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LncRNA ADPGK-AS1 regulated cell proliferation, invasion, migration and apoptosis *via* targeting miR-542-3p in osteosarcoma

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Abstract. – OBJECTIVE: The functions of lncRNAs have been verified to be important biomarkers and regulators for diagnosis and treatment of human diseases. In osteosarcoma (OS), emerging evidence determined that lncRNA was associated with cell progression. However, due to the high incidence and recurrence rate of osteosarcoma, it is important to find an effective treatment for osteosarcoma.

PATIENTS AND METHODS: QRT-PCR was used to detect the expression of ADPGK-AS1 and miR-542-3p in tissues and cells. Western blot was applied to measure the protein expression of CDK4, Cyclin D1, Bcl-2, Bax, Cleaved caspase-3, MMP-2, and MMP-9. MTT assay and flow cytometry were used to measure cell proliferation and apoptosis. Cell invasion and migration were determined using the transwell assay. Moreover, luciferase reporter assay was used to ensure the relation between ADPGK-AS1 and miR-542-3p.

RESULTS: LncRNA ADPGK-AS1 expression was induced while miR-542-3p expression was reduced in OS tissues and cells. Functional experiments showed that inhibition of ADPGK-AS1 could decrease cell proliferation, migration, and invasion, as well as promoted cell apoptosis in OS cells. Also, miR-542-3p has been verified to be a target miRNA of ADPGK-AS1 and miR-542-3p could reverse the effects of ADPGK-AS1 on cell proliferation, apoptosis, migration, and invasion in OS cells.

CONCLUSIONS: ADPGK-AS1 affected cell proliferation, invasion, migration, and apoptosis *via* targeting miR-542-3p in OS, providing a theoretical basis and a new therapeutic target for the diagnosis and treatment of OS.

Key Words:
ADPGK-AS1, MiR-542-3p, Cell Growth, Osteosarcoma.

Introduction

Osteosarcoma (OS) is the most common primary malignant tumor occurring in adolescents and children. Osteosarcoma is highly malignant, with a high recurrence rate and poor prognosis[1,2]. The diagnosis and treatment of osteosarcoma have been significantly improved in recent years, mainly with surgery and chemotherapy. At present, several studies[3-6] have shown that lncRNA can be used as a biomarker and therapeutic target for the treatment of various diseases. Therefore, we are trying to find new ways to treat and diagnose osteosarcoma from lncRNAs.

LncRNA is a non-coding RNA with a length greater than 200 nt. Also, it is an important regulator of cancer development and cellular metabolism[7-9]. Currently, lncRNA has been shown to play an essential role in cell metabolism and cell cycle. In many diseases, lncRNA affects cell proliferation, apoptosis, and metabolism by binding downstream miRNAs to regulate downstream mRNA and protein[10-12]. In addition, lncRNA is used as a biomarker for the early diagnosis and treatment of cancer[6,13,14].

MiRNAs are non-coding RNAs of ~22 nt in length, which together with the upstream lncRNA and downstream target mRNA, constitute a regulatory network that regulates cell growth and tumor formation[15,16]. Moreover, the function of miRNAs has been shown to play a role as a suppressor or promoter in the biological behavior of tumor cells, including proliferation, invasion, metastasis, and apoptosis, and even inflammatory responses, immune responses, as well as drug resistance[17-19].

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Some of the functions of lncRNA ADPGK-AS1 are mentioned in other diseases, including pancreatic cancer and breast cancer[20,21]. However, in OS, the regulation mechanism of ADPGK-AS1 remains unclear. Therefore, in this paper, we further explored its potential regulatory mechanisms by validating the function of ADPGK-AS1 in OS.

Patients and Methods

Patients and Samples

53 pairs of OS tissues and adjacent normal tissues were obtained from 53 patients who undergone resection and did not receive any chemotherapy or radiotherapy in the First Affiliated Hospital of Zhengzhou University. All tissues were collected and stored at −80°C for the following experiments. This research has been approved by the Institutional Ethics Review Board of the First Affiliated Hospital of Zhengzhou University (Zhengzhou, Henan Province, China) and informed consent was obtained from patients.

Cell Cultured and Transfection

OS cell lines (MG63, U2-OS, HOS, and LM7) and normal cell line (hFOB1.19) were purchased from the Institute of the Chinese Academy of Sciences (Shanghai, China). Then, all cell lines were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS) at 37°C with 5% CO_2 in the humid atmosphere to colon cells. Si-ADPGK-AS1, pcDNA-ADPGK-AS1, miR-542-3p, anti-miR-542-3p, and their negative control (si-con, miR-con, anti-miR-con, and pcDNA) were purchased from GeneCopoeia (Guangzhou, China). All vectors and oligos were transfected into MG63 and U2-OS cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

QRT-PCR

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract RNA from tissues and cells following the manufacturer's instructions. TaqMan® MicroRNA real-time PCR Assay reagents (Applied Biosystems, Foster City, CA, USA) and SYBR® Premix Ex Taq™ reagent (TaKaRa, Otsu, Shiga, Japan) were carried out to analyze the expression of miR-542-3p. High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and SYBR® Premix Ex Taq™ reagent (TaKaRa, Otsu, Shiga, Japan) were used to detect the expression of ADPGK-AS1. GAPDH and snRNA U6 were used as endogenous controls. Primers were as follows: ADPGK-AS1: F (5'-GCCGATGTCGACACAAGCG-3'), R (5'-AGCAAATGTGTTCCCATCCCT-3'); GAPDH: F (5'-GCACCGTCAAGGCTGAGAAC-3'), R (5'-TGGTGAAGACGCCAGTGGA-3'); miR-542-3p: F (5'-GCCGCAAAGTGCTTACAGTG-3'), R (5'-TGCAGGGTCCGAGGTAT-3'); U6: F (5'-GCTTCGGCAGCACATATACTAAAAT-3'), R (5'-CGCTTCACGAATTTGCGTGTCAT-3').

Western Blot

Protein was isolated from transfected cells using Radio Immunoprecipitation Assay (RIPA) buffer (Beyotime, Shanghai, China). Then, the equivalent protein was added to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Once blocked with 5% nonfat dry milk for 1 h, the membranes were incubated with primary antibodies CDK4, Cyclin D1, Bcl-2, Bax, Cleaved caspase-3, MMP-2, MMP-9, and β-actin (1:2000; Proteintech, Rosemont, IL, USA). Next, the membranes were incubated with secondary antibodies horseradish peroxidase (HRP)-conjugated secondary antibody for 2 h at room temperature. The bolts were detected and performed using enhanced chemiluminescence (ECL) Western blotting kit (Amersham Biosciences, Little Chalfont, UK).

Luciferase Reporter Assay

ADPGK-AS1-WT and mutation sequences (ADPGK-AS1-MUT) were amplified and inserted into the pGL3 luciferase plasmid (Promega, Madison, WI, USA). The MG63 and U2-OS cells were co-transfected with ADPGK-AS1-WT or ADPGK-AS1-MUT and miR-542-3p or miR-con with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), and the luciferase activities were detected using Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Cell Invasion and Migration

The abilities of cell migration and invasion were detected using transwell chambers (Corning, Corning, NY, USA). Cells were added to the upper chamber, and the RPMI-1640 medium with 10% fetal bovine serum (FBS) was added into the lower chamber. Cell invasion also needed Matrigel (BD Biosciences, Franklin Lakes, NJ, USA).

After 24 h, the migrated and invasive cells in the lower chamber were fixed with 75% methanol and stained with crystal violet at 37°C for 15 min. Then, the microscope was used to calculate cell numbers, and five random fields of view were analyzed for each chamber.

Cell Proliferation and Apoptosis

Cell proliferations were measured using the MTT assay. Briefly, cells were seeded into 96-well cell culture plates (Corning, Corning, NY, USA) at a density of 2×10^4. Then, the plate was incubated at 37°C overnight. After that, 20 ul MTT and 150 μL dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) were also added into each well to incubate, respectively. The spectrophotometric microplate reader (Beyotime Institute of Biotechnology, Haimen, China) was used to detect cell proliferation at a wavelength of 490 nm.

Cell apoptosis was measured using flow cytometry with Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA). First, transfected cells were collected and resuspended in 200 μ binding solution. Second, Annexin V-FITC (10 μl) was added into each well for incubation, and then propidium iodide (PI; 10 μl) was added to stain cells for 15 min at 37°C. Flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) was applied to analyze the cell apoptosis rate.

Statistical Analysis

GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA) was applied to analyze and perform these data. All data were presented as mean ± SD (standard deviation). Student's *t*-test and one-way analysis of variance (ANOVA) were employed for the comparison. Pearson's correlation analysis was used to determine the relation between ADPGK-AS1 and miR-542-3p. *p*-value (**p*) <0.05 was considered statistically significant.

Results

LncRNA ADPGK-AS1 Expression Was Induced While MiR-542-3p Expression Was Reduced in OS Tissues and Cells

To investigate the role of ADPGK-AS1 in OS, ADPGK-AS1 expression was detected in tissues and cells. Also, we predicted that miR-542-3p was a potential miRNA of ADPGK-AS1 with bioinformatics analysis, thus the expression of miR-542-3p was measured. QRT-PCR analysis showed that compared with normal tissues and cells (hFOB1.19), the expression of ADPGK-AS1 was significantly enhanced in OS tissues and cells (MG63, U2-OS, HOS, LM7; Figure 1A and 1D) while the expression of miR-542-3p was inhibited in OS tissues and cells (MG63, U2-OS, HOS, LM7; Figure 1B and 1D). Meanwhile, the results of Pearson's correlation analysis displayed that ADPGK-AS1 expression was negatively related to miR-542-3p expression in OS tissues (Figure 1C). Thus, these results determined that ADPGK-AS1 and miR-542-3p play an important role in OS.

Knockdown of ADPGK-AS1 Inhibited Cell Proliferation in OS Cells

To further explore the effect of ADPGK-AS1 on cell proliferation in OS, si-ADPGK-AS1 was transfected into MG63 and U2-OS cells, which expressed low expression of ADPGK-AS1 (Figure 2A and 2B). MTT assay results showed that cell proliferation of si-ADPGK-AS1 groups was markedly lower than that of si-con and NC groups in MG63, and U2-OS cells. As shown in Figure 2E to 2G, we found that the protein expressions of CDK4 and Cyclin D1 were significantly reduced by si-ADPGK-AS1 transfection in MG63 and U2-OS cells. Therefore, suppression of ADPGK-AS1 inhibited cell proliferation of OS.

Knockdown of ADPGK-AS1 Induced Cell Apoptosis in OS Cells

Cell apoptosis was applied to ensure the cell apoptosis rate in NC, si-con, and si-ADPGK-AS1 groups. Results showed that compared with NC and si-con groups, cell apoptosis was sharply induced in si-ADPGK-AS1 groups in MG63 and U2-OS cells. In addition, Bcl-2 protein expression was inhibited while Bax and Cleaved caspase-3 protein expression were enhanced by the knockdown of ADPGK-AS1 in MG63 and U2-OS cells (Figure 3E to 3F). The knockdown of ADPGK-AS1 induced OS cell apoptosis.

Inhibition of ADPGK-AS1 Suppressed Cell Migration and Invasion in OS Cells

The analysis of transwell determined that cell migration and invasion in si-ADPGK-AS1 groups was significantly lower than that in NC and si-con groups in MG63 and U2-OS cells (Figure 4A to 4D). Additionally, as shown in Figure 4E to 4G,

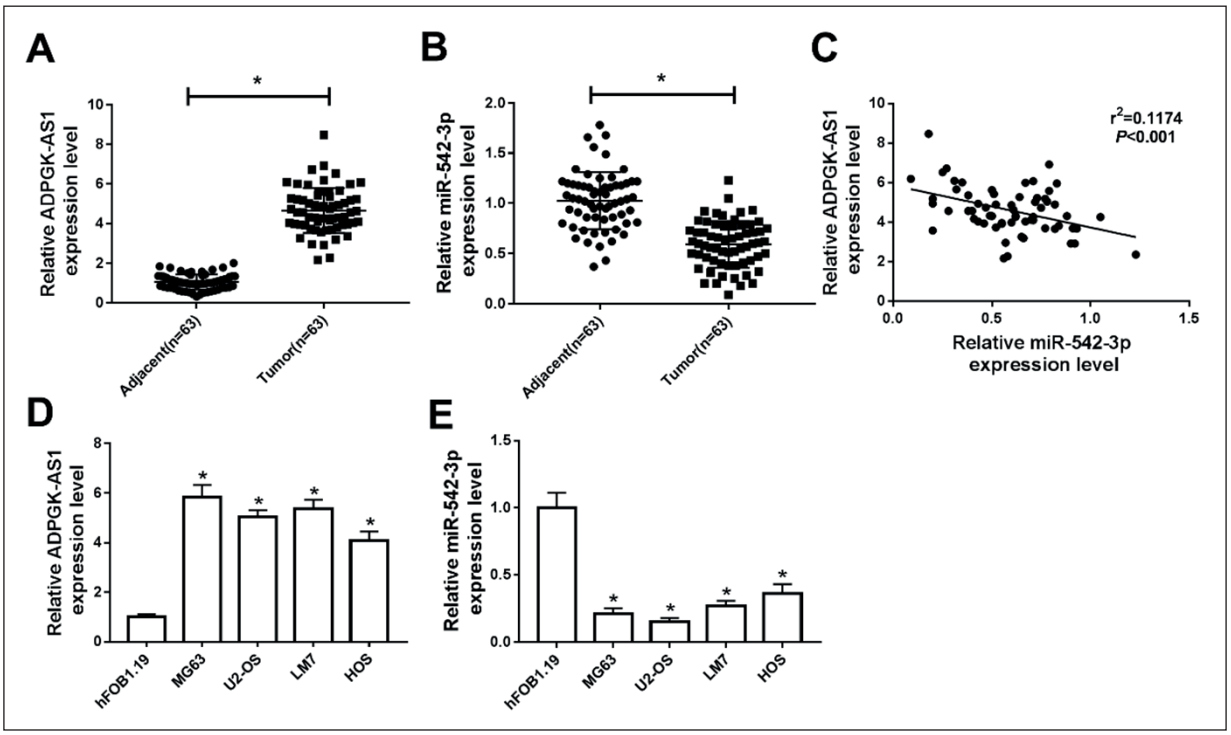

Figure 1. LncRNA ADPGK-AS1 expression was induced while miR-542-3p expression was reduced in OS tissues and cells. **A-B,** Expression of ADPGK-AS1 and miR-542-3p in OS tissues and adjacent tissues were measured with qRT-PCR. **C,** Association analysis of the expression between ADPGK-AS1 and miR-542-3p in 63 OS tissues with Pearson's correlation analysis. **D-E,** Expression of ADPGK-AS1 and miR-542-3p in OS cell lines (MG63, U2-OS, HOS, and LM7) and normal cell line (hFOB1.19) was measured with qRT-PCR. *p<0.05.

MMP-2 and MMP-9 were sharply decreased by si-ADPGK-AS1 transfection in MG63 and U2-OS cells. Thus, these results demonstrated that inhibition of ADPGK-AS1 suppressed cell migration and invasion in OS cells.

ADPGK-AS1 Directly Targeted MiR-542-3p in OS Cells

To confirm the relation between ADPGK-AS1 and mir-542-3p, we found that ADPGK-AS1 has binding sites of miR-542-3p. Then, we performed luciferase assay to ensure that. MG63 and U2-OS cells were co-transfected with a luciferase plasmid containing the ADPGK-AS1 sequence (ADPGK-AS1-WT) or a luciferase plasmid containing the ADPGK-AS1 mutate sequence (ADPGK-AS1-MUT) and miR-542-3p mimic (miR-542-3p) or negative control (Figure 5A and 5B). The results showed that miR-542-3p inhibited the luciferase activity of ADPGK-AS1-WT, not ADPGK-AS1-MUT in MG63 and U2-OS cells (Figure 5C and 5D). Of note, miR-542-2p expression was induced by improving ADPGK-AS1 expression while was inhibited by reducing ADPGK-AS1 expression in MG63 and U2-OS cells (Figure 5E and 5F). Thus, these data showed that ADPGK-AS1 directly targeted miR-542-3p in OS cells.

MiR-542-3p Reversed the Effects of ADPGK-AS1 on Cell Proliferation, Apoptosis, Migration, and Invasion in OS Cells

To verify the regulatory role between ADPGK-AS1 and miR-542-3p in OS cells, reversed experiments were tested. As shown in Figure 6A and 6B, the expression of miR-542-3p was increased by knockdown of ADPGK-AS1, which inhibited by anti-miR-542-3p transfection. MTT assay showed that si-ADPGK-AS1 transfection induced cell proliferation, which impaired by inhibition of miR-542-3p in MG63 and U2-OS cells (Figure 6C and 6D). Furthermore, MG63 and U2-OS cells transfected with si-ADPGK-AS1 could promote cell apoptosis, which were rescued by the downregulation of miR-542-

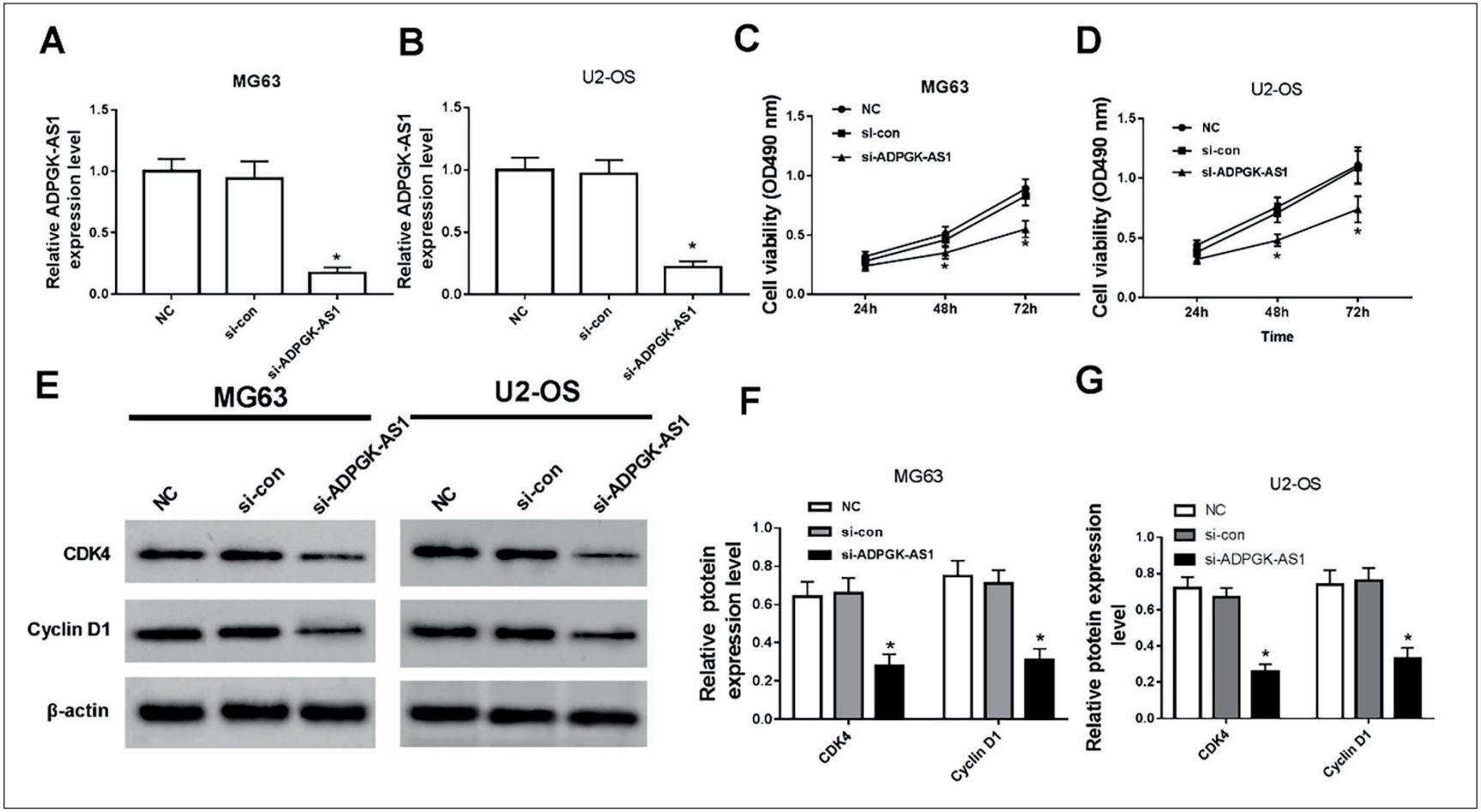

Figure 2. Knockdown of ADPGK-AS1 inhibited cell proliferation in OS cells. **A-B,** Expression of ADPGK-AS1 in NC, si-con, and si-ADPGK-AS1 groups of MG63 and U2-OS cells was detected with qRT-PCR. **C-D,** Cell proliferation in NC, si-con and si-ADPGK-AS1 groups of MG63 and U2-OS cells was measured with MTT assay. **E-G,** Protein expression of CDK4 and Cyclin D1 in NC, si-con and si-ADPGK-AS1 groups of MG63 and U2-OS cells was detected with Western blot. *$p<0.05$.

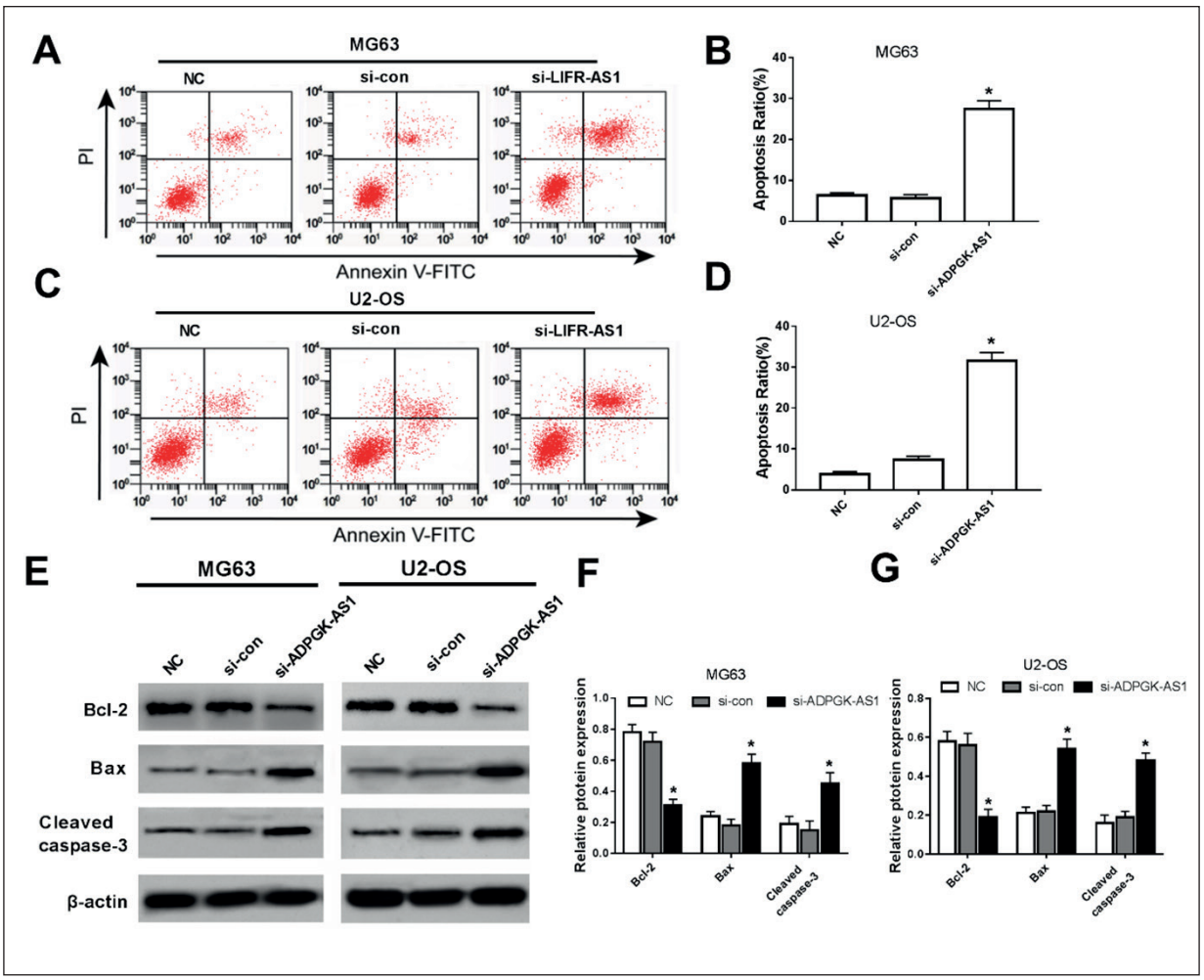

Figure 3. Knockdown of ADPGK-AS1 induced cell apoptosis in OS cells. **A-D,** Cell apoptosis in NC, si-con, and si-ADPGK-AS1 groups of MG63, and U2-OS cells was measured with flow cytometry. **E-G,** Protein expression of Bcl-2, Bax, and Cleaved caspase-3 in NC, si-con, and si-ADPGK-AS1 groups of MG63, and U2-OS cells was measured with Western blot. *$p<0.05$.

3p (Figure 6E and 6F). The inhibition in cell invasion and migration ability caused by knocking down ADPGK-AS1 could be sharply reversed by the suppression of miR-542-3p in MG63 and U2-OS cells (Figure 6G and 6H). These results determined that ADPGK-AS1 regulated OS cell growth and apoptosis *via* the modulation of miR-542-3p.

Discussion

Based on the development of current research methods, the research mechanism of OS can be further revealed. Many lncRNAs have been identified to play an important role in many cancers. LncRNAs regulate the cycle and progression of tumor cells by forming a network with miRNAs[11,22,23]. For example, in cardiomyocytes, lncRNA CARL suppressed anoxia-induced mitochondrial fission and inhibited cell apoptosis by targeting miR-539[24]. In this work, we found that miR-542-3p is a target miRNA of ADPGK-AS1, and that ADPGK-AS1 is highly expressed in OS tissues and cells, while miR-542-3p is under-expressed. Therefore, we hypothesized that the regulation of lncRNAADPGK-AS1 in OS may be through the binding of miR-542-3p.

In OS, the function of some lncRNA has been verified, such as UCA1, HULC, MEG3, and HNF1A-AS1[25-28]. For example, inhibition of lncRNA MEG3 inhibited cell progression and induced cell apoptosis[27]. Zhao et al[28] determined that cell

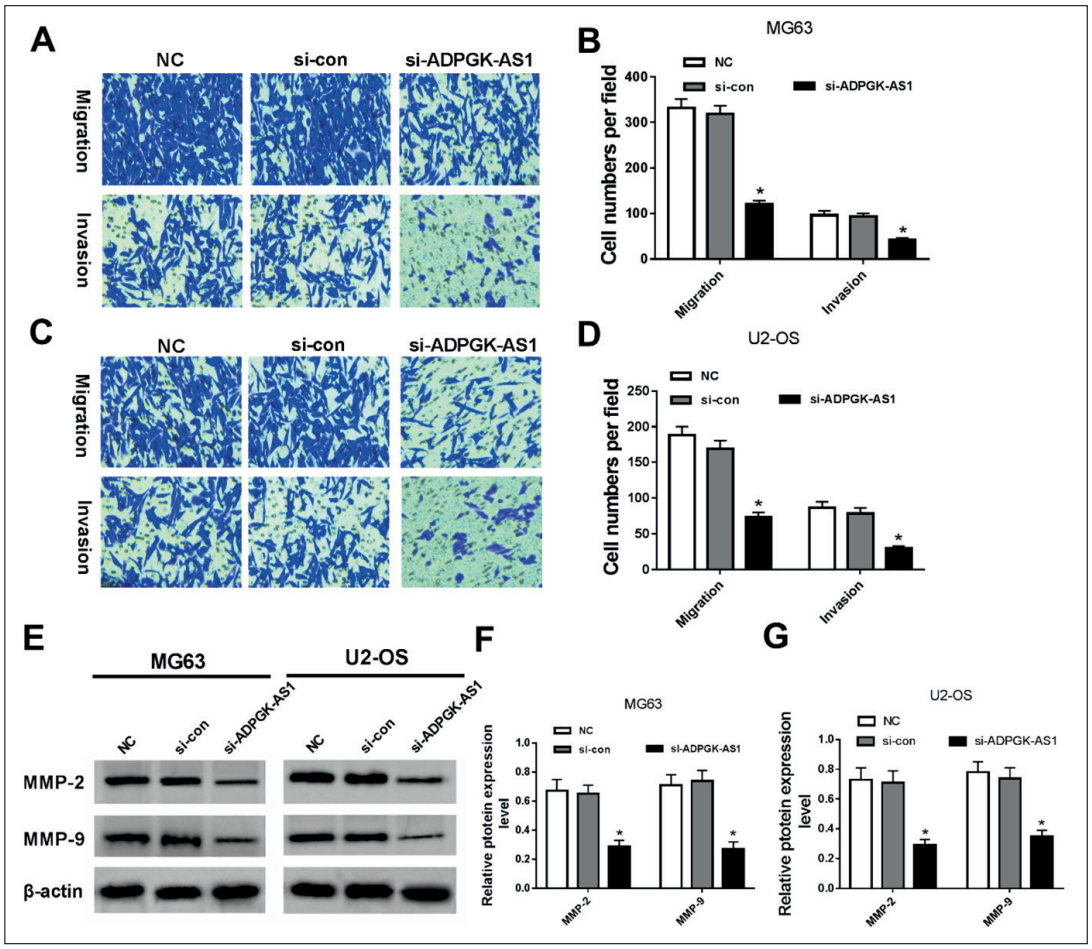

Figure 4. Inhibition of ADPGK-AS1 suppressed cell migration and invasion in OS cells. **A-D,** Cell migration and invasion in NC, si-con, and si-ADPGK-AS1 groups of MG63 and U2-OS cells was measured with transwell assay (100×). **E-G,** Protein expression of MMP-2 and MMP-9 in NC, si-con, and si-ADPGK-AS1 groups of MG63, and U2-OS cells was measured with Western blot. *p<0.05.

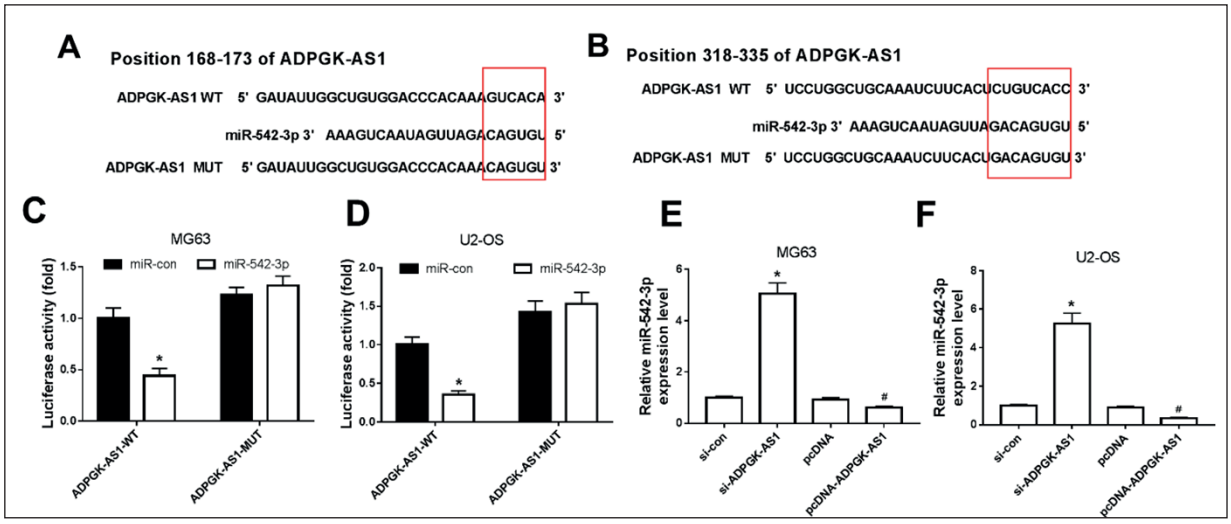

Figure 5. ADPGK-AS1 directly targeted miR-542-3p in OS cells. **A-B,** Schematic representation of the predicted target site for miR-542-3p in ADPGK-AS1. **C-D,** MG63 and U2-OS cells were co-transfected with ADPGK-AS1-WT or ADPGK-AS1-MUT and miR-542-3p or miR-con, and the luciferase activities were detected. **E-F,** Expression of miR-542-3p in si-con, si-ADPGK-AS1, pcDNA, and pcDNA-ADPGK-AS1groups of MG63, and U2-OS cells was detected with qRT-PCR. *p<0.05.

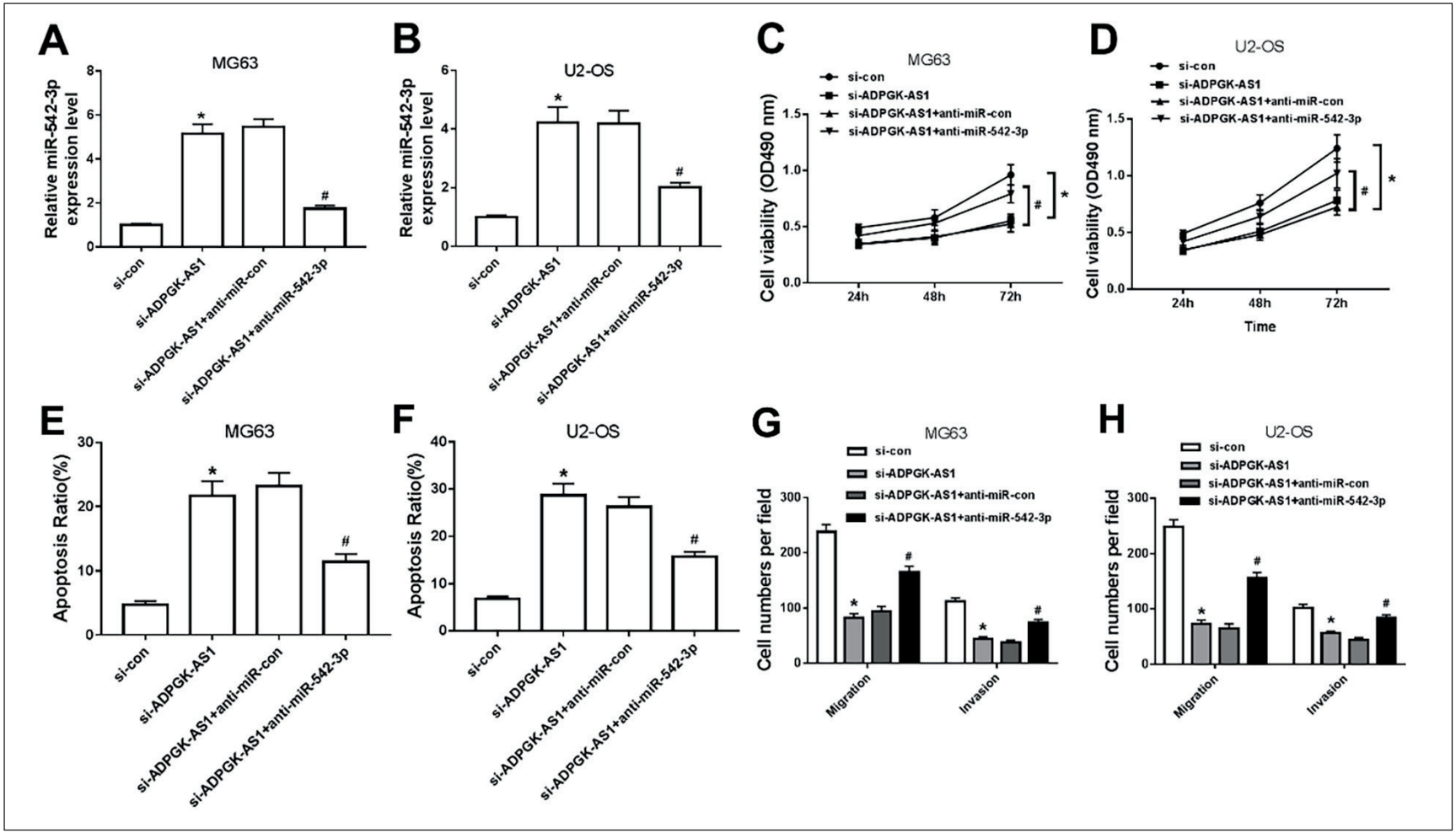

Figure 6. MiR-542-3p reversed the effects of ADPGK-AS1 on cell proliferation, apoptosis, migration and invasion in OS cells. **A-B,** Expression of miR-542-3p in si-con, si-ADPGK-AS1, si-ADPGK-AS1 + anti-miR-con, and si-ADPGK-AS1 + anti-miR-542-3p groups of MG63, and U2-OS cells was detected with qRT-PCR. **C-D,** Cell proliferation in si-con, si-ADPGK-AS1, si-ADPGK-AS1 + anti-miR-con, and si-ADPGK-AS1 + anti-miR-542-3p groups of MG63, and U2-OS cells was measured with MTT assay. **E-F,** Cell apoptosis in si-con, si-ADPGK-AS1, si-ADPGK-AS1 + anti-miR-con, and si-ADPGK-AS1 + anti-miR-542-3p groups of MG63, and U2-OS cells was measured with flow cytometry. **G-H,** Cell migration and invasion in si-con, si-ADPGK-AS1, si-ADPGK-AS1 + anti-miR-con, and si-ADPGK-AS1 + anti-miR-542-3p groups of MG63 and U2-OS cells was measured with transwell assay. *p<0.05.

proliferation and metastasis was enhanced by up-regulating lncRNA HNF1A-AS1 *via* activation of the Wnt/β-catenin signaling pathway. However, studies on the regulatory mechanisms of ADPGK-AS1 in OS are still lacking. The function of ADPGK-AS1 has been shown in some cancers. For example, ADPGK-AS1 was up-regulated in pancreatic cancer and breast cancer tissues and cells, and regulated cell progression and EMT[20,21]. In this study, low expression of ADPGK-AS1 inhibits cell proliferation, migration and metastasis, and promotes apoptosis of OS cells. Therefore, ADPGK-AS1 played an important role in the progression of OS cells.

Increasing evidence indicated that the expression of miR-542-3p is closely related to the formation of cancer and cell progression in many cancers, including hepatocellular carcinoma, bladder cancer, gastric cancer, and OS[29-33]. Wu et al[34] reported that miR-542-3p could inhibit cell proliferation in OS. To verify the above hypothesis, rescue experiments further confirmed that inhibition of ADPGK-AS1 expression reduced cell proliferation, invasion, and metastasis, and promoted apoptosis by suppression of miR-542-3p in OS.

Conclusions

LncRNA ADPGK-AS1 regulated cell growth by targeting miR-542-3p in OS. This study provided a new target for the treatment and diagnosis of OS, revealing the regulatory mechanism of ADPGK-AS1 in OS.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of First Affiliated Hospital of Zhengzhou University. The methods used in this study were performed in accordance with relevant guidelines and regulations. Written consent was obtained from the participants or guardians of participants under 16 years old.

Availability of Data and Materials

All original data and materials are available from the corresponding author upon request.

References

1) Meyers PA, Gorlick R. Osteosarcoma. Pediatr Clin North Am 1997; 44: 973-989.
2) Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. Cancer Treat Res 2009; 152: 3-13.
3) Zhou M, Zhao H, Wang Z, Cheng L, Yang L, Shi H, Yang H, Sun J. Identification and validation of potential prognostic lncRNA biomarkers for predicting survival in patients with multiple myeloma. J Exp Clin Cancer Res 2015; 34: 102.
4) Zhou M, Wang X, Shi H, Cheng L, Wang Z, Zhao H, Yang L, Sun J. Characterization of long non-coding RNA-associated ceRNA network to reveal potential prognostic lncRNA biomarkers in human ovarian cancer. Oncotarget 2016; 7: 12598-12611.
5) Chen L, Yao H, Wang K, Liu X. Long non-coding RNA MALAT1 regulates ZEB1 expression by sponging miR-143-3p and promotes hepatocellular carcinoma progression. J Cell Biochem 2017; 118: 4836-4843.
6) Lu Q, Yu T, Ou X, Cao D, Xie T, Chen X. Potential lncRNA diagnostic biomarkers for early gastric cancer. Mol Med Rep 2017; 16: 9545-9552.
7) Zhang A, Zhang J, Kaipainen A, Lucas JM, Yang H. Long non-coding RNA, a newly deciphered "code" in prostate cancer. Cancer Lett 2016; 375: 323-330.
8) Wang P, Xu J, Wang Y, Cao X. An interferon-independent lncRNA promotes viral replication by modulating cellular metabolism. Science 2017; 358: 1051-1055.
9) Loewen G, Jayawickramarajah J, Zhuo Y, Shan B. Functions of lncRNA HOTAIR in lung cancer. J Hematol Oncol 2014; 7: 90.
10) Li Q, Shen W, Li X, Zhang L, Jin X. The lncRNA n340790 accelerates carcinogenesis of thyroid cancer by regulating miR-1254. Am J Transl Res 2017; 9: 2181-2194.
11) Shen CJ, Cheng YM, Wang CL. LncRNA PVT1 epigenetically silences miR-195 and modulates EMT and chemoresistance in cervical cancer cells. J Drug Target 2017; 25: 637-644.
12) Wu Q, Meng W, Jie Y, Zhao H. LncRNA MALAT1 induces colon cancer development by regulating miR-129-5p/HMGB1 axis. J Cell Physiol 2018; 233: 6750-6757.
13) Ayers D. Long non-coding RNAs: novel emergent biomarkers for cancer diagnostics. J Cancer Res 2013; 1: 31-35.
14) Jiang C, Li X, Zhao H, Liu H. Long non-coding RNAs: potential new biomarkers for predicting tumor invasion and metastasis. Mol Cancer 2016; 15: 62.
15) Braicu C, Catana C, Calin GA, Berindan-Neagoe I. NCRNA combined therapy as future treatment option for cancer. Curr Pharm Des 2014; 20: 6565-6574.

16) Bao AD, Liu CQ, Hong-Mei WU, Liu S, Guan WJ, Yue-Hui MA. Association analysis between function of miRNA and formation of cancer. China Animal Husbandry & Veterinary Medicine 2008.

17) Du T, Zamore PD. MicroPrimer: the biogenesis and function of microRNA. Development 2005; 132: 4645-4652.

18) Zhou X, Ye F, Yin C, Zhuang Y, Yue G, Zhang G. The interaction between miR-141 and lncRNA-H19 in regulating cell proliferation and migration in gastric cancer. Cell Physiol Biochem 2015; 36: 1440-1452.

19) Jiang T, Ye L, Han Z, Liu Y, Yang Y, Peng Z, Fan J. MiR-19b-3p promotes colon cancer proliferation and oxaliplatin-based chemoresistance by targeting SMAD4: validation by bioinformatics and experimental analyses. J Exp Clin Cancer Res 2017; 36: 131.

20) Song S, Yu W, Lin S, Zhang M, Wang H, Guo S, Wang H. LncRNA ADPGK-AS1 promotes pancreatic cancer progression through activating ZEB1-mediated epithelial-mesenchymal transition. Cancer Biol Ther 2018; 19: 573-583.

21) Yang J, Wu W, Wu M, Ding J. Long noncoding RNA ADPGK-AS1 promotes cell proliferation, migration, and EMT process through regulating miR-3196/OTX1 axis in breast cancer. In Vitro Cell Dev Biol Anim 2019; 55: 522-532.

22) Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing miRNA-lncRNA interactions. Methods Mol Biol 2016; 1402: 271-286.

23) Zhu M, Chen Q, Liu X, Sun Q, Zhao X, Deng R, Wang Y, Huang J, Xu M, Yan J, Yu J. LncRNA H19/miR-675 axis represses prostate cancer metastasis by targeting TGFBI. FEBS J 2014; 281: 3766-3775.

24) Wang K, Long B, Zhou LY, Liu F, Zhou QY, Liu CY, Fan YY, Li PF. CARL lncRNA inhibits anoxia-induced mitochondrial fission and apoptosis in cardiomyocytes by impairing miR-539-dependent PHB2 downregulation. Nat Commun 2014; 5: 3596.

25) Li W, Xie P, Ruan WH. Overexpression of lncRNA UCA1 promotes osteosarcoma progression and correlates with poor prognosis. J Bone Oncol 2016; 5: 80-85.

26) Sun XH, Yang LB, Geng XL, Wang R, Zhang ZC. Increased expression of lncRNA HULC indicates a poor prognosis and promotes cell metastasis in osteosarcoma. Int J Clin Exp Pathol 2015; 8: 2994-3000.

27) Wang Y, Kong D. Knockdown of lncRNA MEG3 inhibits viability, migration and invasion and promotes apoptosis by sponging miR-127 in osteosarcoma cell. J Cell Biochem 2017; 119: 669-679.

28) Zhao H, Hou W, Tao J, Zhao Y, Wan G, Ma C, Xu H. Upregulation of lncRNA HNF1A-AS1 promotes cell proliferation and metastasis in osteosarcoma through activation of the Wnt/β-catenin signaling pathway. Am J Transl Res 2016; 8: 3503-3512.

29) Yoon S, Choi YC, Lee S, Jeong Y, Yoon J, Baek K. Induction of growth arrest by miR-542-3p that targets survivin. FEBS Lett 2010; 584: 4048-4052.

30) Tao J, Liu Z, Wang Y, Wang L, Yao B, Li Q, Wang C, Tu K, Liu Q. MiR-542-3p inhibits metastasis and epithelial-mesenchymal transition of hepatocellular carcinoma by targeting UBE3C. Biomed Pharmacother 2017; 93: 420-428.

31) Zhang J, Wang S, Han F, Li J, Yu L, Zhou P, Chen Z, Xue S, Dai C, Li Q. MicroRNA-542-3p suppresses cellular proliferation of bladder cancer cells through post-transcriptionally regulating survivin. Gene 2016; 579: 146-152.

32) Shen X, Si Y, Yang Z, Wang Q, Yuan J, Zhang X. MicroRNA-542-3p suppresses cell growth of gastric cancer cells via targeting oncogene astrocyte-elevated gene-1. Med Oncol 2015; 32: 361.

33) Li Q, Song S, Ni G, Li Y, Wang X. Serum miR-542-3p as a prognostic biomarker in osteosarcoma. Cancer Biomark 2018; 21: 521-526.

34) Wu Y, You J, Li F, Wang F, Wang Y. MicroRNA-542-3p suppresses tumor cell proliferation via targeting Smad2 inhuman osteosarcoma. Oncol Lett 2018; 15: 6895-6902.