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Original Paper

MicroRNA-27a Promotes Proliferation, Migration and Invasion by Targeting *MAP2K4* in Human Osteosarcoma Cells

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Key Words
miR-27a • MG63 cells • Proliferation • Migration • Invasion • *MAP2K4* • Osteosarcoma

Abstract
Background: Osteosarcoma is a high-grade malignant bone neoplasm. Although the introduction of chemotherapy has reduced its mortality, more than 50% of patients develop chemoresistance and have an extremely poor prognosis due to pulmonary metastasis. Several molecular pathways contributing to osteosarcoma development and progression have recently been discovered. Various studies have addressed the genes involved in the metastasis of osteosarcoma. However, the highly complex molecular mechanisms of metastasis are still poorly understood. Recently, the decisive role of microRNAs in the regulation of molecular pathways has been uncovered. miRNAs may function as either oncogenes or tumor suppressors, depending on their target genes. miR-27a, a member of an evolutionarily conserved miRNA family, is abnormally increased in several types of cancers. It has been shown to be upregulated in osteosarcoma and plays a pro-metastatic role in osteosarcoma cell lines. However, the effects of miR-27a on osteosarcoma have not been clearly elucidated. The present study thus addressed the miR-27a sensitive mechanisms in osteosarcoma. ***Methods:*** In this study, three biological programs were used to predict whether *MAP2K4* was a target of miR-27a. A specific miR-27a inhibitor was used to inhibit the endogenous activity of miR-27a in the human osteosarcoma cell line MG63. Cell proliferation, colony formation, migration and invasion assays were performed to assess the effects of miR-27a on the proliferation, metastasis and invasion of MG63 cells. The expression levels of several proteins evolved in the *JNK/p38* signaling pathway were detected using western blot analysis. ***Results:*** The luciferase activity of the wild-type *pGL3-MAP2K4* 3'UTR vector was significantly inhibited after the miR-27a precursor or the control precursor was transfected into the MG63 cells. However, the luciferase activity was not inhibited after transfection of the mutant *pGL3-MAP2K4* 3'UTR vector. The inhibition of miR-27a increased the luciferase activity of the wild-type *pGL3-MAP2K4* 3'UTR

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vector after MG63 cells were transfected with the miR-27a inhibitor or the control inhibitor. Thus, *MAP2K4* is a potential target of miR-27a and can be directly regulated by miR-27a. Inhibition of miR-27a significantly suppressed cell proliferation after 72 hours compared to the negative control group. Inhibition of miR-27a significantly suppressed colony formation of the MG63 cells by 39.6%. Transwell migration and invasion assays demonstrated that the number of migratory and invasive cells transfected with the miR-27a inhibitor was reduced by 63.5% and 69.1%, respectively. After transfection of the miR-27a inhibitor into the MG63 cells, the level of phospho-*JNK1* and phospho-*p38* increased by 25% and 29%, respectively, along with the up-regulation of *MAP2K4* protein. ***Conclusion:*** This is the first study showing that miR-27a can function as an oncogene by targeting *MAP2K4* in the osteosarcoma MG63 cell line. Inhibition of miR-27a increases *MAP2K4* expression, which in turn inhibits cell proliferation and migration through the *JNK/p38* signaling pathway in MG63 cells. These findings may help us understand the molecular mechanism of miR-27a in the tumorigenesis of osteosarcoma and may provide new diagnostic and therapeutic options for the treatment of this neoplasia.

Introduction

Osteosarcoma, which accounts for approximately 60% of malignant bone tumors during the first 2 decades of life, is a high-grade malignant bone neoplasm that is primarily observed in children and adolescents [1, 2]. Osteosarcoma frequently occurs in the long bones and preferentially metastasizes to the lung [1]. The treatment for osteosarcoma is very challenging. Although the introduction of chemotherapy has reduced the mortality, more than 50% of patients who are chemoresistant have an extremely poor prognosis due to pulmonary metastases. The 5-year disease-free survival rate in the patients without metastatic disease can reach up to 60–70%, while in patients with metastatic disease, it is as low as 10–20% [3]. Molecular pathways contributing to osteosarcoma development and progression have recently been discovered. Various studies have been carried out to investigate the genes that are involved in the metastasis of osteosarcoma [4, 5]. This may facilitate better diagnosis, as well as the development of new treatment strategies. Despite the molecular alterations that contribute to the metastasis of osteosarcomas, osteosarcomas are becoming increasingly understood. However, the highly complex molecular mechanisms of metastasis are still poorly understood. Recently, microRNAs have become a new research "hot topic" in the field of molecular pathways.

MicroRNAs (miRNAs), which can regulate gene expression at the post-transcriptional level by inhibiting the translation of an mRNA or by promoting mRNA degradation, are endogenous, noncoding, single-stranded RNAs that are 19-25 nucleotides in length [6]. Currently, more than 1000 miRNAs have been identified in humans, and an individual miRNA may regulate hundreds of protein-coding genes [7]. However, most of the biological functions of these genes remain unknown, and only a few mRNAs that are directly regulated by miRNAs in animals have been verified empirically. Comparison between human cancer tissues and their normal tissue counterparts have revealed distinct miRNA expression profiles. Additionally, miRNAs may function as either oncogenes or tumor suppressors by specifically regulating the expression of their target genes.

Various studies have investigated the role of miRNAs in osteosarcoma using miRNA expression profiles. Ziyan et al. [8] demonstrated that miR-21 is significantly overexpressed in osteosarcoma, and the suppression of miR-21 decreased the invasion and migration ability of the MG63 osteosarcoma cell line. Kobayashi et al. [9] found that miR-199a-3p, miR-127-3p, and miR-376 were significantly decreased in the osteosarcoma cell lines compared to osteoblasts, while the expression levels of miR-151-3p and miR-191 were increased. Lulla et al. [10] found twenty-two differentially expressed miRNAs in osteosarcoma, and miR-135b, miR-150, miR-542-5p, and miR-652 were all highly expressed in osteosarcoma

tumor samples compared to normal osteoblasts. A study by Osaki et al. [11] showed that injection of miR-143 could suppress the lung metastasis of osteosarcoma in mice injected with atelocollagen.

miR-27a, a member of an evolutionarily conserved miRNA family, is abnormally increased in several types of cancers, such as breast cancer [12, 13], gastric adenocarcinoma [14] and colon cancer [15]. Recently, miR-27a was identified as being elevated in osteosarcoma, and it has been shown to play a pro-metastatic role in osteosarcoma cell lines [16]. However, the effects of miR-27a on osteosarcoma have not been clearly elucidated. Therefore, it is of great significance to further study the mechanism of miR-27a in osteosarcoma. In this study, a specific miR-27a inhibitor was used to inhibit endogenous miR-27a activity in the human osteosarcoma cell line MG63. These results showed that the inhibition of miR-27a caused profound suppression of metastasis in the MG63 cell line by targeting the tumor metastasis suppressor gene mitogen activated protein kinase kinase 4 (*MAP2K4*), which acts as an agonist of the JNK/p38 signaling pathway in tumor suppression. These findings may increase the understanding of the functional role of miR-27a in the growth and metastasis of osteosarcoma.

Materials and Methods

Cell culture and transfection

MG63 human osteosarcoma cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) were maintained in RPMI-1640 medium (Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 2.0 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were incubated at 37°C in a humidified incubator supplemented with 5% CO_2 and 95% air.

MG63 cells were allowed to attach overnight in 48-well plates (4×10^4 cells in each well). On the following day, the miR-27a inhibitor or the negative control inhibitor (Ambion, Austin, Texas, USA) were individually transfected into MG63 cells using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After 6 h, the original medium was replaced with fresh medium. The expression of miR-27a was detected by northern blot analysis 48 h after transfection.

Plasmid construction

To obtain a miR-27a expression vector, a 305 bp fragment containing the miR-27a genomic sequence was amplified by PCR from MG63 genomic DNA and cloned into a modified pcDNA6.2-GW/EmGFP vector (Invitrogen, Carlsbad, CA, USA) between the *Xho* Ⅰ and *Bgl* Ⅱ restriction sites. The following PCR primers were used to amplify miR-27a: sense: 5'- CTC GAG AGG AGT TTC CCC TTC CCT GGA GC -3' and antisense: 5'- AGA TCT GGC CCT AGG CAG ATG GTG GCA -3'. The *Xho* Ⅰ and *Bgl* Ⅱ sites are underlined. The construct was verified by DNA sequencing.

Luciferase reporter assays

The 3'UTR of human *MAP2K4*, which contains a miR-27a binding site, was PCR-amplified from MG63 genomic DNA (PCR primers, sense: 5'- GCC GCT CGG CTC TTC ACT CC -3' and antisense: 5'- CCA CAA GCT GTC CAC TGA TGC CG -3') and cloned downstream of the luciferase reporter gene in a modified pGL3-Control vector (Ambion, Austin, TX). The resulting plasmid was designated *pGL3-MAP2K4* 3'UTR. To generate the *pGL3-MAP2K4* 3'UTR-Mut construct, the seed regions were mutated from ACUGUGA to UGACACU, thus removing all complementation to nucleotides 1-7 of miR-27a (QuickchangeXL Mutagenesis Kit; Stratagene, La Jolla, CA).

MG63 cells were seeded into 24-well plates (1×10^5 per well). After 24 h, the cells were co-transfected with the pGL3 vector containing the 3'UTR fragment of *MAP2K4*, a *Renilla* vector and pcDNA6.2-miR-27a or pcDNA6.2-miR-neg, using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Firefly and *Renilla* Luciferase activities were measured 24 h after transfection using the Dual Luciferase Reporter Assay System (Promega, WI, USA). Firefly luciferase activity was normalized to *Renilla* luciferase expression for each sample.

Knock-down of MAP2K4 in the MG63 cell line

This analysis was performed using the MG63 cell line and the miR-27a knockdown MG63 cell line described above. Reagents for transient gene knockdown were purchased from Dharmacon: small interfering RNA (siRNA) against MAP2K4 (SMARTPool for MAP2K4; product L-003574-00; Dharmacon, Lafayette, CO) and non-targeting control siRNA (siCO; product D-001810-0X; Dharmacon). For RNA interference studies, the cells were transfected with siRNAs against MAP2K4 or with the non-targeting control siRNAs, as indicated, with DharmaFECT (Dharmacon) as previously described [17].

Cell proliferation assay

Cell proliferation capacity was evaluated using a methyl thiazole tetrazolium (MTT) assay, as previously described [17]. Briefly, MG63 cells were seeded into 96-well plates at a density of 3000 cells/well and were transfected with 50 nM miR-27a inhibitor or a negative control inhibitor. After 24 h culture, 20 µl of 5 mg/ml MTT (dimethyl thiazolyl diphenyl tetrazolium; Sigma, Deisenhofen, Germany) reagent was added to each well, and the cells were incubated for another 4 h at 37°C. Optical density (OD) was assessed by measuring the absorbance at 450 nm with a microtiter plate reader (Bio-Rad Labs, Sunnyvale, CA).

Colony formation assay

MG63 cells were treated with the miR-27a inhibitor or the negative control inhibitor for 24 h and were then seeded for colony formation in 24-well plates at a density of 500 cells per well. After 14 days, the colonies were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet solution.

Migration and invasion assay

MG63 cells were transfected with 50 nM miR-27a inhibitor or the negative control for 24 h. To measure cell invasion, 8 µm pore polycarbonate membrane inserts (Transwell; Costar, Cambridge, MA) were placed into the wells of 24-well culture plates, separating the upper and the lower chambers. The filter of the top chamber was coated with 50 µl of diluted Matrigel following the standard procedure and was incubated at 37°C for 2 h. Then, 1×10^5 cells per well were added to the top chamber of the transwell insert in serum-free media. The bottom chamber was filled with media containing 10% FBS. After 24 h of incubation at 37°C with 5% CO_2, the number of cells that had migrated through the pores was quantified by counting five random fields under a microscope (Zeiss, Oberkochen, Germany). The same experiments were independently repeated three times. The procedure for the transwell migration assay was the same as the transwell invasion assay, except that the filter in the top chamber was not coated with Matrigel.

Western blotting analysis

Total cellular proteins were extracted from MG63 cells transfected with the miR-27a inhibitor or a negative control. Extracted proteins (20 µg) were separated on 15% SDS-PAGE gels and then transferred onto PVDF membranes (Invitrogen, Carlsbad, CA, USA) by a constant voltage of 100 V for 1 h. After blocking with BSA for 1 h, a mouse anti-human antibody for MAP2K4 (1:1000), phospho-JNK1 (1:200), phospho-p38 (1:1000) or β-actin (1:500) (Abcam, Cambridge, MA) was added and incubated on a shaking table at room temperature for 2 h. Each membrane was washed three times in TBST and incubated with a secondary rabbit anti-mouse IgG (1:1000; Abcam, Cambridge, MA), conjugated with horseradish peroxidase in TBST with 0.5% BSA for 2 h at room temperature. Band signals were acquired in the linear range of the scanner and were analyzed using the Quantity One software.

Statistical analyses

All data are expressed as the mean ± SD. Differences between the groups were assessed by unpaired, two-tailed Student's *t*-test, and $p< 0.05$ was considered significant.

Results

Inhibition of miR-27a suppresses proliferation of MG63 cells

To evaluate the effects of miR-27a on the proliferation of MG63 osteosarcoma cells, MG63 cells were transfected with a specific inhibitor of miR-27a. An MTT assay was performed

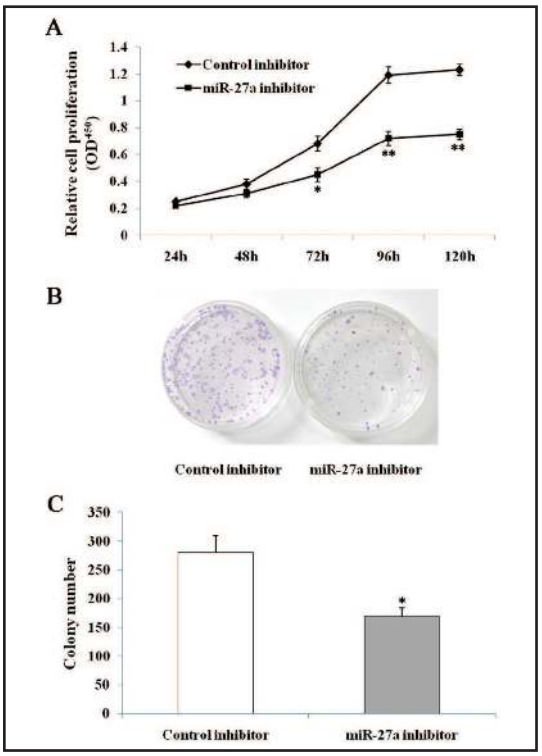

Fig. 1. Inhibition of miR-27a suppresses the proliferation and colony formation of MG63 cells. (A) The proliferation of MG63 cells transfected with either the control inhibitor or the miR-27a inhibitor was detected using the MTT assay at 24, 48, 72, 96 and 120 h. The values represent the mean ± SD of three replicates (*p<0.05; **p<0.01). (B) Cell colony formation was measured using a soft agar colony formation assay. (C) The number of colonies was counted 14 days after seeding the transfected cells. The values represent the mean ± SD of three replicates (*p<0.05).

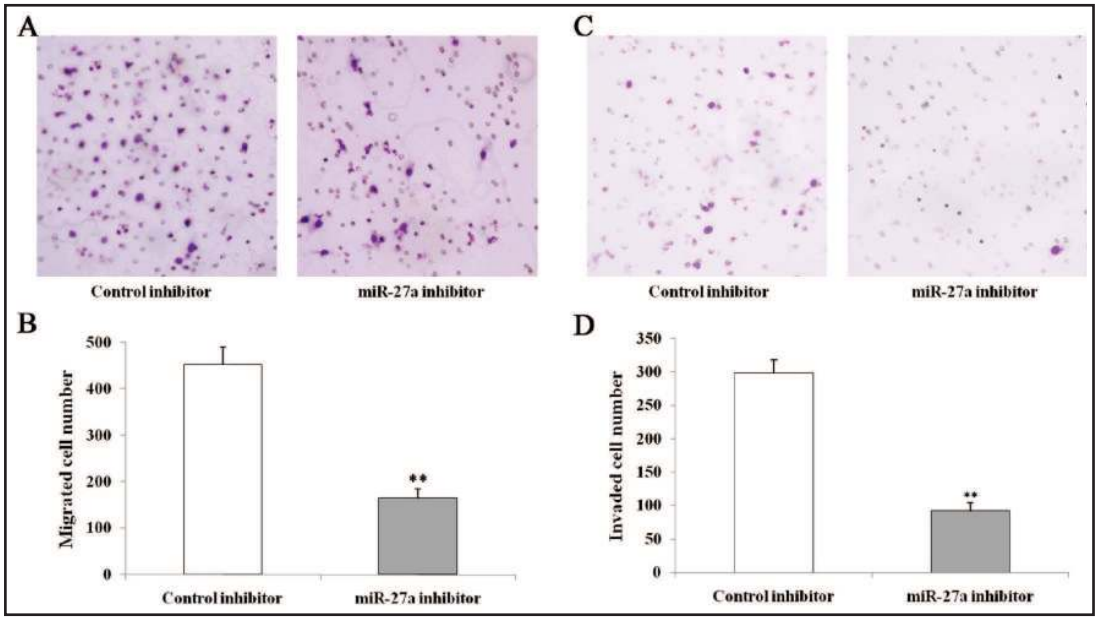

Fig. 2. Inhibition of miR-27a inhibits the migration and invasion of MG63 cells. (A, C) Transwell assays were employed to evaluate the migratory and invasive ability of the cells. (B, D) Quantitative results for the migratory and invasive ability of each group are shown as the number of migrated and invaded cells 24 h after incubation. The values represent the mean ± SD of three replicates (**p<0.01).

following the procedure described in the Methods section every 24 h. It was observed that, after 72 hours, cell proliferation was significantly suppressed by the inhibition of miR-27a compared to the negative control group (Fig. 1A). The role of miR-27a in colony formation was also evaluated. The miR-27a inhibitor-transfected cells formed fewer clones than the negative control group (Fig. 1B). Statistical analysis indicated that the inhibition of miR-27a suppressed the colony formation of MG63 cells by 39.6% (Fig. 1C).

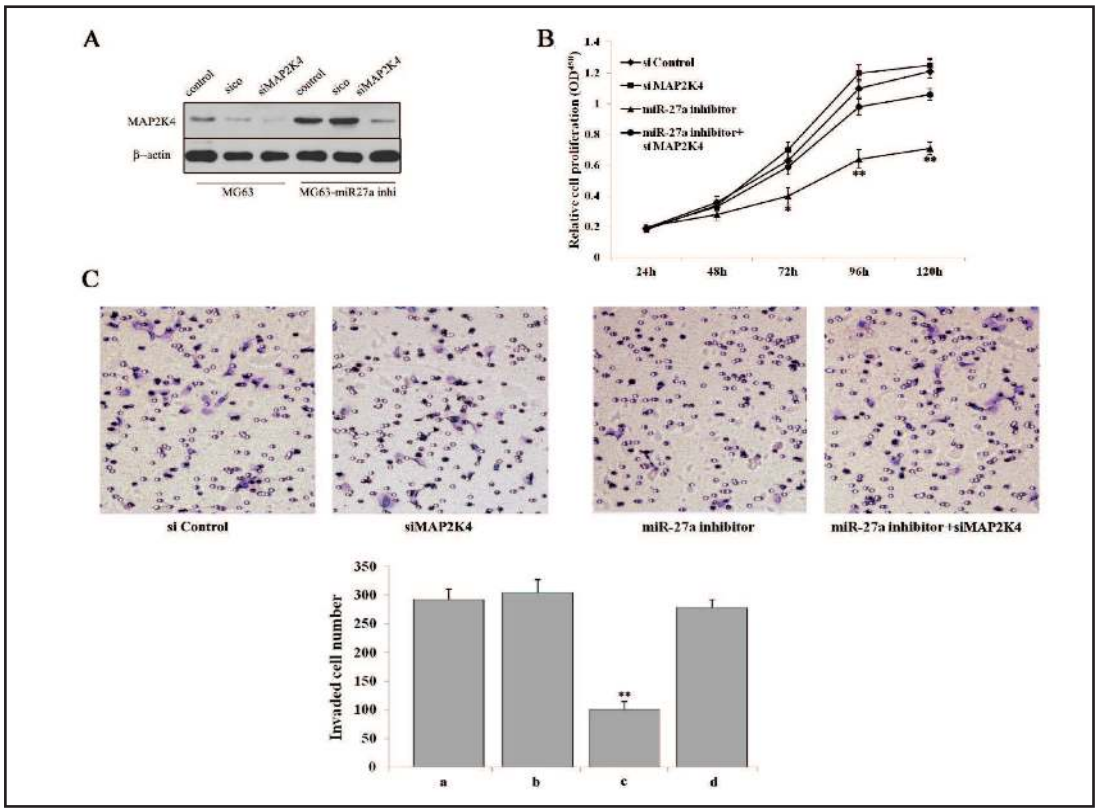

Fig. 3. Knockdown of MAP2K4 expression neutralized the effect of miR-27a Inhibition. (A) Western blot measurements were performed after silencing MAP2K4 by transfection of siRNA in MG63 cells and the miR-27a inhibition MG63 cells. (B) The proliferation of MG63 cells transfected with control siRNA, MAP2K4 siRNA, miR-27a inhibitor, or both MAP2K4 siRNA and the miR-27a inhibitor was detected using an MTT assay at 24, 48, 72, 96 and 120 h. The values represent the mean ± SD of three replicates (*p<0.05; **p<0.01). (C) Transwell assays were employed to evaluate the invasive ability of the cells, and quantitative results for the invasive ability of each group are shown as the number of invaded cells 24 h after incubation. The values represent the mean ± SD of three replicates (**p<0.01). (a: control siRNA, b: MAP2K4 siRNA, c: miR-27a inhibitor, d: transfected with both MAP2K4 siRNA and miR-27a inhibitor).

Inhibition of miR-27a inhibits the migration and invasion of MG63 cells

Transwell migration and invasion assays were performed to determine whether miR-27a was involved in the migratory and invasive behaviors of MG63 cells. The results demonstrated that transfection with the miR-27a inhibitor reduced the migration and invasion of the cells by 63.5% and 69.1%, respectively (Fig. 2B, D), as a significantly lower number of the transfected cells migrated than with the negative control inhibitor (Fig. 2A, C). These results strongly indicated that miR-27a played an oncogenic role in the promotion of migration and invasion of osteosarcoma *in vitro*.

MAP4K4 silencing prevents the effect of miR-27a Inhibition on MG63 cells

To decrease the expression of *MAP2K4*, the MG63 cell lines were transfected with *MAP2K4*-specific siRNA or a control siRNA. In both the MG63 cell line and the MG63 cell line transfected with miR-27a inhibitor, the cells transfected with *MAP2K4*-specific siRNA contained substantially lower levels of *MAP2K4* protein (Fig. 3 A) than the cells transfected with control siRNA.

To evaluate the effects of MAP4K4 on the proliferation, migration and invasion of MG63 osteosarcoma cells, MTT and transwell assays were performed following the procedures described in the Methods section. During this time course, the MG63 cell line showed a

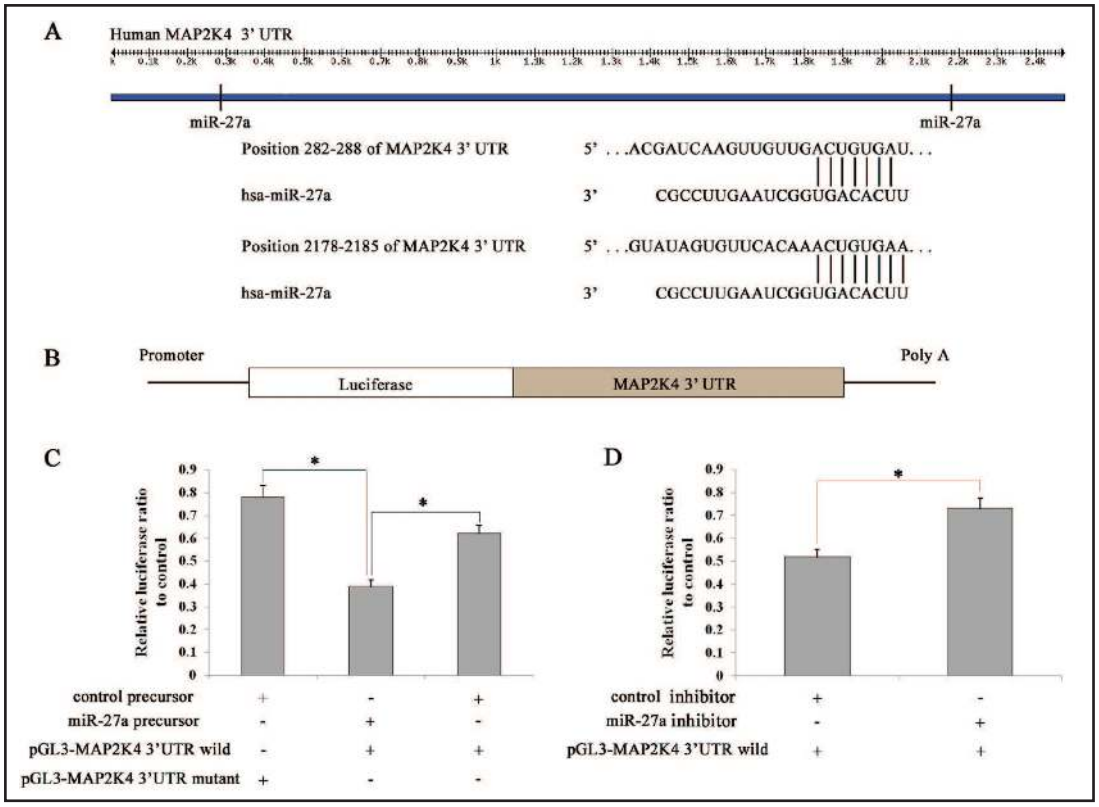

Fig. 4. Identification of the miR-27a targets using the dual luciferase reporter gene assay. (A) Specific locations of the binding sites in the *MAP2K4* 3'UTR. Alignment between the predicted miR-27a target sites and miR-27a. The conserved 7 bp "seed" sequence for miR-27a mRNA pairing is indicated. (B) Diagram of the luciferase reporter gene construct containing the *MAP2K4* 3'UTR that was cloned from *MAP2K4* mRNA in MG63 cells. (C, D) Direct effect of miR-27a on the *MAP2K4* 3'UTR luciferase activity of the wild-type *pGL3-MAP2K4* 3'UTR vector. Activity was significantly inhibited after transfection of the miR-27a precursor or the control precursor, but the activity of the mutant-type *pGL3-MAP2K4* 3'UTR vector was not changed (C). Inhibition of miR-27a increased the luciferase activity of the wild-type *pGL3-MAP2K4* 3'UTR vector after transfection of the MG63 cells with the miR-27a inhibitor or the control inhibitor (D). The values represent the mean ± SD of three replicates (*p<0.05).

reduction in cell number and invasion when treated with the miR-27a inhibitor. The data showed that there was no significant difference between the cells treated with the MAP2K4 siRNA and the cells treated with control siRNA. However, it was observed that knockdown of MAP2K4 significantly suppressed the effect of miR-27a inhibition on cell proliferation and invasion in the MG63 cells (Fig. 3 BC).

miR-27a directly targets the MAP2K4 3'UTR

TargetScan (http://www.targetscan.org) analysis was conducted to predict the target mRNAs of miR-27a. As a result, two potential binding sites of miR-27a were predicted in the 3'UTR of the *MAP2K4* mRNA (Fig. 4A), and these sites were confirmed by miRanda and PicTar. Alignment between the predicted miR-27a target sites and the conserved 7-bp "seed" sequence for miR-27a is shown in Fig. 3A. To confirm the specific regulation of *MAP2K4* through the two predicted binding sites, the *MAP2K4* 3'UTR sequence was cloned into the pGL3 vector downstream of the luciferase reporter gene (Fig. 4B). Mutations in the putative binding sites were prepared as described in the Materials and Methods section. As expected, the luciferase activity of the wild-type *pGL3-MAP2K4* 3'UTR vector was significantly inhibited after transfecting MG63 cells with the miR-27a precursor or the control precursor,

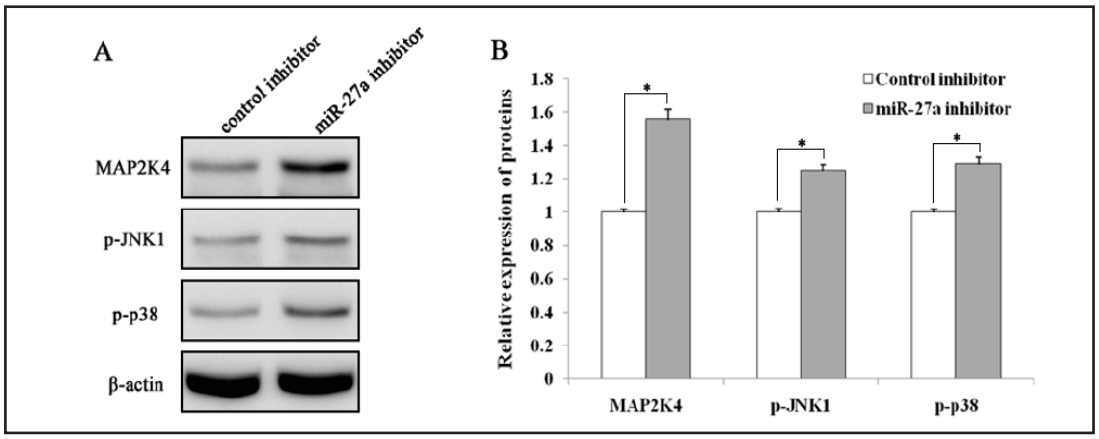

Fig. 5. Inhibition of miR-27a increased *MAP2K4* expression and enhanced the phosphorylation of *JNK1* and *p38*. (A) The protein levels of *MAP2K4* and *phospho-JNK1/p38* were detected using western blot analysis after transfection for 48 h. (B) ImageJ 2.1.4.6 was used to calculate the relative quantity of *MAP2K4* and *phospho-JNK1/p38*. The values represent the mean ± SD of three replicates (*p<0.05).

but the luciferase activity of the mutant *pGL3-MAP2K4* 3'UTR vector was not affected (Fig. 4C). Meanwhile, the inhibition of miR-27a increased the luciferase activity after transfection with the miR-27a inhibitor or the control inhibitor using the wild-type *pGL3-MAP2K4* 3'UTR vector (Fig. 4D). These results demonstrated that *MAP2K4* is a potential target of miR-27a and can be directly regulated by miR-27a.

Inhibition of miR-27a increases the expression of MAP2K4 in MG63 cells

To determine whether miR-27a played a functional role in modifying endogenous *MAP2K4* expression, MG63 cells were transfected with the miR-27a inhibitor or the control inhibitor, and the results showed that the protein level of *MAP2K4* was increased in the miR-27a inhibitor-treated cells (Fig. 5A), indicating that miR-27a can affect *MAP2K4* protein levels.

MAP2K4 can regulate various cellular activities such as proliferation, differentiation, and apoptosis by activating the *JNK/p38* signaling pathway, thus inhibiting tumor metastasis [18]. Therefore, the effect of miR-27a inhibition on the phosphorylation of *JNK1* and *p38* was also measured. As shown in Fig. 4A, after transfecting the MG63 cells with the miR-27a inhibitor, the level of *phospho-JNK1* and *phospho-p38* increased by 25% and 29%, respectively, along with an increase in the level of *MAP2K4* protein (Fig. 5B). This suggests that miR-27a may be involved in the *JNK/p38* signaling pathway by affecting *MAP2K4* expression.

Discussion

Uncontrolled cell proliferation and aggressive tumor cell metastasis are two essential steps during cancer progression. More and more emerging evidence has indicated that miRNAs may play regulatory roles in cell proliferation and metastasis of cancer in humans [19]. Therefore, to understand the functional mechanism of miRNAs, it is paramount to identify the targets that are involved in the regulation process. Recently, overexpression of miR-27a was observed in osteosarcoma samples [16]. Our study demonstrated that the proliferation, migration and invasion of MG63 cells can be significantly suppressed by the inhibition of miR-27a *in vitro*. These results indicate that miR-27a may play an oncogenic role in the development of osteosarcoma.

Previous studies have identified several tumor suppressors as targets of miR-27a, including *ZBTB10* in breast cancer [13], gastric cancer [20] and colon cancer [15], *Sprouty2* in pancreatic cancer [21], and *FOXO1* in breast cancer [12]. For the first time, *MAP2K4* was

identified as a direct functional target of miR-27a in osteosarcoma MG63 cells. For this identification, it was shown that the 3'UTR of *MAP2K4* contained two binding sites that matched the miR-27a seed sequence. Furthermore, over-expression of miR-27a led to a decrease in the luciferase activity of the wild type *MAP2K4* 3'UTR in MG63 cells, whereas a site mutation abolished the miR-27a regulation. Additionally, the inhibition of miR-27a increased the luciferase activity of the wild type 3'UTR of *MAP2K4* and upregulated *MAP2K4* expression. Therefore, it was found that miR-27a acts directly on the 3'UTR of *MAP2K4* in MG63 cells.

*MAPK*s are a family of conserved Ser/Thr protein kinases that transmit extracellular signals into the cytoplasm. The different *MAPK*s can be divided into 3 main subgroups, including the extracellular signal-regulated protein kinase (*ERK*), *c-Jun* N-terminal kinase/stress-activated protein kinases (*JNK/SAPK*), and p38 [22]. *MAP2K4*, also known as *MKK4*, can directly phosphorylate *JNK* or *p38* with dual specificity [23], which contributes to tumorigenesis and metastasis [24-28]. A large body of evidence has indicated the possible roles of *MAP2K4* in cancer, including pancreatic cancer [18, 29], ovarian cancer [30], and lung adenocarcinoma [31]. The expression of *MAP2K4* was low in the osteosarcoma MG63 cells, which were examined in this study.

To explore the role of MAP2K4 in the functions of miR-27a, we knocked down MAP2K4 in MG63 cells using siRNA and assessed cellular proliferation and invasion. The expression of MAP2K4 in the siRNA-transfected cells was also evaluated. Compared to the control group, highly efficient gene silencing was observed in the MG63 cell line after transfection with the miR-27a inhibitor. The results also showed that MAP2K4 inactivation correlated with the increased proliferation and invasiveness of the MG63 cell line, as well as neutralized the effect of miR-27a inhibition. This finding supports a tumor suppressor role for MAP2K4 in osteosarcoma and suggests that MAP2K4 is probably the central link involved in the miR-27a functional pathway.

It was found that *MAP2K4* expression and the *phospho-JNK1/p38* levels can be increased by the inhibition of miR-27a, meaning that miR-27a may participate in the modulation of malignant biological behavior, and a decrease in *MAP2K4* by miR-27a provides a proliferative advantage or performs anti-apoptotic function in tumor cells by permitting the deactivation of the *JNK/p38* pathway.

In summary, this is the first study to propose that miR-27a functions as an oncogene targeting *MAP2K4* in the osteosarcoma MG63 cell line. Inhibition of miR-27a increased *MAP2K4* expression, which in turn inhibits cell proliferation and migration through the *JNK/p38* signaling pathway in MG63 cells. These findings may help us understand the molecular function of miR-27a in osteosarcoma tumorigenesis and may provide new diagnostic and therapeutic options for treatment of this neoplasia.

Conflict of Interest

None declared.

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