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MicroRNA-342-3p Inhibits the Proliferation, Migration, and Invasion of Osteosarcoma Cells by Targeting Astrocyte-Elevated Gene-1 (AEG-1)

Shaokun Zhang,* Lidi Liu,* Zhenshan Lv,* Qiao Li,* Weiquan Gong,* and Hong Wu†

*Department of Spine Surgery, The First Hospital of Jilin University, Changchun, P.R. China
†Department of Ophthalmology, The Second Hospital of Jilin University, Changchun, P.R. China

Recent studies suggest that microRNAs (miRNAs) are critical regulators in many types of cancer, including osteosarcoma. miR-342-3p has emerged as an important cancer-related miRNA in several types of cancers. However, the functional significance of miR-342-3p in osteosarcoma is unknown. The aims of this study were to investigate whether miR-342-3p is dysregulated in osteosarcoma and to explore the biological function of miR-342-3p in regulating cellular processes of osteosarcoma cells. We found that miR-342-3p expression was significantly decreased in osteosarcoma tissues and cell lines. Overexpression of miR-342-3p inhibits the proliferation, migration, and invasion of osteosarcoma cells. In contrast, the inhibition of miR-342-3p exhibited the opposite effect. Astrocyte-elevated gene-1 (AEG-1) was identified as one of the target genes of miR-342-3p in osteosarcoma cells by bioinformatics analysis, dual-luciferase reporter assay, real-time quantitative polymerase chain reaction, and Western blot analysis. Overexpression of miR-342-3p also inhibited the Wnt and nuclear factor κB signaling pathways. Moreover, overexpression of AEG-1 partially rescued the inhibitory effects of miR-342-3p mediated on the proliferation, migration, and invasion of osteosarcoma cells. Overall, our results show that miR-342-3p inhibits the proliferation, migration, and invasion of osteosarcoma cells through targeting AEG-1, suggesting a potential target for the development of miRNA-based therapy for osteosarcoma.

Key words: Astrocyte-elevated gene-1 (AEG-1); miR-342-3p; Osteosarcoma; Wnt; Nuclear factor κB (NF-κB)

INTRODUCTION

Osteosarcoma is the most common malignant bone tumor with a high morbidity in children and adolescents[1,2]. Osteosarcoma is a refractory disease because of its high local aggressiveness and its potential for rapid metastasis[3]. Despite the advances in treatment strategies over the past few decades, the prognosis and survival rate of osteosarcoma are still poor[3]. The pathogenesis of osteosarcoma is related to aberrant genetic and epigenetic alterations that lead to dysregulation of oncogenes or tumor suppressor genes[4]. However, a good understanding of the pathogenesis of osteosarcoma remains a challenge. Therefore, it is essential to gain a better understanding of the underlying mechanism of osteosarcoma to help with the development of novel strategies for the diagnosis, prognosis, and treatment of osteosarcoma patients.

MicroRNAs (miRNAs) are a group of short, noncoding RNAs, usually 18–25 nucleotides in length, that negatively regulate gene expression through base pairing to the 3′-untranslated region (3′-UTR) of the target genes[5,6]. It has been documented that miRNAs play a critical role in various activities, such as development, cellular differentiation programs, and oncogenesis[7]. In particular, miRNAs modulate various cellular processes in cancer, including proliferation, apoptosis, migration, and invasion[8,9]. The dysregulation of miRNA expression has been suggested to be an important mechanism for tumorigenesis[10,11]. Thus, miRNAs have a therapeutic potential in the treatment of cancer[12,13]. Increasing evidence has also reported that various miRNAs are dysregulated in osteosarcoma and participate in the development and progression of osteosarcoma[14–17]. Thus, miRNAs have emerged as potential and promising targets for the prognosis, diagnosis, and treatment of osteosarcoma. However, the precise role of miRNAs in osteosarcoma remains largely unknown.

Astrocyte-elevated gene-1 (AEG-1), also known as metadherin, has been suggested as an oncogene in recent years[18–21]. AEG-1 is originally induced in human fetal astrocytes and characterized as a human immunodeficiency virus-1- and tumor necrosis factor-α-inducible gene[22]. AEG-1 is found to be highly expressed in numerous cancers, including lung cancer[23], hepatocellular carcinoma[24], and glioma[25]. AEG-1 is associated with various oncogenic signaling pathways such as Wnt and nuclear factor κB (NF-κB), which regulate cancer growth and metastasis[26]. AEG-1 also plays an important role in osteosarcoma. AEG-1

Address correspondence to Hong Wu, Department of Ophthalmology, The Second Hospital of Jilin University, No. 218 Ziqiang Street, Changchun 130041, P.R. China. E-mail: hongwujlu@163.com

is highly expressed in osteosarcoma tissues and is positively correlated with clinical stage, metastasis, and poor survival of osteosarcoma patients[27,28]. AEG-1 promotes osteosarcoma cell proliferation, metastasis, and chemoresistance associated with activation of the Wnt, endothelin, Jun N-terminal kinase, and NF-κB signaling pathways[29–31]. Therefore, AEG-1 may serve as a potential and promising therapeutic target for osteosarcoma treatment.

miR-342-3p has been reported to be an important cancer-related miRNA in several types of cancers[32,33]. However, the functional significance of miR-342-3p in osteosarcoma is unknown. In this study, we showed that miR-342-3p expression was significantly decreased in osteosarcoma tissues and cell lines. Overexpression of miR-342-3p inhibits the proliferation, migration, and invasion of osteosarcoma cells. AEG-1 was identified as one of the target genes of miR-342-3p in osteosarcoma cells. miR-342-3p also regulates the Wnt and NF-κB signaling pathways by targeting AEG-1. Overall, our results show that miR-342-3p inhibits the proliferation, migration, and invasion of osteosarcoma cells through the downregulation of AEG-1, suggesting a potential therapeutic target for the development of miRNA-based therapy for osteosarcoma treatment.

MATERIALS AND METHODS

Tissue Specimen Selection

Paired osteosarcoma tissue samples and adjacent nontumor tissues were obtained from 20 patients undergoing surgery at The First Hospital of Jilin University. The resected tissues were immediately snap frozen in liquid nitrogen and stored at −80°C before use. All patients agreed and signed the written informed consent prior to sample collection. This study was reviewed and approved by the Human Research and Ethics Committee of The First Hospital of Jilin University and conducted in accordance with the Declaration of Helsinki.

Cell Lines and Cell Culture

The normal human osteoblast cell line hFOB1.19 and human osteosarcoma cell lines (MG63, U2OS, SOSP-9607, and HOS) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, P.R. China) and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS), streptomycin (100 μg/ml), and penicillin (100 U/ml). All cells were incubated at 37°C in a humidified incubator of 5% CO_2.

Cell Transfection

The miR-342-3p mimics, miR-342-3p inhibitor, and scramble negative control (NC) were synthesized by GenePharma (Shanghai, P.R. China). The AEG-1 cDNA without 3′-UTR was cloned into pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA) to generate the pcDNA3.1-AEG-1 vector. Cell transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted using TRIzol (Invitrogen) and miRNeasy mini kit (Qiagen, Dusseldorf, Germany) according to the manufacturers' protocols. RNA was converted to cDNA using Moloney murine leukemia virus reverse transcriptase (Takara, Dalian, P.R. China) and TaqMan MicroRNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). PCR was performed using a SYBR Green PCR kit (Applied Biosystems) on a 7900HT system (Applied Biosystems). The PCR conditions were as follows: 95°C for 5 min; 35 cycles at 95°C for 5 s; 55°C for 30 s; 72°C for 30 s; and 72°C for 5 min. GAPDH and U6 were used as internal controls. Relative gene expression was calculated by the $2^{-\Delta\Delta}$Ct method. Fold changes of gene expression were obtained by normalization with the control group. The primers used were as follows: miR-342-3p, 5′-TGCGGTCTCACACAGAAATCGCAC-3′ (forward) and 5′-CCAGTGCAGGGTCCGAGGT-3′ (reverse); U6, 5′-CTCGCTTCGGCAGCACA-3′ (forward) and 5′-AACGCTTCACGAATTTGCGT-3′ (reverse); AEG-1, 5′-CGAGAAGCCCAAACCAAATG-3′ (forward) and 5′-TGGTGGCTGCTTTGCTGTT-3′ (reverse); cyclin D1, 5′-CCGTCCATGCGGAAGATC-3′ (forward) and 5′-GAAGACCTCCTCCTCGCACT-3′ (reverse); matrix metalloproteinase-2 (MMP-2), 5′-AGGCCAAGTGGTCCGTGTGA-3′ (forward) and 5′-TAGGTGGTGGAGCACCAGAG-3′ (reverse); GAPDH, 5′-GACTCATGACCACAGTCCATGC-3′ (forward) and 5′-AGAGGCAGGGATGATGTTCTG-3′ (reverse).

Cell Proliferation Assay

Cell proliferation was detected by the cell counting kit-8 (CCK-8; Sigma-Aldrich, St. Louis, MO, USA). Briefly, cells were seeded into 96-well plates at a concentration of 5,000 cells per well and cultured for 48 h after transfection. Cells were then incubated in 10% CCK-8 reagent at 37°C. Absorbance at 450 nm was measured using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

Cell Cycle Assay

Cells were serum starved for 24 h to synchronize the cell cycle. After transfection for 48 h, cells were digested with trypsin, washed with phosphate-buffered saline (PBS), and then fixed with 70% ethanol. Cells were incubated with 100 μg/ml of propidium iodide (Sigma-Aldrich) and

10 μg/ml of RNase A for 30 min in the dark. Cell cycle distribution was detected by a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA), and data were analyzed by CellQuest software.

Cell Migration and Invasion Assays

Cells transfected with miR-342-3p mimic or miR-342-3p inhibitor were harvested 48 h after transfection and subjected to cell invasion and migration assays. Cells in serum-free DMEM were seeded into the top chamber of the Transwell chamber, whereas DMEM containing 10% FBS was added to the bottom chamber. Cells were cultured for 24 h at 37°C. Cells remaining on the top side of the membrane were removed, and cells in the bottom chamber were stained with 0.1% crystal violet (Sigma-Aldrich). Cells were counted at 10× magnification under a microscope (Olympus, Tokyo, Japan). For the invasion assay, Transwell inserts were precoated with Matrigel matrix (BD Biosciences), and the assay was performed in the same manner as the migration assay.

Dual-Luciferase Reporter Assay

To detect the targeting relationship between miR-342-3p and AEG-1 3′-UTR, the AEG-1 3′-UTR containing the seed-matched sequences or mutated sequences of miR-342-3p was cloned into pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA). The miR-342-3p mimic or miR-342-3p inhibitor and pmirGLO-AEG-1 (WT) or mirGLO-AEG-1 (MT) were cotransfected into the MG63 cells using Lipofectamine 2000. To detect Wnt activity, cells were cotransfected with miR-342-3p mimic or miR-342-3p inhibitor and TOPFlash firefly luciferase reporter vector along with phRL-TK *Renilla* luciferase vectors (Promega). To detect NF-κB activity, cells were cotransfected with miR-342-3p mimic or miR-342-3p inhibitor and pNF-κB-luciferase vector (Promega) along with phRL-TK *Renilla* luciferase vectors (Promega). Relative luciferase activity was detected 48 h after transfection using a dual-luciferase assay kit (Promega).

Western Blot

Cells were lysed, and protein concentrations were measured using a bicinchoninic acid kit (Beyotime, Haimen, P.R. China). Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Millipore, Boston, MA, USA). After blocking with 5% nonfat milk for 1 h, the membrane was immunoblotted with primary antibodies (anti-AEG-1 and anti-GAPDH; Abcam, Cambridge, UK) overnight at 4°C followed by horseradish peroxidase-linked secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The signal was detected using an enhanced chemiluminescence system (Millipore). Gray values of protein bands were measured by Image-Pro Plus 6.0 software.

Data Analysis

Data were reported as the mean±standard deviation. The difference between groups was analyzed using Student's *t*-test or one-way ANOVA followed by the Bonferroni test. Statistical analyses were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). A value of $p<0.05$ was regarded as statistically significant.

RESULTS

miR-342-3p Is Decreased in Osteosarcoma Tissues and Cell Lines

To determine the potential relevance of miR-342-3p in osteosarcoma, we first examined the expression changes in osteosarcoma tissues by RT-qPCR. miR-342-3p was significantly decreased in osteosarcoma tissues compared with adjacent nontumor tissues (Fig. 1A). We further detected the miR-342-3p expression in four osteosarcoma cell lines and in a normal human osteoblast cell line. We found that the expression levels of miR-342-3p in osteosarcoma cell lines were much lower than in normal cell lines (Fig. 1B). This indicates a suppressed expression of miR-342-3p in osteosarcoma.

miR-342-3p Inhibits Proliferation of Osteosarcoma Cells

To investigate the biological function of miR-342-3p, we performed gain-of-function and loss-of-function experiments by transfection of miR-342-3p mimic or miR-342-3p inhibitor into MG63 and U2OS cells. We then detected their effects on osteosarcoma cell proliferation using CCK-8. The results showed that miR-342-3p overexpression significantly inhibited osteosarcoma cell proliferation, whereas miR-324-3p inhibition markedly promoted the proliferation of osteosarcoma cells (Fig. 2A). Furthermore, we detected the role of miR-342-3p on cell cycle progression of osteosarcoma cells by flow cytometry. miR-342-3p overexpression induced an increase in G_0/G_1 phase arrest, whereas miR-342-3p inhibition showed the opposite effect (Fig. 2B). This suggests that miR-342-3p inhibits the proliferation of osteosarcoma cells.

miR-342-3p Inhibits Migration and Invasion of Osteosarcoma Cells

To further investigate the antitumor effect of miR-342-3p on osteosarcoma cells, we analyzed the role of miR-342-3p in regulating osteosarcoma cell migration and invasion. The results showed that both migration (Fig. 3A) and invasion (Fig. 3B) of osteosarcoma cells were significantly reduced by miR-342-3p overexpression. However, miR-342-3p inhibition markedly

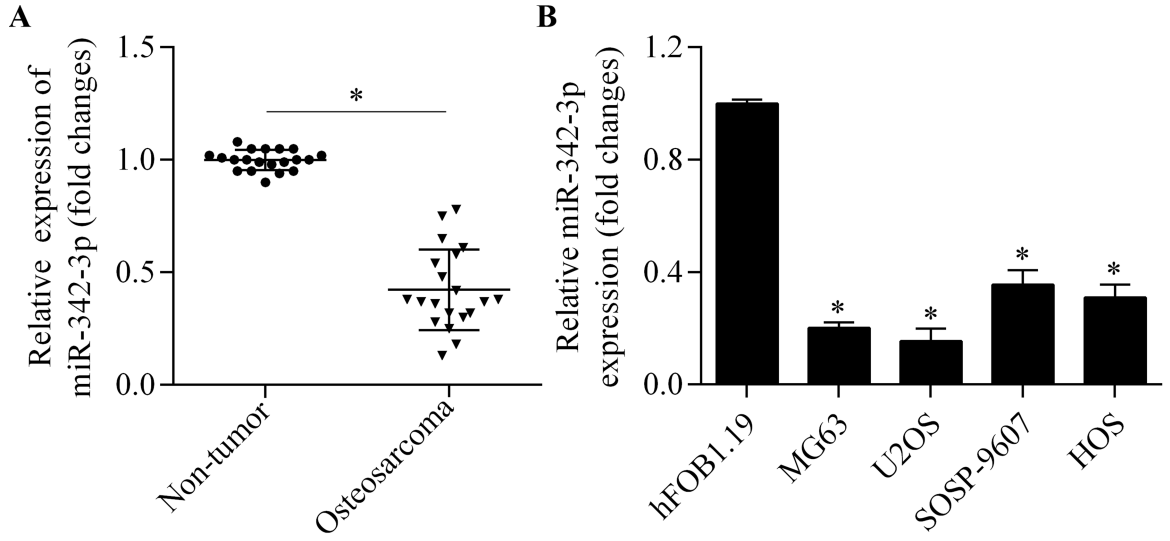

Figure 1. Downregulation of miR-342-3p in osteosarcoma. (A) Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of miR-342-3p expression in osteosarcoma tissues and adjacent nontumor tissues. *$p<0.05$. (B) RT-qPCR analysis of miR-342-3p expression in four osteosarcoma cell lines (MG63, U2OS, SOSP-9607, and HOS) and normal human osteoblast cell line hFOB1.19. *$p<0.05$ versus hFOB1.19.

increased the migration (Fig. 3A) and invasion (Fig. 3B) of osteosarcoma cells. Overall, these results suggest that miR-342-3p impedes the migration and invasion of osteosarcoma cells.

miR-342-3p Targets the 3′-UTR of AEG-1 and Regulates AEG-1 Expression

To identify the functional target of miR-342-3p in osteosarcoma cells, we predicted the targets of miR-342-3p using bioinformatics analysis. AEG-1 was among the predicted target genes of miR-342-3p. AEG-1 is highly expressed in osteosarcoma and relates to tumorigenesis, metastasis, and chemoresistance of osteosarcoma cells[27,29,30]. The putative binding sites are shown in Figure 4A. To test whether AEG-1 is a direct target gene of miR-342-3p, we cloned AEG-1 3′-UTR and its corresponding mutant counterparts into a pmirGLO vector. These vectors were then cotransfected into MG63 cells with miR-342-3p mimic or

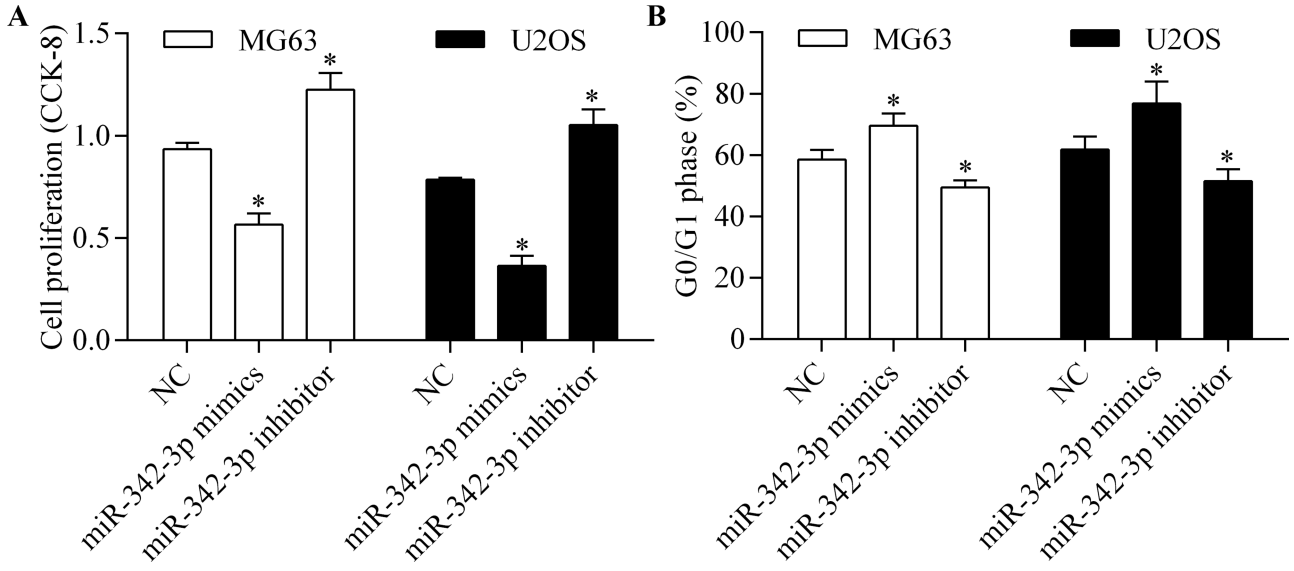

Figure 2. miR-342-3p inhibits osteosarcoma cell proliferation. MG63 and U2OS cells were transfected with miR-342-3p mimics or miR-342-3p inhibitor for 48 h. (A) Cell proliferation was assessed by cell counting kit-8 (CCK-8). (B) Cell cycle distribution was detected by flow cytometry. *$p<0.05$ versus NC.

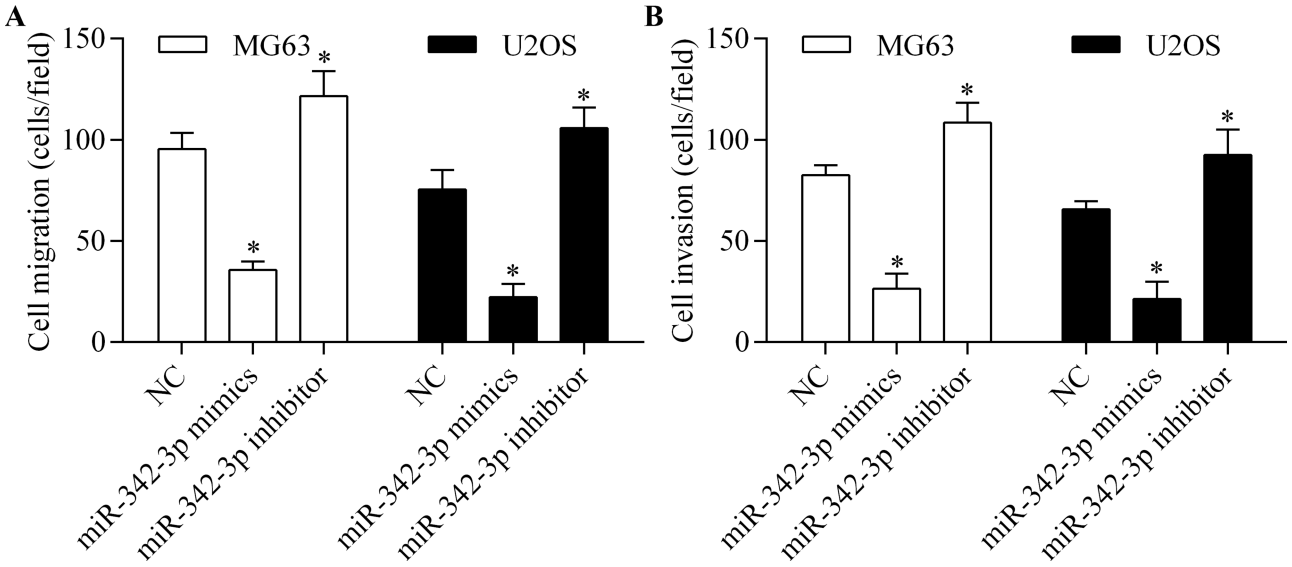

Figure 3. miR-342-3p impedes the migration and invasion of osteosarcoma cells. Cell migration (A) and invasion (B) of MG63 and U2OS cells were detected by Transwell assay. Cells were transfected with miR-342-3p mimic or miR-342-3p inhibitor for 48 h and then subjected to Transwell assay for 24 h. *$p<0.05$ versus NC.

miR-342-3p inhibitor. We found that miR-342-3p overexpression significantly reduced the luciferase activity in cells transfected with the wild-type (WT) 3′-UTR of AEG-1 but not in cells with mutant (MT) 3′-UTR (Fig. 4B). In contrast, miR-342-3p inhibition significantly increased the luciferase activity in cells transfected with the WT 3′-UTR of AEG-1 but not in cells with MT 3′-UTR (Fig. 4B). To test whether AEG-1 expression is regulated by miR-342-3p, we detected the effect of miR-342-3p on AEG-1 expression. We found that miR-342-3p overexpression led to the inhibition of AEG-1 mRNA and protein expression (Fig. 5A–D). In contrast, miR-342-3p inhibition significantly promoted AEG-1 expression (Fig. 5A–D). Taken together, these results indicate that miR-342-3p targets the 3′-UTR of AEG-1 and regulates AEG-1 expression.

miR-342-3p Inhibits the Wnt and NF-κB Signaling Pathways

To further determine the molecular mechanism of miR-342-3p in regulating osteosarcoma cell proliferation, migration, and invasion, we detected the role of miR-342-3p on the Wnt and NF-κB signaling pathways. The results showed that miR-342-3p overexpression significantly inhibited Wnt pathway activity (Fig. 6A) and downstream target gene cyclin D1 expression (Fig. 6B). Moreover, miR-342-3p overexpression markedly suppressed NF-κB pathway activity (Fig. 6C) and downstream target gene MMP-2 expression (Fig. 6D). Overall, these results suggest that miR-342-3p inhibits osteosarcoma cell proliferation, migration, and invasion associated with inhibition of the Wnt and NF-κB signaling pathways.

Overexpression of AEG-1 Rescues miR-342-3p-Induced Antitumor Effects

To further investigate whether miR-342-3p inhibits osteosarcoma cell proliferation, migration, and invasion through AEG-1, cells were cotransfected with miR-342-3p mimics and AEG-1 vector. Western blot showed that the reduced expression of AEG-1 induced by miR-342-3p overexpression was markedly restored by AEG-1 vector

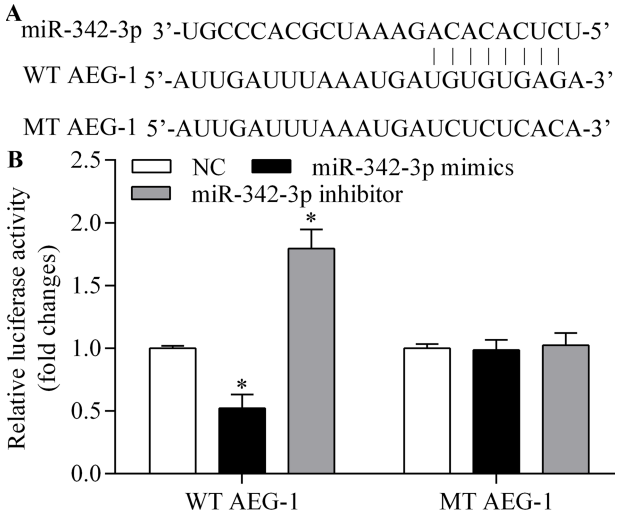

Figure 4. miR-342-3p targets the 3′-untranslated region (3′-UTR) of AEG-1. (A) Schematic diagram of miR-342-3p predicted binding sites for AEG-1 3′-UTR. (B) Dual-luciferase assay of AEG-1 WT and MT 3′-UTR with miR-342-3p mimic or miR-342-3p inhibitor in MG63 cells. *$p<0.05$ versus NC.

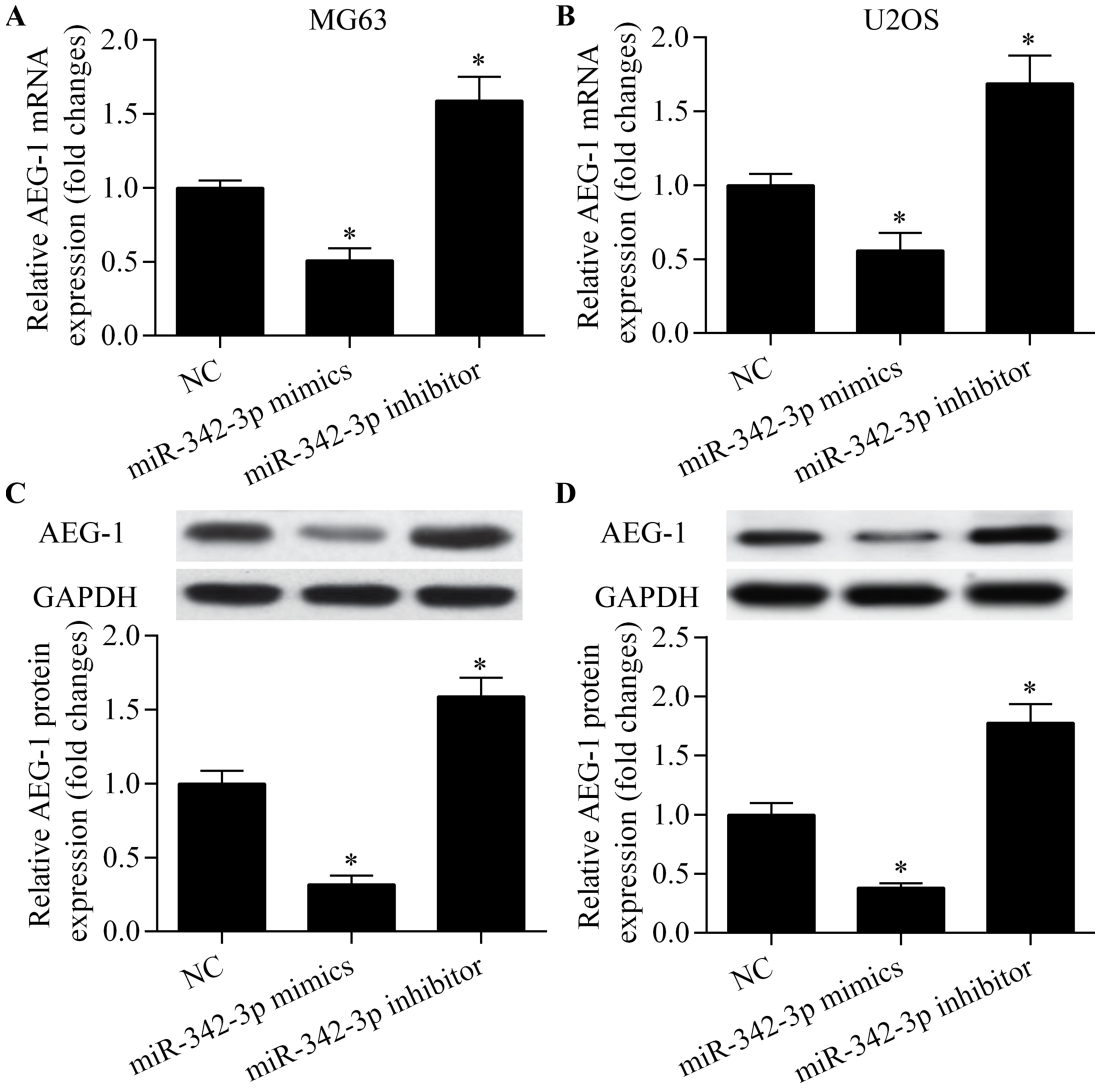

Figure 5. miR-342-3p regulates AEG-1 expression. RT-qPCR of AEG-1 mRNA expression in MG63 (A) and U2OS (B) cells transfected with miR-342-3p mimic or miR-342-3p inhibitor for 48 h. Western blot of AEG-1 protein expression in MG63 (C) and U2OS (D) cells transfected with miR-342-3p mimic or miR-342-3p inhibitor for 48 h. *$p < 0.05$ versus NC.

transfection (Fig. 7A and B). The inhibitory effects of miR-342-3p overexpression on the Wnt (Fig. 7C and D) and NF-κB (Fig. 7E and F) signaling pathways were significantly reversed by AEG-1 overexpression. Furthermore, AEG-1 overexpression partially rescued cell proliferation (Fig. 8A and B), migration (Fig. 8C and D), and invasion (Fig. 8E and F). These results suggest that miR-342-3p inhibits osteosarcoma cell proliferation, migration, and invasion through the downregulation of AEG-1.

DISCUSSION

Altered miRNA expression has been well documented in nearly all human diseases, especially in cancer[10,11]. miRNAs are involved in regulating proliferation, apoptosis, migration, and invasion[8,9]. Increasing evidence suggests that miRNAs also participate in the tumorigenesis of osteosarcoma[14–17]. Therefore, miRNAs have emerged as potential and promising targets for the prognosis, diagnosis, and treatment of osteosarcoma. However, the precise role of miRNAs in osteosarcoma needs to be further studied. In this study, we found that the expression level of miR-342-3p was decreased in osteosarcoma tissue and cell lines. Overexpression of miR-342-3p inhibited osteosarcoma cell proliferation, migration, and invasion. Moreover, AEG-1 was identified as one of the target genes of miR-342-3p, and AEG-1 overexpression abolished the roles of miR-342-3p in osteosarcoma cells. Taken together, our results show that miR-342-3p acts as a tumor suppressor in osteosarcoma progression and development through targeting AEG-1.

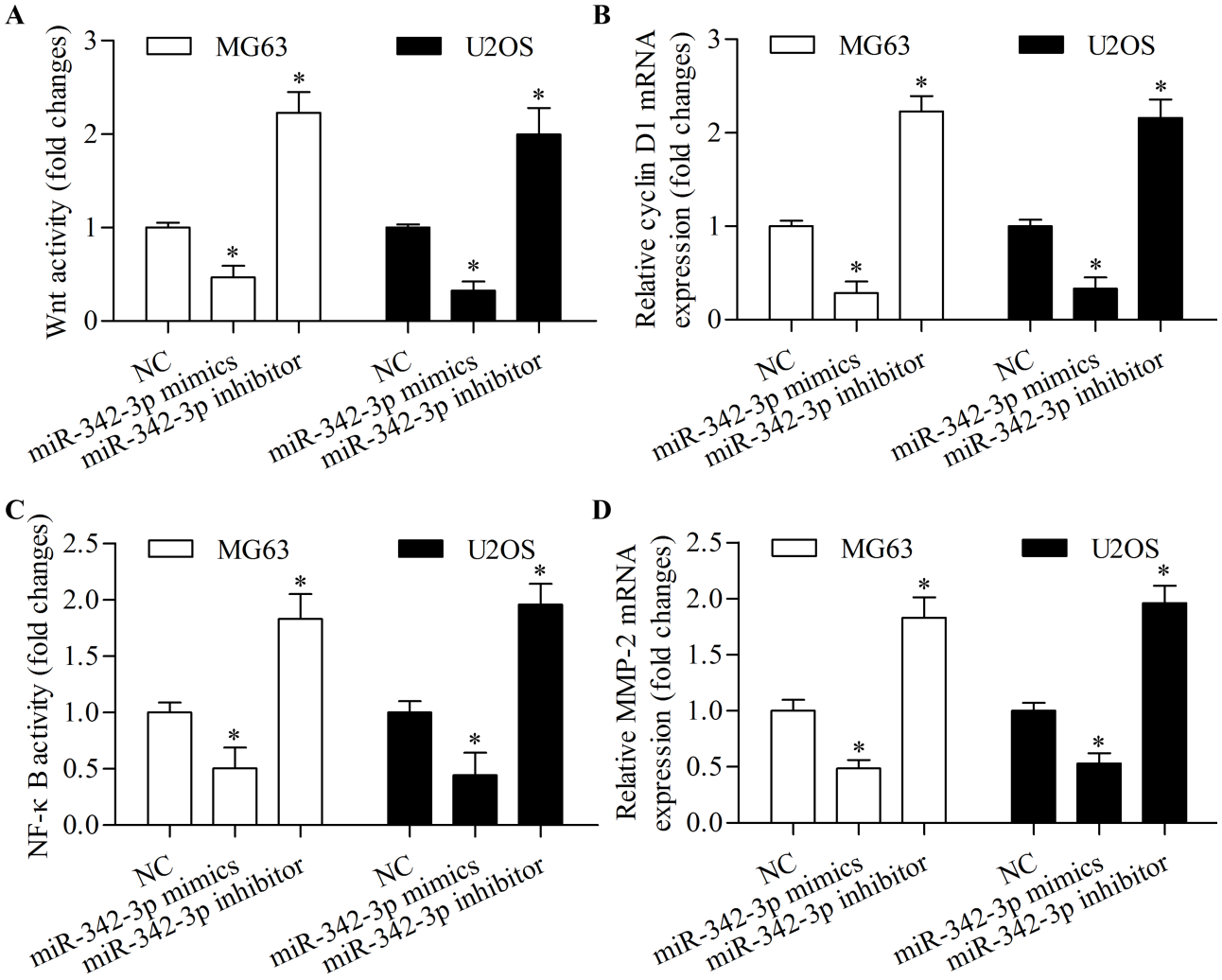

Figure 6. miR-342-3p inhibits the Wnt and nuclear factor κB (NF-κB) signaling pathways. (A) Wnt luciferase assay in MG63 and U2OS cells transfected with miR-342-3p mimic or miR-342-3p inhibitor and TOPFlash luciferase reporter vector for 48 h. (B) RT-qPCR of cyclin D1 mRNA expression in MG63 and U2OS cells transfected with miR-342-3p mimic or miR-342-3p inhibitor for 48 h. (C) NF-κB luciferase assay in MG63 and U2OS cells transfected with miR-342-3p mimic or miR-342-3p inhibitor and NF-κB reporter vector for 48 h. (D) RT-qPCR of MMP-2 mRNA expression in MG63 and U2OS cells transfected with miR-342-3p mimic or miR-342-3p inhibitor for 48 h. *$p<0.05$ versus NC.

miR-342-3p has been reported as a cancer-related miRNA involved in various cancers. miR-342-3p has been suggested to be a predictive biomarker for glioma[34], colon cancer[35,36], and pancreatic cancer[37]. Li et al. reported that miR-342-3p inhibits the proliferation, migration, and invasion of human cervical cancer by targeting the forkhead box protein M1[38]. A recent study showed that the long noncoding RNA H19 promotes the expression of forkhead box protein M1 by competitively binding endogenous miR-342-3p in gallbladder cancer[39]. miR-342-3p inhibits hepatocellular carcinoma cell proliferation by targeting the inhibitor of NF-κB kinase and transforming growth factor-β-activated kinase 1-binding protein 2/3 of the NF-κB signaling pathway[40]. In non-small cell lung cancer, miR-342-3p is found to be decreased, and the overexpression of miR-342-3p inhibits cell proliferation and invasion through targeting the Ras-related protein Rap-2b[33]. miR-342-3p also inhibits the proliferation of lung cancer cells through inhibiting Myc transcriptional activity via targeting E2F transcription factor 1[32]. mR-342-3p is decreased in extranodal natural killer/T-cell lymphoma, nasal type, and may contribute to the pathogenesis by regulating T-lymphoma invasion and metastasis-inducing factor 1[41]. These reports suggest an antitumor role for miR-342-3p. Our results showed that miR-342-3p was decreased in osteosarcoma and that the overexpression of miR-342-3p inhibited osteosarcoma proliferation, migration, and invasion. Our data support a tumor suppressive role for miR-342-3p.

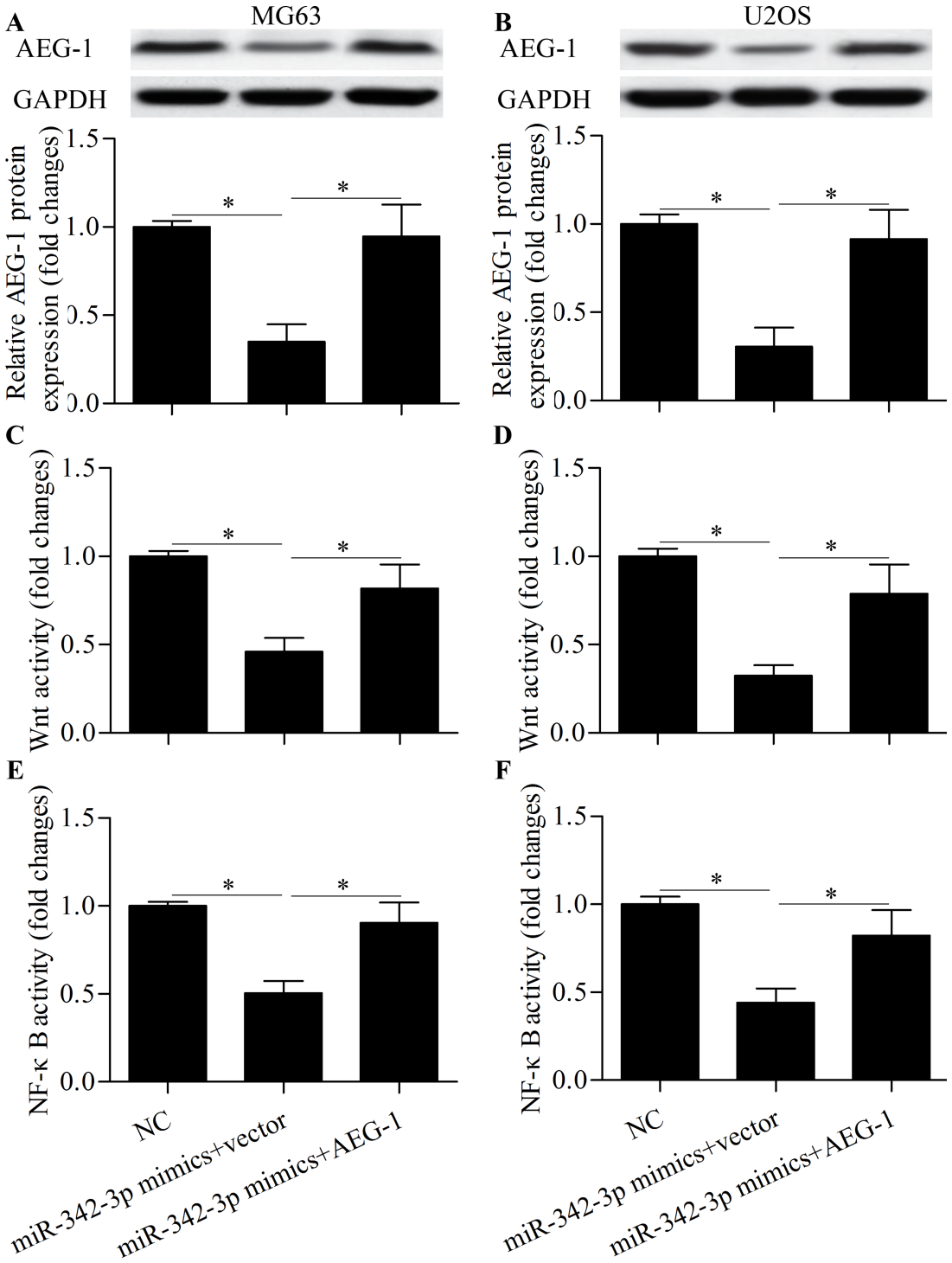

Figure 7. Overexpression of AEG-1 reverses the inhibitory effect of miR-342-3p on the Wnt and NF-κB signaling pathways. Western blot of AEG-1 protein expression in MG63 (A) and U2OS (B) cells transfected with miR-342-3p mimic and AEG-1 vector for 48 h. Wnt luciferase assay in MG63 (C) and U2OS (D) cells transfected with miR-342-3p mimic, AEG-1 vector, and TOPFlash luciferase reporter vector for 48 h. NF-κB luciferase assay in MG63 (E) and U2OS (F) cells transfected with miR-342-3p mimic, AEG-1 vector, and NF-κB reporter vector for 48 h. *$p < 0.05$.

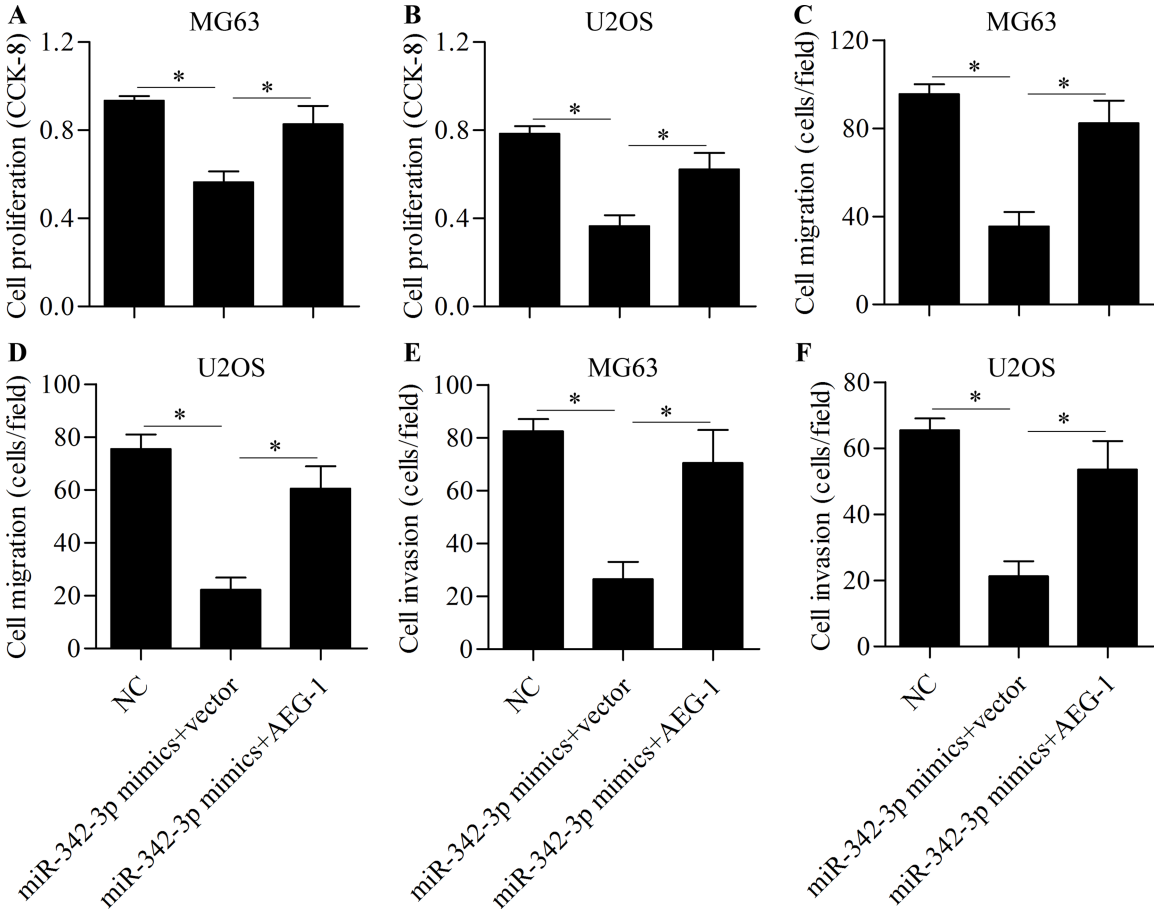

Figure 8. Overexpression of AEG-1 rescues miR-342-3p-induced antitumor effects. Cells were transfected with miR-342-3p mimic and AEG-1 vector for 48 h. Cell proliferation of MG63 (A) and U2OS (B) cells was detected by CCK-8. Cell migration of MG63 (C) and U2OS (D) cells was detected by Transwell migration assay. Cell invasion of MG63 (E) and U2OS (F) cells was detected by Transwell invasion assay. *$p<0.05$.

AEG-1 is a widely studied oncogene in various cancers[18–21]. AEG-1 is also implicated in the tumorigenesis of osteosarcoma. AEG-1 is highly expressed in osteosarcoma tissue, and the overexpression of AEG-1 is strongly associated with clinical stage, metastasis, and poor survival of osteosarcoma patients[27]. Similarly, Tang et al. reported that the overexpression of AEG-1 was significantly associated with metastasis and poor survival of osteosarcoma patients and that the knockdown of AEG-1 inhibited proliferation, migration, and invasion of osteosarcoma cells[28]. AEG-1 promotes the carcinogenesis and invasiveness of osteosarcoma by upregulating MMP-2[27]. Liu et al. reported that AEG-1 promoted osteosarcoma metastasis and chemoresistance by endothelin-1/endothelin A receptor signaling[29]. AEG-1 has been reported to promote osteosarcoma cell invasion through activation of Jun N-terminal kinase/MMP-2 signaling[30]. Furthermore, Wnt/cyclin D1 and NF-κB/MMP-2 signaling are also involved in AEG-1-mediated carcinogenesis and invasiveness of osteosarcoma[31,42]. Thus, AEG-1 serves as a potential therapeutic target for osteosarcoma treatment. In this study, we identified that AEG-1 was a target gene of miR-342-3p. Our findings showed that overexpression of miR-342-3p inhibited proliferation, migration, and invasion of osteosarcoma cells through inhibiting AEG-1 as well as Wnt/cyclin D1 and NF-κB/MMP-2 signaling. Our study suggests that miR-342-3p acts as a tumor suppressor gene, and the decreased expression of miR-342-3p may contribute to the progression and metastasis of osteosarcoma through AEG-1-mediated oncogenic signaling.

A growing body of evidence has suggested that AEG-1 undergoes epigenetic regulation by miRNAs during cancer development and progression[43,44]. Numerous tumor suppressive miRNAs such as miR-137 and miR-375 block tumorigenesis by targeting AEG-1[43,45]. Thus, inhibiting AEG-1 by miRNAs represents a promising therapeutic

strategy for cancer treatment. Interestingly, a recent study revealed that AEG-1 is epigenetically regulated by miR-506 in osteosarcoma cells and that overexpression of miR-506 inhibits tumor growth of osteosarcoma through downregulation of AEG-1[31]. In the present study, we showed that miR-342-3p was a novel regulator for AEG-1 in osteosarcoma. We found that inhibiting AEG-1 by miR-342-3p suppressed the proliferation, migration, and invasion of osteosarcoma cells. Our study suggests that inhibiting AEG-1 by miR-342-3p may be a promising strategy for the treatment of osteosarcoma.

In conclusion, our study reveals that miR-342-3p functions as a tumor suppressor through inhibiting cancer cell proliferation, migration, and invasion by targeting AEG-1 in human osteosarcoma. These findings provide novel insights into understanding the molecular pathogenesis of osteosarcoma. Targeting miR-332-3p may be a potential and promising strategy for the development of novel miRNA-based anticancer therapies for osteosarcoma.

ACKNOWLEDGMENT: The authors declare no conflicts of interest.

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