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Down-regulation of MIAT suppresses osteosarcoma progression by acting as a ceRNA for miR-141-3p to regulate SIX1-mediated PI3K/AKT pathway

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Abstract. – OBJECTIVE: Osteosarcoma (OS) is a frequent bone malignancy. Long non-coding RNA myocardial infarction associated transcript (MIAT) has been reported to be involved in the development of human cancers, including OS. However, the mechanism underlying MIAT in OS progression remains largely unclear.

PATIENTS AND METHODS: The expression levels of MIAT and sineoculis homeobox homolog 1 (SIX1) in OS tissues and cells were detected by quantitative real-time polymerase chain reaction and Western blot. Cell viability, apoptosis, migration and invasion of OS cells were determined by MTT, flow cytometry and transwell assays, respectively. The target interaction among MIAT, miR-141-3p and SIX1 was analyzed by bioinformatics analysis and luciferase reporter assay. Phosphatidylinositide 3-kinases (PI3K)/protein kinase B (AKT) pathway was evaluated by Western blot.

RESULTS: MIAT and SIX1 expression levels were enhanced in OS tissues and cells. Knockdown of MIAT or SIX1 repressed cell viability, migration and invasion but promoted apoptosis in OS cells. Moreover, overexpression of SIX1 reversed the inhibitive role of MIAT silence in OS progression. Furthermore, MIAT could increase SIX1 expression by competitively sponging miR-141-3p. Besides, inhibition of MIAT blocked PI3K/AKT pathway by decreasing SIX1 in OS cells.

CONCLUSIONS: MIAT silence suppresses OS progression through inactivating PI3K/AKT signaling by sponging miR-141-3p to regulate SIX1, indicating a novel target for the treatment of OS.

Key Words:
Osteosarcoma, MIAT, SIX1, PI3K/AKT pathway, miR-141-3p.

Introduction

Osteosarcoma (OS) is a common bone tumor with high cancer-related deaths in children and adolescence[1]. Various strategies for the treatment of OS have been developed in recent years[2]. However, the survival rate and outcomes of patients remain unsatisfactory. Thus, it is desirable to elucidate the underlying mechanism of OS development and find out a novel target for the treatment of OS.

Long non-coding RNAs (lncRNAs) with over 200 nucleotides in length are regarded as essential targets for therapy of human cancers[3]. Moreover, lncRNAs could act as promising targets for the development and therapeutics of OS[4]. Myocardial infarction associated transcript (MIAT) is a crucial carcinogenic lncRNA, which promotes cell proliferation and metastasis in multiple human cancers, such as hepatocellular carcinoma, breast cancer, non-small cell lung cancer, gastric cancer, colorectal cancer and pancreatic carcinoma[5-10]. What's more, MIAT plays a promoting role in OS development[11,12]. Nevertheless, the underlying mechanism of MIAT in OS progression is still uncertain.

Sineoculis homeobox homolog 1 (SIX1) is an oncogene which promotes cell proliferation, migration and invasion in human cancers, including cervical cancer, oral squamous cell carcinoma and colorectal cancer[13-15]. More importantly, SIX1 is also reported to regulate OS cell processes, such as proliferation, apoptosis, migration and invasion[16,17]. Meanwhile, SIX1 could activate phosphatidylinositide 3-kinases (PI3K)/protein kinase

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B (AKT) pathway in cervical cancer and OS[18,19]. And the activation of PI3K/AKT pathway is positively correlated with OS development[20-23]. However, it is unclear whether there is an interaction between MIAT and SIX1-mediated PI3K/AKT signaling. In the current study, we measured the expression levels of MIAT in OS tissues and cells. Moreover, we explored the role of MIAT in OS progression and potential interaction between MIAT and miR-141-3p, SIX1 as well as PI3K/AKT pathway.

Patients and Methods

Patients and Tissues

Twenty-five OS patients who did not receive other therapy were recruited from Huishan District People's Hospital and they all have signed the informed consents. Patient cancer tissues and corresponding normal tissues were collected by surgical resection and stored at -80°C. This work was approved by the Ethics Committee of Huishan District People's Hospital.

Cell Culture and Transfection

The human OS cell lines (HOS, Saos-2,143B, U2OS and MG63) and normal human osteoblastic cell line hFOB were purchased from ATCC (Manassas, VA, USA). Roswell Park Memorial Institute-1640 (RPMI-1640) medium plus 10% fetal bovine serum (FBS) and antibiotics were used for cell culture at 37°C with 5% CO_2 and it was replaced every two or three days.

pcDNA3.1 empty vector (pcDNA-control) was purchased from (Thermo Fisher Scientific, Waltham, MA, USA). pcDNA3.1-based MIAT overexpression vector (pcDNA-MIAT) and SIX1 overexpression vector (pcDNA-SIX1) were generated by our laboratory. shRNA against MIAT (sh-MIAT-1 (sense: 5'-CACCGCAGTCCAGGGTCTATTTACACTCGAGTGTAAATAGACCCTGGACTGC-3', anti-sense: 5'-AAAAGCAGTCCAGGGTCTATTTACACTCGAGTGTAAATAGACCCTGGACTGC-3') and sh-MIAT-2 (sense: 5'- CACCGCATAATTAGGGTACACTTAGCTCGAGCTAAGTGTACCCTAATTATGC-3', anti-sense: 5'- AAAAGCATAATTAGGGTACACTTAGCTCGAGCTAAGTGTACCCTAATTATGC-3')), shRNA against SIX1 (sh-SIX1-1 (sense: 5'-CACCGGGTCTACTTTCAAGAGAACTCTCGAGAGTTCTCTTGAAAGTAGACCC-3', anti-sense: 5'-AAAAGGGTCTACTTTCAAGAGAACTCTCGAGAGTTCTCTTGAAAGTAGACCC-3') and sh-SIX1-2 (sense: 5'- CACCGCGCATAGCACTTTCCCTTTCCTCGAGGAAAGGGAAAGTGCTATGCGC-3', anti-sense: 5'-AAAAGCGCATAGCACTTTCCCTTTCCTCGAGGAAAGGGAAAGTGCTATGCGC-3')), shRNA negative control (sh-NC) (sense: 5'-CACCGTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGAATTTTTTG-3', anti-sense: 5'-GATCCAAAAAATTCTCCGAACGTGTCACGTTCTCTTGAAACGTGACACGTTCGAGAAC-3'), miR-141-3p mimic (miR-141-3p) (sense: 5'-UAACACUGUCUGGUAAAGAUGG-3', anti-sense: 5'-CCAUCUUUACCAGACAGUGUUA-3') and miRNA negative control (miR-NC) (sense: 5'-UUUGUACUACACAAAAGUACUG-3', anti-sense: 5'-CAGUACUUUUGUGUAGUACAAA-3') were generated from GenePharm (Shanghai, China). U2OS and MG63 cells were transfected with 40 nM oligonucleotides or 500 ng vectors using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Transfection efficacy was analyzed after transfection for 24 h.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA of OS tissues or cells was isolated using Triquick reagent (Solarbio, Beijing, China) and 1 μg RNA (A260/280: 1.8-2.0) was used for reverse transcription using the Universal RT-PCR Kit (Solarbio). The product was diluted by 1:5 and used for qRT-PCR using SYBR Green mix (Solarbio). The qRT-PCR parameters were: 95°C for 1 min, 35 cycles of 95°C for 10 s and 60°C for 30 s. The primers used for qRT-PCR assay were as follows: MIAT (Forward, 5'-GGACGTTCACAACCACACTG-3'; Reverse, 5'-TCCCACTTTGGCATTCTAGG-3'); SIX1 (Forward, 5'-AAGGAGAAGTCGAGGGGTGT-3'; Reverse, 5'-TGCTTGTTGGAGGAGGAGTT-3'). The relative expression levels of MIAT and SIX1 mRNA were normalized to GAPDH (Forward, 5'-CGGAGTCAACGGATTTGGTCGTAT-3'; Reverse, 5'- AGCCTTCTCCATGGTGGTGAAGAC-3'). The data were analyzed by $2^{-\Delta\Delta Ct}$ method[24].

Western Blot

After treatment of RIPA lysis, cell lysates were centrifuged and then quantified using BCA protein assay kit (Beyotime, Shanghai, China). Protein (30 μg) was used for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and membranes transfer. The polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) were

blocked with 5% non-fat milk, incubated with primary antibodies against SIX1 (ab236761, 1:1000 dilution, 32 kDa, Abcam, Cambridge, MA, USA), PI3K (#13666, 1:1000 dilution, 85 kDa, Cell Signaling Technology, Danvers, MA, USA), p-PI3K (Tyr199) (#4228, 1:1000 dilution, 60 kDa, Cell Signaling Technology), AKT (#2920, 1:2000 dilution, 60 kDa, Cell Signaling Technology) or p-AKT (Ser473) (#4051, 1:1000 dilution, 60 kDa, Cell Signaling Technology) at 4°C, and then interacted with horseradish peroxidase (HRP)-labeled IgG (ab6728, 1:10000 dilution, Abcam) for 2 h. BeyoECL Plus (Beyotime, Shanghai, China) was used to develop the band signaling and the antibody against GAPDH (ab125247, 1:5000 dilution, 37 kDa, Abcam) was used as an endogenous control.

MTT

The viability of MG63 and U2OS cells was analyzed via MTT assay. Transfected cells (5000/well) were seeded into 96-well plates and cultured at 37°C with 5% CO_2 for 0, 24, 48 or 72 h. Then 10 μl 5 mg/ml MTT solution (Beyotime) was added to each well and incubated for another 4 h. Subsequently, the medium was replaced with dimethyl sulfoxide (DMSO) reagent (Beyotime) to dissolve the produced formazan. The absorbance of each well at 490 nm was measured with a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow Cytometry

Cell apoptosis was determined by flow cytometry. After the transfection, MG63 and U2OS cells were cultured for 72 h and then incubated with Annexin V-FITC and PI (Beyotime, Shanghai, China). A flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) was used to analyze the stained cells. Cells with Annexin V-FITC positive and PI negative or positive were regarded at early or late apoptotic phase, respectively. The apoptotic rate of MG63 and U2OS cells was presented as the percentage of cells at early and late apoptotic phages.

Transwell Assay

For cell migration assay, transfected MG63 and U2OS cells (2×10^4/well) in 200 μl RPMI-1640 medium without serum were seeded into the upper trans-well chamber. 500 μl medium containing 10% FBS as a chemoattractant was placed into the lower chamber. After culturing for 24 h, migrated cells were fixed with 4% paraformaldehyde and incubated with 1% crystal violet. For cell invasion assay, chambers were pro-coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) and the procedures were similar to those in migration assay. The migrated or invasive cells were observed under a microscope (Olympus, Tokyo, Japan).

Bioinformatics Analysis and Luciferase Reporter Assay

Bioinformatics analysis was performed by using miRcode (http://mircode.org/) and TargetScan (http://www.targetscan.org/vert_71/). The sequences of MIAT or 3' UTR sequences of SIX1 containing the wild-type (WT) (AGUGUU) or mutant (MUT) (UCACAA) binding sites of miR-141-3p were inserted into pGL3-promoter vectors (Promega, Madison, WI, USA) to generate luciferase reporter vectors WT-MIAT, MUT-MIAT, SIX1 3' UTR-WT and SIX1 3' UTR-MUT, respectively. MG63 and U2OS cells were co-transfected with miR-141-3p or miR-NC and luciferase reporter vectors for 48 h, followed by luciferase activity assay using a luciferase reporter assay kit (Promega, Madison, WI, USA).

Statistical Analysis

GraphPad Prism 7 software (La Jolla, CA, USA) was applied to statistical and diagram analyses. The experiments were repeated three times. The linear relationship between the abundances of SIX1 mRNA and MIAT in OS samples was analyzed via Spearman's correlation coefficient. The difference was compared by Student's *t*-test for two groups or one-way ANOVA followed by Tukey's post-hoc test for multiple groups. $p<0.05$ was considered statistically significant.

Results

The Expression Levels of MIAT and SIX1 are Enhanced in Osteosarcoma

To explore the levels of MIAT and SIX1 in OS tissues, tumor tissues and adjacent normal samples were collected from 25 patients. As shown in Figure 1A and 1B, the expression levels of MIAT and SIX1 mRNA were significantly increased in OS tissues compared with those in normal group. Moreover, the abundances of MIAT and SIX1 in OS tissues displayed a significantly positive correlation (r=0.5724, p=0.0028) (Figure 1C). Be-

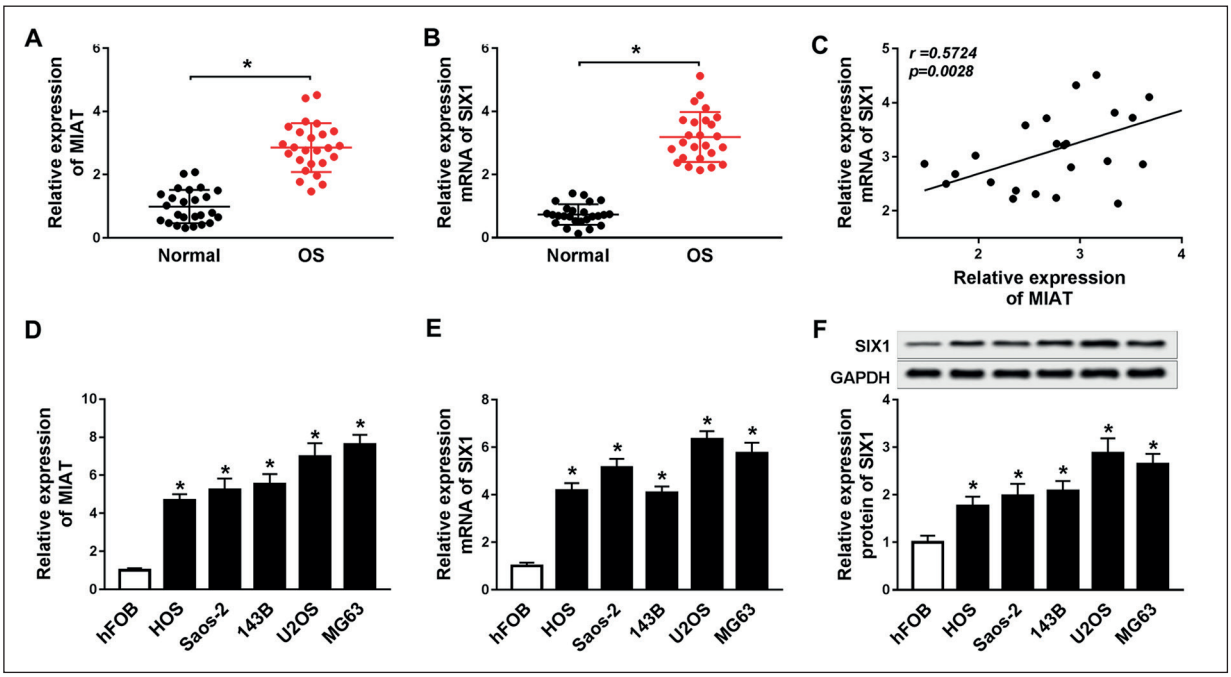

Figure 1. MIAT and SIX1 expression levels are increased in osteosarcoma. **A-B,** qRT-PCR was used to measure the levels of MIAT and SIX1 in osteosarcoma (OS) tissues and adjacent normal tissues, n=25. **C,** The relationship between levels of SIX1 and MIAT in OS tissues was analyzed. **D-F,** The abundances of MIAT and SIX1 were detected in OS cells and normal cells by qRT-PCR and Western blot. *$p<0.05$, *vs.* normal group for A and B; *vs.* hFOB group for D-F.

sides, the expressions of MIAT and SIX1 were also examined in OS cell lines (HOS, Saos-2, 143B, U2OS and MG63) and control cells (hFOB). Results showed that their expression levels were markedly enhanced in OS cells in comparison to those in hFOB cells (Figure 1D-1F). The U2OS and MG63 cells with relative higher level of MIAT were used for further study.

Knockdown of MIAT Inhibits OS Progression

To explore the function of MIAT on OS progression, its abundance in MG63 and U2OS cells was knocked down by using two shRNA against MIAT and sh-MIAT-1, which exhibited a higher transfection efficacy than sh-MIAT-2 (Figure 2A). Furthermore, the viability of MG63 and U2OS cells at 72 h was significantly decreased by knockdown of MIAT compared with that by treatment of sh-NC (Figure 2B and 2C). In addition, the silence of MIAT led to stronger apoptosis production in MG63 and U2OS cells at 72 h (Figure 2D). Besides, transwell assay for 24 h described that interference of MIAT evidently reduced the abilities of migration and invasion in MG63 and U2OS cells (Figure 2E and 2F).

Interference of SIX1 Suppresses OS Progression

To investigate the biological role of SIX1 in OS development, MG63 and U2OS cells were transfected with sh-SIX1-1, sh-SIX1-2 or sh-NC. After the transfection, the expression of SIX1 at mRNA and protein levels was notably decreased in the two cells (Figure 3A and 3B). Moreover, down-regulation of SIX1 resulted in a great viability inhibition in MG63 and U2OS cells at 72 h (Figure 3C and 3D). Additionally, the apoptotic rate of MG63 and U2OS cells at 72 h was conspicuously increased by knockdown of SIX1 (Figure 3E). Furthermore, silencing SIX1 induced significant loss of migration and invasion abilities in MG63 and U2OS cells at 24 h (Figure 3F and 3G).

Restoration of SIX1 Attenuates the Effect of MIAT Inhibition on OS Progression

To explore whether SIX1 is involved in MIAT-mediated OS progression, the effect of MIAT on SIX1 expression was investigated in MG63 and U2OS cells. As displayed in Figure 4A and 4B, the expression of SIX1 at mRNA and protein levels was significantly reduced by

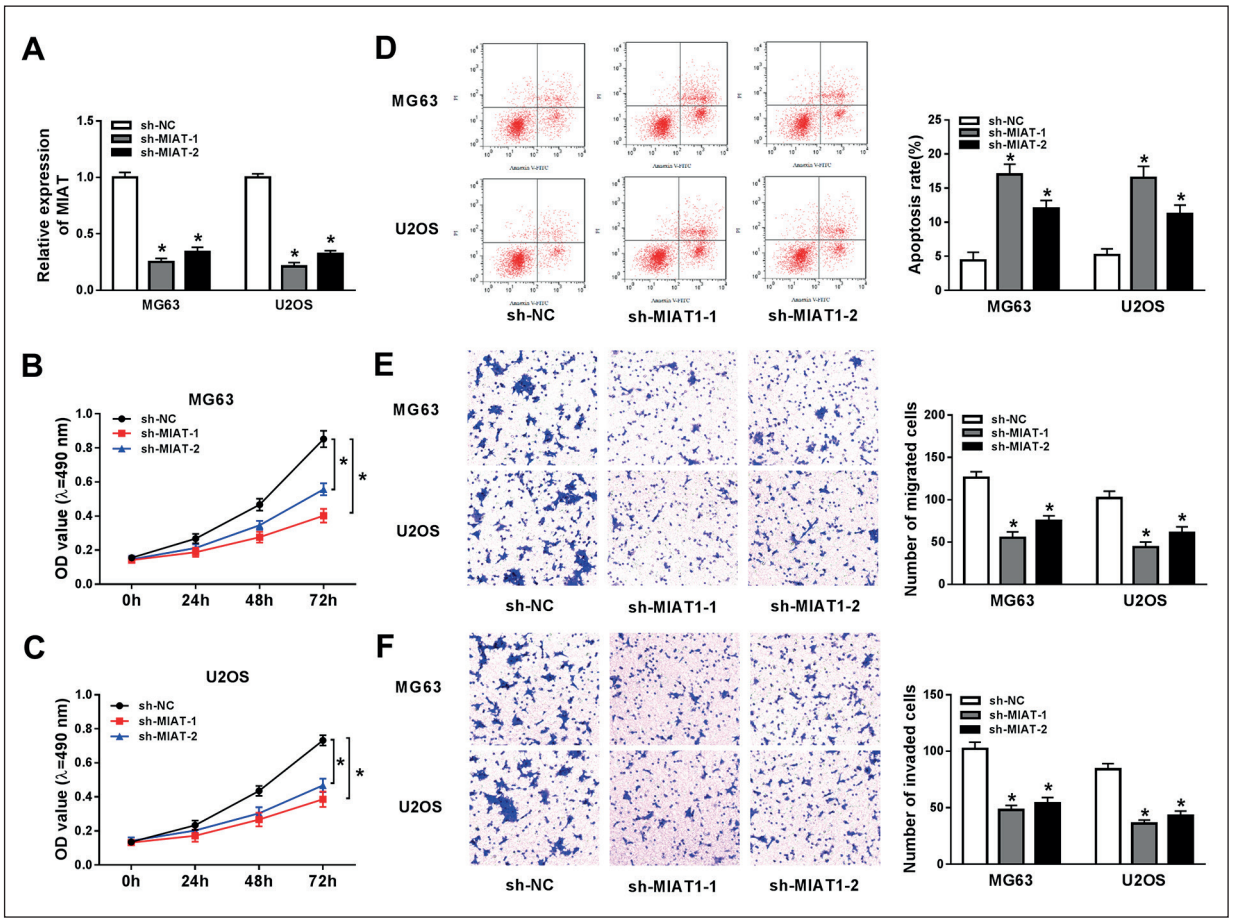

Figure 2. Knockdown of MIAT promotes apoptosis and inhibits cell viability, migration and invasion in OS cells. MIAT abundance change **(A)**, cell viability **(B-C),** apoptosis **(D)**, migration **(E)** and invasion **(F)** in MG63 and U2OS cells transfected with sh-MIAT-1, sh-MIAT-2 or sh-NC were measured by qRT-PCR, MTT, flow cytometry and trans-well assays, respectively. *$p<0.05$, *vs.* sh-NC group.

MIAT knockdown and increased by MIAT overexpression in MG63 and U2OS cells. Moreover, overexpression of SIX1 weakened silencing MIAT-mediated viability suppression in MG63 and U2OS cells at 72 h (Figure 4C and 4D). Meanwhile, the cell apoptosis that induced MIAT knockdown was mitigated by SIX1 introduction in the two cells (Figure 4E). Besides, the silence of MIAT-induced reduction of migration and invasion was significantly restored by addition of SIX1 in MG63 and U2OS cells at 24 h (Figure 4F and 4G).

MIAT Regulates SIX1 Expression by Competitively Sponging MiR-141-3p

To explore how MIAT regulates SIX1 expression, the potential miRNAs were explored by bioinformatics analysis. As shown in Figure 5A and 5B, miRcode predicted the binding sites of MIAT and miR-141-3p and TargetScan predicted the binding sites of miR-141-3p and SIX1. Moreover, luciferase reporter analysis revealed that overexpression of miR-141-3p led to 72% and 68% reduced luciferase activity in MG63 and U2OS cells, respectively, in WT-MIAT group (Figure 5C and 5D). Meanwhile, the luciferase activity was decreased 57% and 52% in MG63 and U2OS cells, respectively, by addition of miR-141-3p in SIX1 3' UTR-WT group (Figure 5E and 5F). However, the luciferase activity was not changed in MG63 and U2OS cells when mutating the binding sites of miR-141-3p in MUT-MIAT and SIX1 3' UTR-MUT groups (Figure 5C-5F). In addition, the mRNA and protein levels of SIX1 in MG63 and U2OS cells were notably inhibited by miR-141-3p overexpression, which was restored by the introduction of MIAT (Figure 5G-5J).

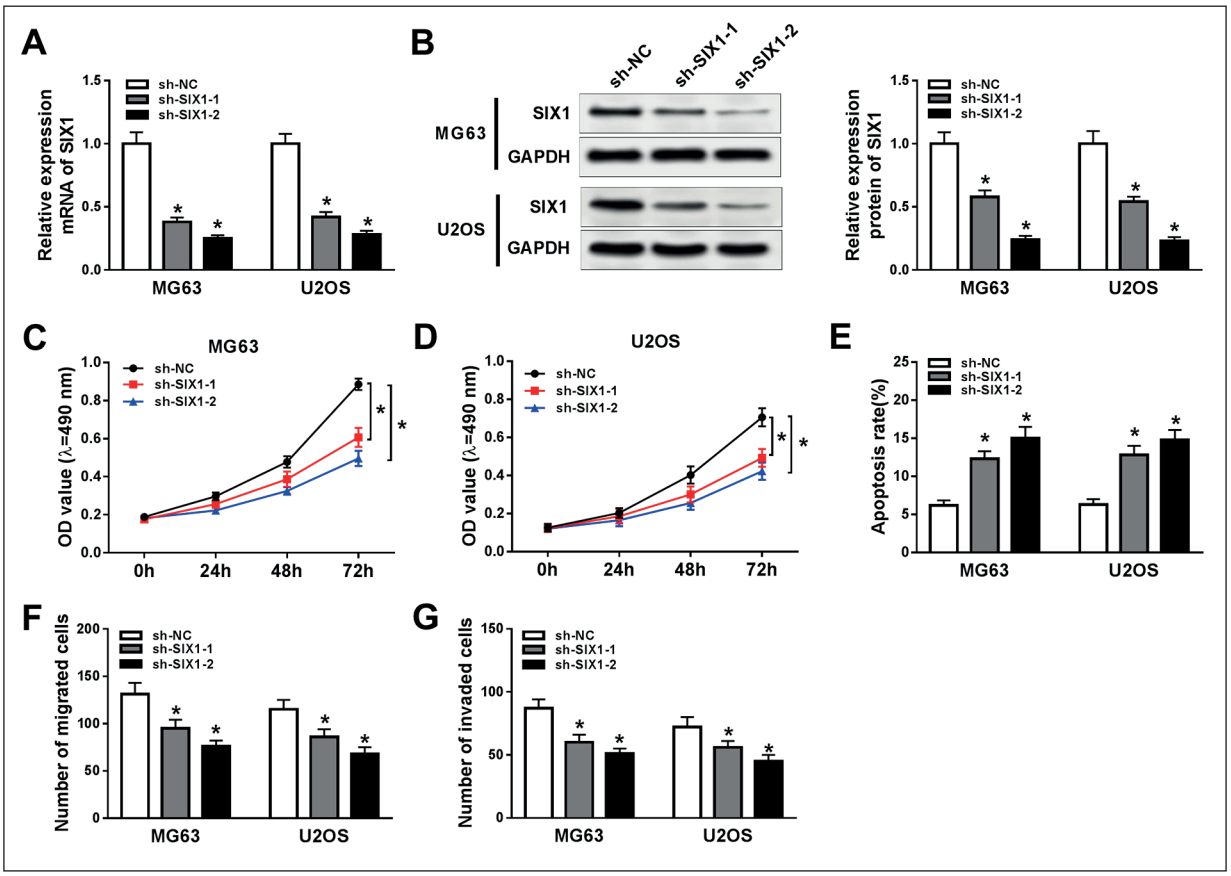

Figure 3. Interference of SIX1 induces apoptosis and suppresses cell viability, migration and invasion in OS cells. XIST mRNA and protein levels **(A-B)**, cell viability **(C-D)**, apoptosis **(E)**, migration **(F)** and invasion **(G)** in MG63 and U2OS cells transfected with sh-SIX1-1, sh-SIX1-2 or sh-NC were measured by qRT-PCR, Western blot, MTT, flow cytometry and trans-well assays, respectively. *p<0.05, *vs.* sh-NC group.

MIAT Knockdown Blocks PI3K/AKT Pathway by Increasing SIX1

To further elucidate the signaling pathway involved in this research, the expression levels of proteins associated with PI3K/AKT pathway were measured in MG63 and U2OS cells. As shown in Figure 6A and 6B, knockdown of MIAT significantly inhibited phosphorylation of PI3K and AKT in MG63 and U2OS cells, while this event was abrogated by overexpression of SIX1. However, the expression levels of total PI3K and AKT were unchanged.

Discussion

LncRNAs play important roles in the regulation of progression, prognosis and treatment of OS[25]. In the current research, we investigated the effect of MIAT on viability, apoptosis, migration and invasion in OS *in vitro*. Moreover, this is the first to provide the regulatory network of MIAT/miR-141-3p/SIX1 and interaction between MIAT and PI3K/AKT signaling in OS.

Our research displayed that MIAT expression was enhanced and its silence led to the suppression of viability, migration and invasion and promotion of apoptosis production in OS, which is similar to previous reports[11,12]. This reflected that MIAT could function as an essential target for the treatment of OS. However, the regulatory mechanism remains poorly understood. Previous study demonstrated that SIX1 was associated with Enneking stage and tumor size of patients with OS and indicated poor outcome of patients with OS[26]. Here we found that SIX1 was highly expressed and its knockdown suppressed cell viability, migration and invasion but induced apoptosis production in OS cells, which

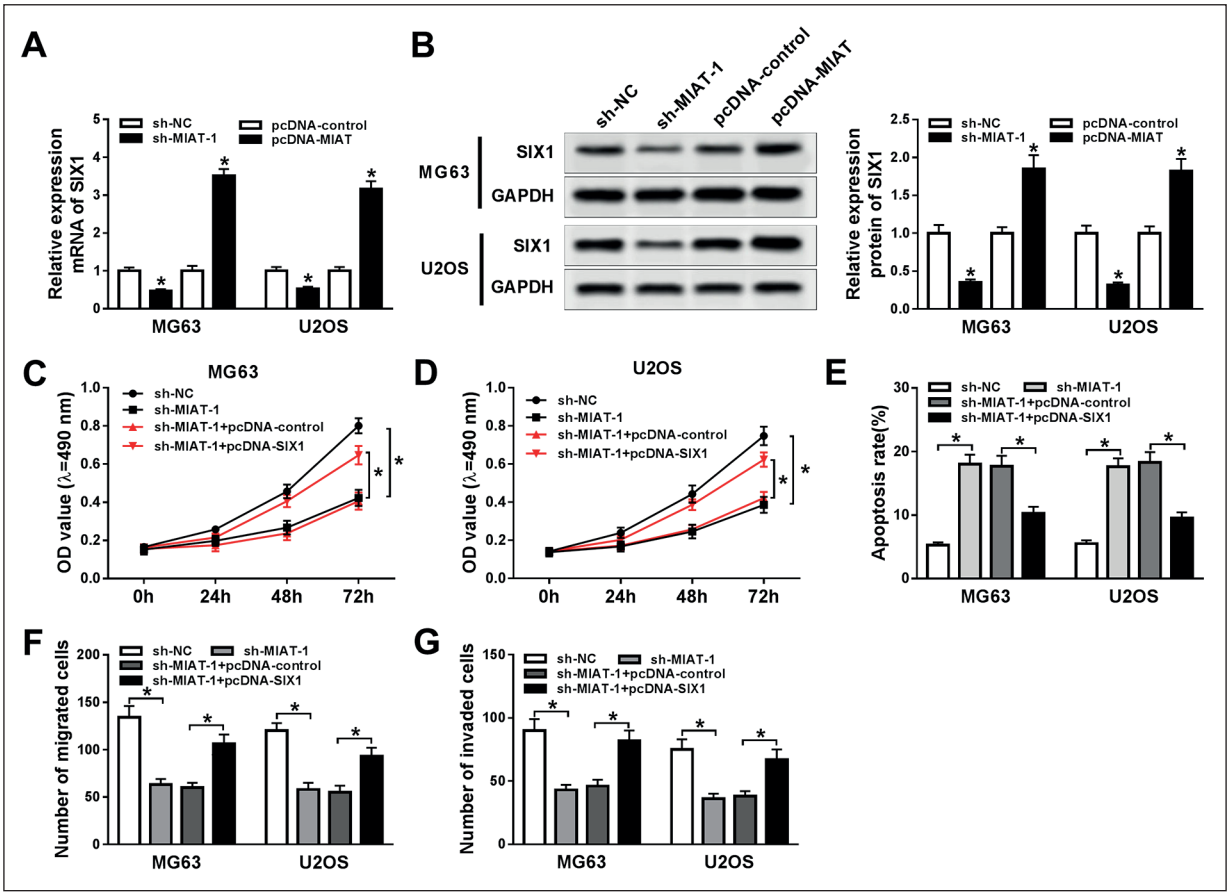

Figure 4. Overexpression of SIX1 reverses the effect of MIAT silence on OS progression *in vitro*. **A-B,** The expression change of SIX1 mRNA and protein was detected in MG63 and U2OS cells transfected with sh-NC, sh-MIAT-1, pcDNA-control or pcDNA-MIAT by qRT-PCR and Western blot. Cell viability **(C-D)**, apoptosis **(E)**, migration **(F)** and invasion **(G)** in MG63 and U2OS cells transfected with sh-NC, sh-MIAT-1, sh-MIAT-1 and pcDNA-control or pcDNA-SIX1 were determined by MTT, flow cytometry and trans-well assays, respectively. *p<0.05, sh-MIAT-1 group *vs.* sh-NC group and pcDNA-MIAT group *vs.* pcDNA-control group for A and B; sh-MIAT-1 group *vs.* sh-NC group and sh-MIAT-1 + pcDNA-SIX1 group *vs.* sh-MIAT-1 + pcDNA-control group for C-G.

is consistent with former works[16,17]. In addition, SIX1 overexpression abrogated the anti-cancer role of MIAT silence in OS, uncovering that SIX1 is responsible for MIAT-mediated OS progression.

Then we investigated how MIAT regulated SIX1 in OS progression. The competing endogenous RNA (ceRNA) network is the key mechanism for the interaction among lncRNA-miRNA-mRNA in human cancers[7]. Previous studies indicated that MIAT could regulate OS progression by serving as ceRNA or miRNA sponge[11,12,28]. To explore a novel ceRNA network, we performed the bioinformatics analysis using miRcode and TargetScan and found that miR-141-3p has the complementary sequences of MIAT and SIX1. The former work has confirmed MIAT as a sponge of miR-141-3p in gastric cancer [8]. Meanwhile, miR-141-3p was reported as a tumor suppressor by decreasing cell proliferation and migration but promoting apoptosis in OS[29,30]. This stimulated us to form the ceRNA hypothesis of MIAT/miR-141-3p/SIX1. The luciferase reporter assay validated the target association between MIAT and miR-141-3p as well as miR-141-3p and SIX1. Furthermore, introduction of MIAT could restored the SIX1 levels decreased by miR-141-3p, indicating that miR-141-3p might be required for the ceRNA cross talk of MIAT and SIX1.

Moreover, SIX1 is an activator of PI3K/AKT pathway, which could promote OS progression[19]. In the present study, we found that MIAT knockdown inhibited activation of PI3K/AKT

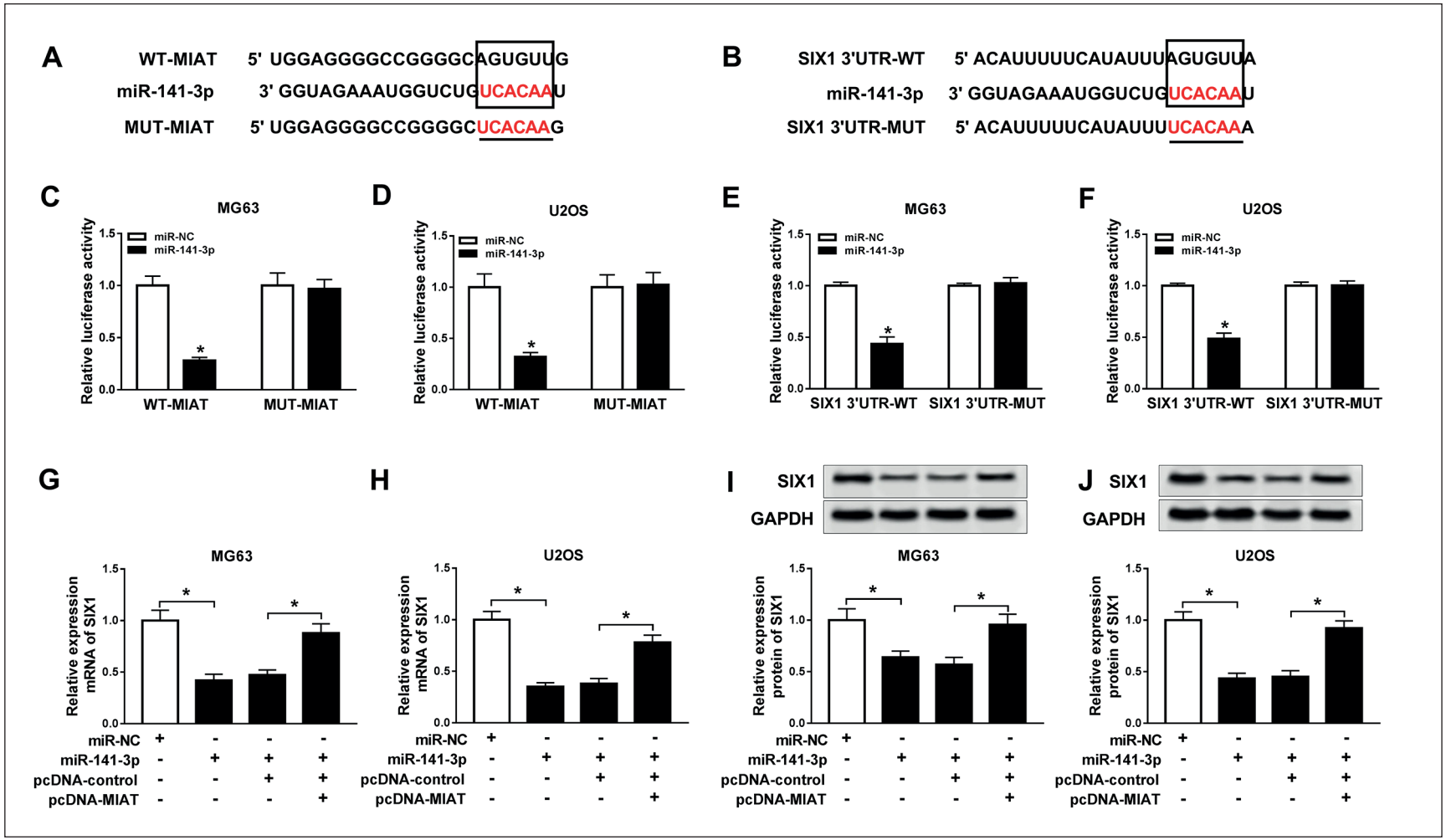

Figure 5. MIAT targets miR-141-3p to regulate SIX1 expression in OS cells. **A,** The binding sites of MIAT and miR-141-3p were predicted by miRcode. **B,** TargetScan provided the binding sites of miR-141-3p and SIX1. **C-D,** Luciferase activity was measured in MG63 and U2OS cells co-transfected with WT-MIAT or MUT-MIAT and miR-141-3p or miR-NC. **E-F,** Luciferase reporter assay was performed in MG63 and U2OS cells to validate the association between miR-141-3p and SIX1. **G-J,** qRT-PCR and Western blot assays were conducted to detected SIX1 mRNA and protein levels in MG63 and U2OS cells transfected with miR-NC, miR-141-3p, miR-141-3p and pcDNA-control or pcDNA-MIAT. *p<0.05, *vs.* miR-NC group for C-F; miR-141-3p group *vs.* miR-NC group and miR-141-3p + pcDNA-MIAT group *vs.* miR-141-3p + pcDNA-control group for G-J.

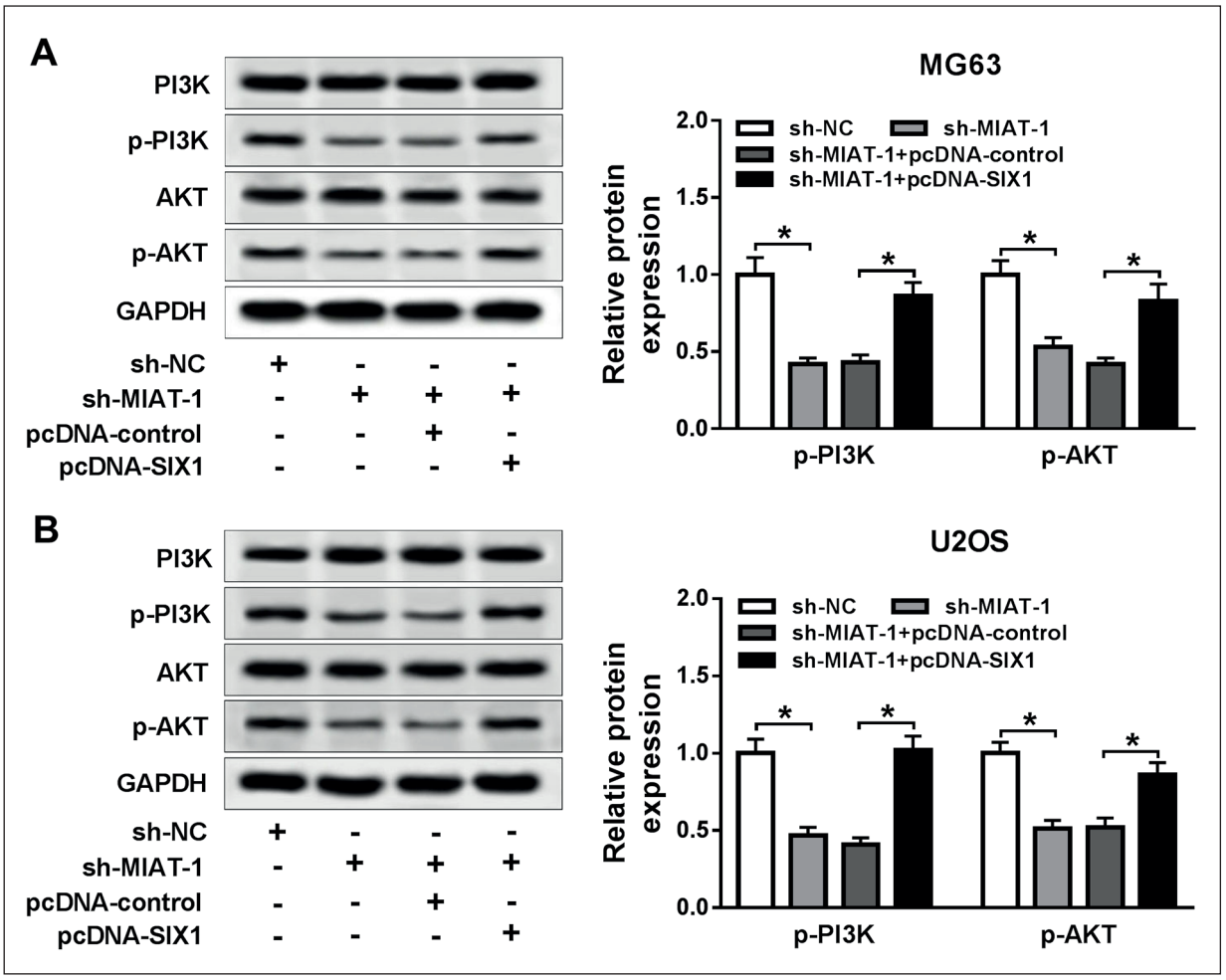

Figure 6. MIAT silence inhibits PI3K/AKT signaling by regulating SIX1. **A-B,** The protein levels of PI3K, p-PI3K, AKT and p-AKT in MG63 and U2OS cells transfected with sh-NC, sh-MIAT-1, sh-MIAT-1 and pcDNA-control or pcDNA-SIX1 were detected by Western blot. *$p<0.05$, sh-MIAT-1 group *vs.* sh-NC group and sh-MIAT-1 + pcDNA-SIX1 group *vs.* sh-MIAT-1 + pcDNA-control group.

pathway in OS cells, which is also in agreement with that in other cell lines reported by previous studies[31-33]. Meanwhile, it was re-activated via overexpression of SIX1, suggesting that MIAT could activating PI3K/AKT signaling by increasing SIX1. Furthermore, PI3K/AKT signal is an important pathway involved in cancer progression, activation of which could induce promotion of proliferation, migration and invasion and inhibition of apoptosis in OS[34,35]. Hence, we concluded that MIAT might promote OS progression through regulating SIX1-mediating PI3K/AKT pathway by acting as a ceRNA for miR-141-3p. The novelty of this study is that here we were the first to indicate that MIAT could regulate PI3K/AKT pathway in OS by SIX1. Moreover, we first validated the ceRNA network of MIAT/miR-141-3p/SIX1. However, to better elucidate this view, an inhibitor of PI3K/AKT pathway might be helpful, which would be introduced in future studies. Furthermore, the exact role of miR-141-3p should be explored in the future.

Conclusions

This research highlighted a novel mechanism for MIAT in OS progression and demonstrated that MIAT knockdown inhibited OS cell viability, migration and invasion and promoted cell apoptosis, possibly via inhibiting PI3K/AKT pathway by the ceRNA network of MIAT/miR-141-3p/SIX1.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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