3p as an oncogene promoted colon cancer progression (24).

To further investigate the underlying mechanisms of miR-208a-3p-mediated regulation of OS progression, the downstream targets of miR-208a-3p were examined. Several genes have been identified to be directly targeted by miR-208a-3p, including SFRP1, cadherin 9 and thyroid hormone receptor associated protein 1 (19,34,35). The present study verified that PTEN was a direct target of miR-208a-3p in OS cells. Downregulation of PTEN, a well-known tumor suppressor protein, may result in tumor progression (36,37). As a tumor suppressor, PTEN has been associated with several biological processes, including maintenance of genomic stability, cell survival, migration, proliferation and metabolism (38). Recently, a number of studies revealed that PTEN downregulation was associated with poor prognosis in several types of cancers, including breast, prostate and non-small cell lung cancers (39-41). Moreover, loss of PTEN expression has been implicated in the promotion of OS cell proliferation and metastasis (42). In the present study, bioinformatics analysis and luciferase assays revealed that PTEN was a direct target of miR-208a-3p. Subsequently, the detection of PTEN mRNA and protein expression levels in OS cells following transfection with miR-208a-3p mimics or inhibitors demonstrated that miR-208a-3p mimics downregulated while miR-208a-3p inhibitor upregulated PTEN expression.

In summary, the present study revealed that miR-208a-3p was upregulated in OS tissues and cell lines, and promoted cell proliferation, migration and invasion of OS cells via targeting PTEN. Overall, these findings indicate that miR-208a-3p, acting as an oncogene, promotes OS progression and may be considered a diagnostic biomarker for OS.

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Availability of data and materials

All datasets used and/or generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YF and ZB contributed to study design, data collection, statistical analysis, data interpretation and manuscript preparation. YW, LY, KB, YS, YL and CF contributed to data collection and statistical analysis. BL, ZL and FZ contributed to experimental design, manuscript revision and data proofreading. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The research was approved by the Medical Ethics Committee of The First Affiliated Hospital of Harbin Medical University (Harbin, China; approval no. 201516). Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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