

MicroRNA-635 inhibits the malignancy of osteosarcoma by inducing apoptosis

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Abstract. Recent evidence has suggested that microRNAs (miRs), which are a class of non-coding RNAs, serve diverse roles in tumorigenesis. However, the role of miR-635 in osteosarcoma (OS) remains unknown. The present study revealed that miR-635 may be a tumor suppressive miR. The expression of miR-635 was significantly decreased in OS specimens. In addition, the proliferation and invasion of OS cells transfected with miR-635 may be effectively attenuated. Transfection of cells with miR-635 may further inhibit tumor growth *in vivo*. miR-635 may antagonize tumorigenesis of OS possibly by inducing apoptosis, as demonstrated by flow cytometric analysis and caspase-3 kinase assays. The data of the present study suggested that miR-635 may be a novel tumor suppressor and may serve as a putative diagnostic marker for patients with OS.

Introduction

Tumor cells maintain themselves by self-renewal via cancer stem cells (1). Osteosarcoma (OS) is one of the most malignant tumors with non-hematological features and contributes to ~two-thirds of common subtypes of bone sarcomas (2). Due to the development of current therapeutic strategies, the survival rate for a fraction of patients with OS without metastases has reached ≥60% (3). However, metastatic OS poses a serious threat to ~40% cases and these patients exhibit a low survival rate (3). Understanding the underlying mechanisms of how OS develops and identifying effective therapeutics is required.

MicroRNAs (miR) are small non-coding RNAs of ~22 nucleotides that modulate gene expression by base-pairing with transcripts (4). The imperfect binding of miRs to targets may either promote its degradation or inhibit translation (5).

A recent report demonstrated that the miRs are located in coding and non-coding regions in the genome (6). >1,800 miR sequences have been identified and the majority are involved in the regulation of various biological processes (7). Aberrant expression of miRs is frequently observed in different types of tumor tissue and contributes to tumorigenesis (8). For example, a previous study revealed that miR-34 mediates tumor suppression in OS via the p53 signaling pathway and OS cells frequently downregulate miR-34 expression (9). miR-539 has additionally been reported to be a tumor suppressor in OS and the inhibitory effect is mediated by targeting matrix metalloproteinase-8 (10). A recent report suggested that the miR-138 decreases the tumorigenic potential of OS by targeting differentiated embryonic chondrocyte gene 2 (11). However, certain miRs may additionally serve roles in tumor progression. For example, the miR-17-92 cluster is positively associated with OS development (12). Another report revealed that miR-20a may advance the metastasis of OS by targeting Fas for degradation (13). Numerous studies have focused on the role of miRs in OS; however, little information is available for miR-635, particularly in OS.

In the present study, the physiological significance of miR-635 in OS was investigated. The results demonstrated that the expression levels of miR-635 are frequently downregulated in OS tissues compared with healthy adjacent tissues. In addition, miR-635 may function as a tumor suppressor in OS by inhibiting proliferation and migration, and inducing apoptosis. This data suggested novel roles for miR-635 and may aid the development of efficient therapeutics that target OS cells.

Materials and methods

Cell culture and specimens. The OS cell lines MG-63 and HOS, and the control OS cell line, hFOB, were obtained from Shanghai Institute of Cell Biology (Shanghai, China). MG-63, HOS and hFOB cells were maintained in RPMI-1640 medium (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The medium was supplemented with 3% fetal bovine serum (FBS; Sigma-Aldrich; Merck KGaA) and cells were incubated at 20°C and 5% CO₂. The OS specimens were obtained from surgical archives at The Third Affiliated Hospital, Xinxiang Medical University (Xinxiang, China) from June 2014 to October 2015 (Table I). Written formal consent was obtained from all patients. The research on human specimens was

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reviewed and approved by the Research Ethics Committee of The Third Affiliated Hospital, Xinxiang Medical University (no. 2014L00145).

Plasmid transfection. The miR-635 and negative control sequences were synthesized and cloned into the pcDNA3.1 vector (Tiangen Biotech Co., Ltd., Beijing, China). A total of 0.2 μ g negative control (3'-GTACGACGACCATGGCTGTA-5') and pcDNA-miR-635 (3'-UUGGGCACUGAAACA AUGUCC-5') was subsequently transfected into MG-63 and HOS cells at a density of 1×10^5 cell/ml in 12-well plates using Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. After 48 h, the culture was replaced with fresh medium, prior to measuring the expression levels of miR-635.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from MG-63, hFOB and HOS cells, or human specimens, with TRIzol reagent (Sigma-Aldrich; Merck KGaA). A total of 10 μ g cDNA was synthesized by reverse transcription using the SYBR Premix Taq[™] toolkit (Takara Biotechnology Co., Ltd., Dalian, China), according to the manufacturer's protocol. The TaqMan microRNA RT-qPCR kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to quantify miR-635 expression. GAPDH was used as the control. The primer sequences were: Sense, 5'-GATTAAGCGTTGAGAGTG-3' and anti-sense, 5'-CTGAGCTTAGTCCCGATT-3' for human miR-635; sense, 5'-CTCACTTTGATCTGCATTGT-3' and anti-sense, 5'-ATGCAACTGCTATGTGAT-3', for human GAPDH. Reactions were performed using the ABI PRISM[®] 7000 Sequence Detection system (Applied Biosystems) according to the manufacturer's protocol. The expression of miR-635 was calculated using the $2^{-\Delta\Delta C_q}$ method (14). The cycling conditions were as follows: 55°C for 2 min, predenaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 20 sec and annealing at 60°C for 1 min. Experiments were performed in triplicate.

Proliferation assay. The Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was utilized to determine the proliferation of HOS and MG-63 cells. Following treatment for 24 h, cells were re-suspended and 10^5 cells/well were seeded into a 96-well plate for 5 days. A total 20 μ l MTT solution was added to the culture to a final concentration of 10 mg/ml at 20°C for 4 h. The crystalline formazan product was dissolved in 150 μ l 15% SDS solution for one day and the optical density was measured at a wavelength of 490 nm using the Spectramax M5 microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA), following the manufacturer's protocol (15).

Flow cytometry for apoptosis detection. HOS and MG-63 cells were seeded into 6-well plates (Qiagen GmbH, Hilden, Germany) and washed with cold PBS solution 24 h post transfection. Apoptosis was quantified by double staining with the Annexin V/propidium iodide (PI) Apoptosis Detection toolkit (Sigma-Aldrich; Merck KGaA) following the manufacturer's protocol. A BD FACSCalibur instrument (BD Biosciences,

Franklin Lakes, NJ, USA) was used to visualize and Cell Quest version 3.1 software (BD Biosciences) was used to analyze the data.

Transwell invasion assay. The upper chamber was coated with Matrigel (Invitrogen, Thermo Fisher Scientific, Inc.) overnight. The lower chambers were replenished with RPMI-1640 medium containing 5% FBS. After 24 h, 1×10^5 cells were transfected with the pcDNA-miR-635 or negative control plasmids and starved in 100 ml serum-free medium, prior to being moved to the upper chamber. The upper chamber was removed after 24 h and invasive cells in the lower chamber were fixed with 5% paraformaldehyde and stained using crystal violet solution for 1 h at 20°C. Cells were counted using a Leica microscope fluorescent microscope (model DM-IRB; Leica Microsystems GmbH, Wetzlar, Germany). Three randomly selected fields were counted for each assay. Image were captured using x200 magnification.

In vivo implantation. BALB/c nude mice (6-8 weeks old, average weight: 19.5 g; 12 male and 12 female) were purchased from the Shanghai Institute of Cell Biology (Shanghai, China). Mice were housed at 20°C, with 50-55% humidity and a light-dark cycle of 12 h. *Ad libitum* access to food and water was provided. Animal protocols and experiments were in accordance with the General Guide for the Use of Laboratory Animals and approved by the Ethics Committee for Animal Experiments at The Third Affiliated Hospital, Xinxiang Medical University (no. 2014D00035). MG-63 cells transfected with pcDNA3.1 were cultured for an additional 24 h. Subsequently, 1×10^6 MG-63 cells were implanted subcutaneously into the nude mice. The volume of tumor *in vivo* was monitored every 2 days. After 20 days, all mice were sacrificed and the tumor weight was obtained.

Caspase-3 activity assay. MG-63 and HOS cells were transfected with pcDNA-miR-635 or negative control plasmids. After transfection, the activity of caspase-3 was quantified using a colorimetric caspase-3 assay kit (EMD Millipore, Billerica, MA, USA), according to the manufacturer's protocol.

Statistical analysis. All statistics were analyzed using SPSS software (version 15.0; SPSS, Inc., Chicago, IL, USA) and the Student's t-test. The χ^2 test was used to determine the correlation between miR-635 expression and different clinicopathological features. Data are presented as the mean \pm standard deviation. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression level of miR-635 is reduced in OS tissues and cell lines. RT-qPCR was utilized to quantify the expression levels of miR-635 in OS tissues and paired normal adjacent tissues (NATs) from 63 patients. Compared with NATs, miR-635 was significantly downregulated in OS tissues ($P < 0.001$; Fig. 1A). In addition, reduced expression of miR-635 was associated with advanced tumor node metastasis (TNM) stage ($P < 0.01$; Fig. 1B). Compared with the expression in

Table I. Association between miR-635 expression and clinicopathological features.

| Clinicopathological features | No. | miR-635 expression | | P-value |
|------------------------------|-----|--------------------|-------------|---------|
| | | Low, n (%) | High, n (%) | |
| Age | | | | 0.280 |
| <60 | 29 | 15 (51.7) | 14 (48.3) | |
| ≥60 | 34 | 14 (41.2) | 20 (58.8) | |
| Gender | | | | 0.254 |
| Male | 30 | 12 (40.0) | 18 (60.0) | |
| Female | 33 | 17 (51.5) | 16 (48.5) | |
| Tumor differentiation | | | | 0.004 |
| Well + Moderate | 25 | 6 (24.0) | 19 (76.0) | |
| Poor | 38 | 23 (60.5) | 15 (39.5) | |
| Metastasis | | | | 0.005 |
| Absent | 36 | 11 (30.6) | 25 (69.4) | |
| Present | 27 | 18 (66.7) | 9 (33.3) | |
| TNM stage | | | | 0.001 |
| 0/I | 26 | 5 (19.2) | 21 (80.8) | |
| II/III/IV | 37 | 24 (64.9) | 13 (35.1) | |

miR, microRNA; TNM, tumor node metastasis.

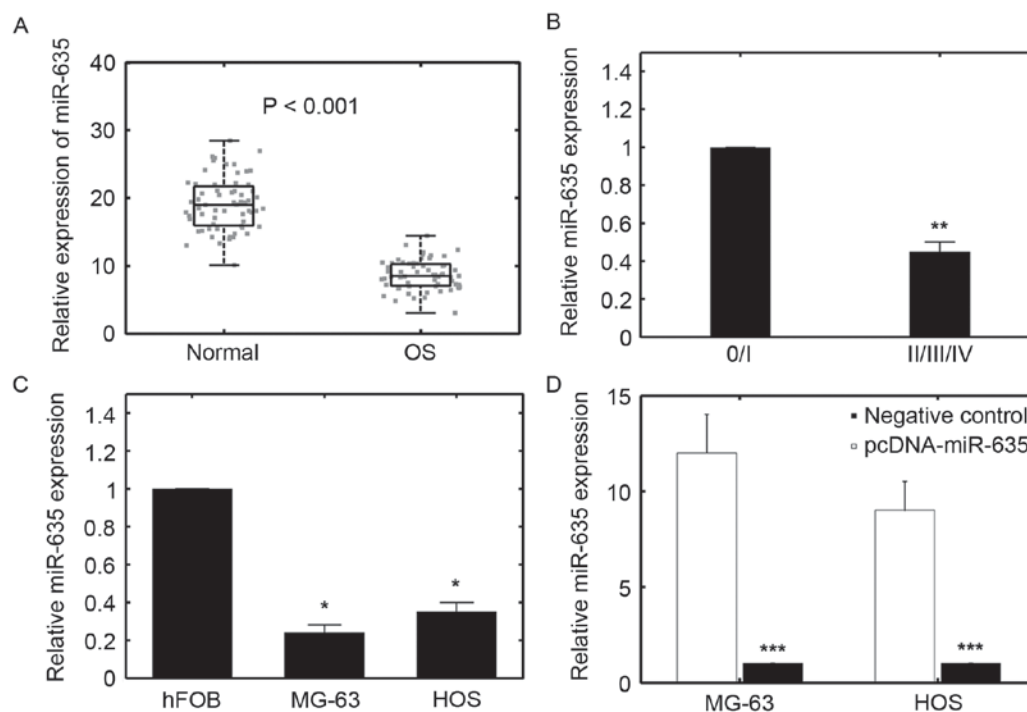


Figure 1. Expression levels of miR-635 are downregulated in osteosarcoma. (A) The expression levels of miR-635 in 63 paired osteosarcoma and normal adjacent human tissues. $P < 0.001$ vs. normal tissues. (B) The association between miR-635 expression and tumor node metastasis stage $^{**}P < 0.01$ vs. stage 0/I. (C) The expression levels of miR-635 in hFOB (control), MG-63 and HOS cells. $^{*}P < 0.01$ vs. hFOB. (D) The expression levels of MG-63 and HOS cells transfected with pcDNA-miR-635 or negative control plasmids. $^{***}P < 0.001$ vs. pcDNA-miR-635-transfected cells. Data are presented as the mean \pm standard deviation. miR, microRNA.

normal osteoblastic hFOB cells, miR-635 was significantly decreased in MG-63 and HOS cells (both $P < 0.01$; Fig. 1C). In addition to TNM stage, miR-635 was significantly associated with differentiation and metastasis (Table I). These results

suggested that miR-635 may suppress OS cells and function as a potential tumor suppressor. To investigate this, MG-63 and HOS cells were transfected with pcDNA-miR-635 or negative control plasmids and the efficiency of transfection

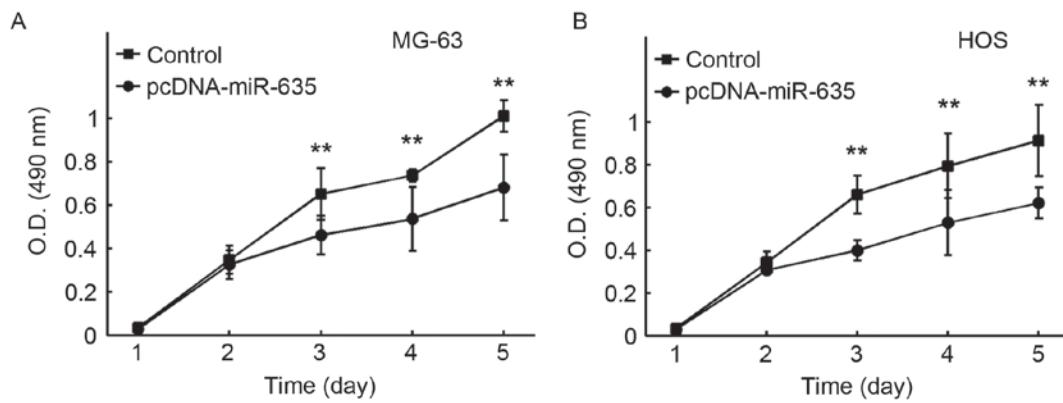


Figure 2. Overexpression of miR-635 inhibits the proliferation of osteosarcoma cells. (A) Cell Counting Kit-8 proliferation assay in MG-63 cells transfected with pcDNA-miR-635 or negative control plasmids. (B) The proliferation of HOS cells transfected with pcDNA-miR-635 or negative control plasmids. **P<0.01 vs. pcDNA-miR-635-transfected cells. Data are presented as the mean \pm standard deviation. miR, microRNA; O.D., optical density.

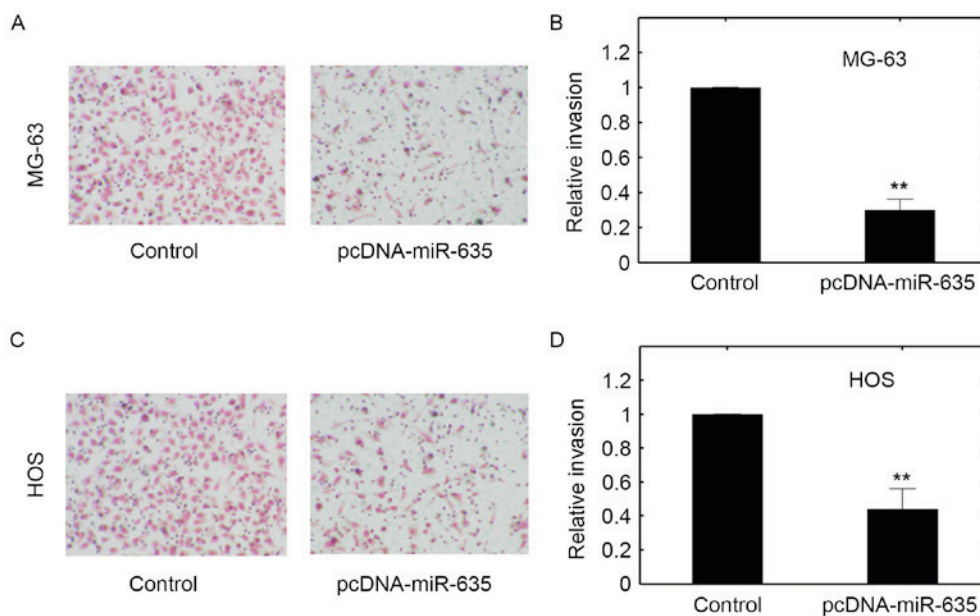


Figure 3. Overexpression of miR-635 inhibits invasion of osteosarcoma cell lines. (A) Transwell invasion assay for MG-63 cells transfected with pcDNA-miR-635 or negative control plasmids. (B) Quantification of the invasion assay in MG-63 cells. (C) Invasion of HOS cells transfected with pcDNA-miR-635 or negative control plasmids. (D) Quantification of the invasion assay in HOS cells. Data are presented as the mean \pm standard deviation. **P<0.01 vs. pcDNA-miR-635-transfected cells. miR, microRNA.

was determined by RT-qPCR. Results demonstrated that the ectopic transfection significantly upregulated miR-635 expression levels in MG-63 and HOS cells (P<0.01; Fig. 1D).

miR-635 transfection suppresses proliferation of OS cells. The effect of miR-635 overexpression on the proliferation of OS cells was investigated. The CCK-8 assay was utilized to evaluate the proliferation of HOS and MG-63 cells. Overexpression of miR-635 significantly decreased the proliferation of MG-63 cells (P<0.01; Fig. 2A) and HOS cells (P<0.01; Fig. 2B) after 3 days. These results suggested that miR-635 inhibited OS cell proliferation.

Overexpression of miR-635 inhibits OS cell invasion. As miR-635 overexpression inhibited OS cell proliferation, it was determined whether invasion is attenuated by miR-635

transfection. A transwell invasion assay was used to quantify the invasive capacity of MG-63 and HOS cells transfected with the pcDNA-miR-635 or negative control plasmids. The results demonstrated that miR-635 decreased the migration of MG-63 cells (Fig. 3A). Quantification results revealed that the decrease in migration was statistically significant (P<0.01; Fig. 3B). Similar results were observed in HOS cells (Fig. 3C and D). These results suggested that miR-635 suppresses cell invasion in OS cell lines *in vitro*.

miR-635 inhibits OS growth *in vivo*. To further clarify the role of miR-635 *in vivo*, xenograft studies in mice were performed. A total of 1×10^6 MG-63 cells were transfected with pcDNA-miR-635 or negative control and were subcutaneously injected into nude mice. Tumor growth was evaluated every 2 days. miR-635 transfection significantly reduced the rate of tumor growth (P<0.05; Fig. 4A) and the weight of solid tumors

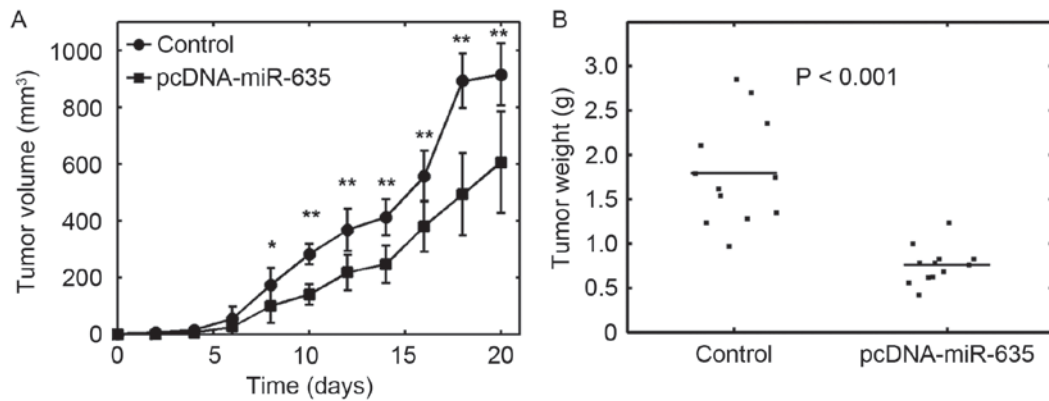


Figure 4. Overexpression of miR-635 inhibits osteosarcoma progression *in vivo*. (A) The tumor volume in nude mice injected with MG-63 cells that were transfected with pcDNA-miR-635 or negative control plasmids. *P<0.05, **P<0.01 vs. pcDNA-miR-635-transfected cells. (B) At the end of the experiment, solid tumors were resected and weighed. The graph demonstrates tumor weight in nude mice injected with MG-63 cells that were transfected with pcDNA-miR-635 or negative control plasmids. P<0.001 vs. pcDNA-miR-635-transfected cells. Data are presented as the mean \pm standard deviation. miR, microRNA.

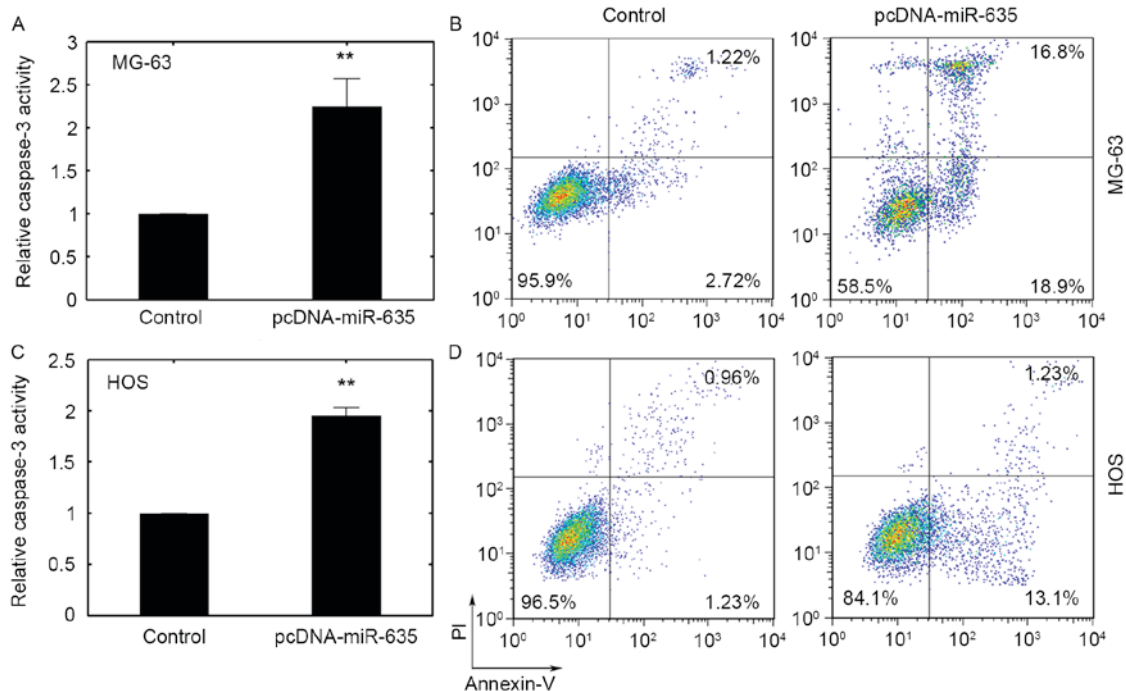


Figure 5. Overexpression of miR-635 induces apoptosis in osteosarcoma cells. (A) Caspase-3 activity in MG-63 cells transfected with pcDNA-miR-635 or negative control plasmids. (B) Annexin V/PI staining in MG-63 cells transfected with pcDNA-miR-635 or negative control plasmids. (C) Caspase-3 activity in HOS cells transfected with pcDNA-miR-635 or negative control plasmids. **P<0.01 vs. pcDNA-miR-635-transfected cells. (D) Annexin V/PI staining in HOS cells transfected with pcDNA-miR-635 or negative control plasmids. Data are presented as the mean \pm standard deviation. miR, microRNA; PI, propidium iodide.

(P<0.001; Fig. 4B). These results suggested that miR-635 inhibits OS tumor development *in vivo*.

miR-635 induces apoptosis in OS. To investigate the potential mechanism of miR-635-induced inhibition of proliferation and invasion in OS cells, it was determined whether miR-635 serves a role in apoptosis. MG-63 and HOS cells were transfected with pcDNA-miR-635 or negative control plasmid prior to the performance of apoptotic assays. The results revealed that miR-635 significantly increased caspase-3 activity in MG-63 cells (P<0.01; Fig. 5A). MG-63 cells were additionally subjected to flow cytometric analysis, which demonstrated that miR-635 induced apoptosis, as determined by Annexin V and

PI staining (Fig. 5B). Similar results were observed in HOS cells (Fig. 5C and D). These results suggested that overexpression of miR-635 induces apoptosis in OS cells.

Discussion

The present study revealed that miR-635 functions as a tumor suppressor in OS. Decreased expression of miR-635 was observed in human OS specimens and cell lines. In addition, miR-635 inhibited the proliferation of OS cell lines. Transwell assays have been demonstrated to be an effective strategy to measure the migratory capacity of tumor cells transfected with miRs (16-18). The present study demonstrated that

overexpression of miR-635 decreased the invasion of OS cells. The growth of solid tumors was substantially decreased in cells transfected with the miR-635-containing plasmid. Mechanistic studies suggested that miR-635 exerts these effects by inducing apoptosis. Therefore, miR-635 may prevent tumor cell progression via apoptotic pathways.

The association between miR expression and tumor development has been an active area of research in recent years. miRs have diverse functions in a cell type and tumor specific manner (19). Understanding the underlying mechanisms of miR-mediated tumor promotion or inhibition may aid early diagnosis and the development of effective targeted therapeutics against tumors.

Various studies have focused on the role of miRs in OS cells. However, few reports have demonstrated the effect of miR-635 in OS. A recent report demonstrated that miR-635 may accelerate invasion of A375 melanoma cells (20). However, the present study revealed a tumor suppressive role for miR-635. This suggested that miRs serve different roles in a cell-specific manner. An earlier report demonstrated that miR-635 is implicated in mesial temporal lobe epilepsy (mTLE) (21). A candidate target of miR-635 is intercellular cell adhesion molecule-1 (ICAM1) (21). The expression level of miR-635 altered by >2.0-fold, which suggested that it is highly regulated (21). However, western blot analysis failed to identify a significant role for miR-635 in downregulating ICAM1, suggesting that miR-635 may not serve a significant role in mTLE. These studies do not provide sufficient information about the function of miR-635 and therefore the exact function of miR-635 requires further investigation. The present study demonstrated that miR-635 may function as a tumor suppressor in OS, as determined by experiments with human specimens and an *in vitro* study in mice. miR-635 may mediate its effects via apoptotic pathways. However, the exact target(s) of miR-635 was not clarified in the present study and this requires further investigation in future studies. For example, in order to determine the putative target of miR-635, bioinformatics tools may be used and targets may be categorized based on their physiological roles (22). By identifying the putative target(s), investigations into whether the regulation of this target(s) is restricted to miR-635 or shared by other miRs may be determined. These future studies may aid the understanding of the underlying signaling pathways involved in this. Whether miR-635 has a similar function in other tumor types remains to be determined.

In conclusion, the present study identified a novel role for miR-635 in OS. miR-635 may be a novel tumor suppressive miR in OS. Downregulation of miR-635 may favor OS progression, possibly by reducing apoptosis. The underlying mechanism of miR-635 in OS requires further investigation and may provide an insight into miR-targeted intervention.

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References

- Shackleton M, Quintana E, Fearon ER and Morrison SJ: Heterogeneity in cancer: Cancer stem cells versus clonal evolution. *Cell* 138: 822-829, 2009.
- Heare T, Hensley MA and Dell'Orfano S: Bone tumors: Osteosarcoma and Ewing's sarcoma. *Curr Opin Pediatr* 21: 365-372, 2009.
- Levings PP, McGarry SV, Currie TP, Nickerson DM, McClellan S, Ghivizzani SC, Steindler DA and Gibbs CP: Expression of an exogenous human Oct-4 promoter identifies tumor-initiating cells in osteosarcoma. *Cancer Res* 69: 5648-5655, 2009.
- Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
- Lim EL, Trinh DL, Scott DW, Chu A, Krzywinski M, Zhao Y, Robertson AG, Mungall AJ, Schein J, Boyle M, *et al*: Comprehensive miRNA sequence analysis reveals survival differences in diffuse large B-cell lymphoma patients. *Genome Biol* 16: 18, 2015.
- Feng B, Zhang K, Wang R and Chen L: Non-small-cell lung cancer and miRNAs: Novel biomarkers and promising tools for treatment. *Clin Sci (Lond)* 128: 619-634, 2015.
- van Rooij E: The art of microRNA research. *Circ Res* 108: 219-234, 2011.
- Liu X, Zhang J, Xie B, Li H, Shen J and Chen J: MicroRNA-200 family profile: A promising ancillary tool for accurate cancer Diagnosis. *Am J Ther* 23: e388-e397, 2016.
- He C, Xiong J, Xu X, Lu W, Liu L, Xiao D and Wang D: Functional elucidation of MiR-34 in osteosarcoma cells and primary tumor samples. *Biochem Biophys Res Commun* 388: 35-40, 2009.
- Jin H and Wang W: MicroRNA-539 suppresses osteosarcoma cell invasion and migration *in vitro* and targeting Matrix metalloproteinase-8. *Int J Clin Exp Pathol* 8: 8075-8082, 2015.
- Jiang B, Mu W, Wang J, Lu J, Jiang S, Li L, Xu H and Tian H: MicroRNA-138 functions as a tumor suppressor in osteosarcoma by targeting differentiated embryonic chondrocyte gene 2. *J Exp Clin Cancer Res* 35: 69, 2016.
- Li X, Yang H, Tian Q, Liu Y and Weng Y: Upregulation of microRNA-17-92 cluster associates with tumor progression and prognosis in osteosarcoma. *Neoplasia* 61: 453-460, 2014.
- Huang G, Nishimoto K, Zhou Z, Hughes D and Kleiner ES: miR-20a encoded by the miR-17-92 cluster increases the metastatic potential of osteosarcoma cells by regulating Fas expression. *Cancer Res* 72: 908-916, 2012.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.
- Wang T, Chen T, Niu H, Li C, Xu C, Li Y, Huang R, Zhao J and Wu S: MicroRNA-218 inhibits the proliferation and metastasis of esophageal squamous cell carcinoma cells by targeting BMI1. *Int J Mol Med* 36: 93-102, 2015.
- Rajendiran S, Parwani AV, Hare RJ, Dasgupta S, Roby RK and Vishwanatha JK: MicroRNA-940 suppresses prostate cancer migration and invasion by regulating MIEN1. *Mol Cancer* 13: 250, 2014.
- Savita U and Karunakaran D: MicroRNA-106b-25 cluster targets β -TRCP2, increases the expression of Snail and enhances cell migration and invasion in H1299 (non small cell lung cancer) cells. *Biochem Biophys Res Commun* 434: 841-847, 2013.
- Xu S, Zhao N, Hui L, Song M, Miao ZW and Jiang XJ: MicroRNA-124-3p inhibits the growth and metastasis of nasopharyngeal carcinoma cells by targeting STAT3. *Oncol Rep* 35: 1385-1394, 2016.
- Leonardo TR, Schultheisz HL, Loring JF and Laurent LC: The functions of microRNAs in pluripotency and reprogramming. *Nat Cell Biol* 14: 1114-1121, 2012.
- Weber CE, Luo C, Hotz-Wagenblatt A, Gardyan A, Kordaß T, Holland-Letz T, Osen W and Eichmüller SB: miR-339-3p is a tumor suppressor in melanoma. *Cancer Res* 76: 3562-3571, 2016.
- Kan AA, van Erp S, Derijck AA, de Wit M, Hessel EV, O'Duibhir E, de Jager W, Van Rijen PC, Gosselaar PH, de Graan PN and Pasterkamp RJ: Genome-wide microRNA profiling of human temporal lobe epilepsy identifies modulators of the immune response. *Cell Mol Life Sci* 69: 3127-3145, 2012.
- Kozomara A and Griffiths-Jones S: miRBase: Annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res* 42 (Database issue): D68-D73, 2014.