European Review for Medical and Pharmacological Sciences 2021; 25: 678-686

LncRNA FOXD2-AS1 knockdown inhibits the resistance of human osteosarcoma cells to cisplatin by inhibiting miR-143 expression

Q.-Q. ZHANG[1], S.-L. XU[2], C. DING[1], C.-C. MA[1], T.-S. YUAN[1], C.-C. HUA[1], X.-H. WANG[1]

[1]Department of Orthopedics, Bozhou People's Hospital, Bozhou, China
[2]Department of Orthopedics, The First Affiliated Hospital of Anhui Medical University, Hefei, China

Abstract. – OBJECTIVE: The aim of this study was to explore the effect of long non-coding ribonucleic acid (lncRNA) FOXD2-adjacent opposite strand RNA 1 (FOXD2-AS1) on the sensitivity of osteosarcoma cells to cisplatin and its possible underlying mechanism. Our findings might help to provide a certain reference for clinically preventing the drug resistance of osteosarcoma cells.

MATERIALS AND METHODS: Cisplatin with a certain concentration gradient was used to induce the stable acquired resistance of human osteosarcoma U2-OS cell line. Subsequently, the expression level of lncRNA FOXD2-AS1 was determined in osteosarcoma cells in non-resistance group (Control group) and Cisplatin-resistance group (Cisplatin-RES group), respectively. Next, the cell line with stable lncRNA FOXD2-AS1 knockdown was constructed in Cisplatin-RES group using small interfering RNA (siRNA). The effects of stable knockdown of lncRNA FOXD2-AS1 on the proliferation of human osteosarcoma cells and the half maximal inhibitory concentration (IC50) of cisplatin were detected by Cell Counting Kit-8 (CCK-8) assay. 5-ethynyl-2'-deoxyuridine (EdU) staining was performed to measure deoxyribonucleic acid (DNA) replication level in each group of cells. The protein expression levels of apoptosis-associated genes B-cell lymphoma 2 (Bcl-2) and Bcl-2 associated X protein (Bax) in each group of cells were measured via Western blotting. The migration and invasion abilities of cells in each group were determined using wound-healing assay and transwell assay. In addition, the expression of micro RNA (miR)-143 in each group of cells was detected via Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

RESULTS: Compared with Control group, the expression level of lncRNA FOXD2-AS1 rose significantly in cells in Cisplatin-RES group ($p<0.05$). Knockdown of FOXD2-AS1 evidently decreased the IC50 of cisplatin in human osteosarcoma cells ($p<0.05$). According to EdU staining results, the knockdown of FOXD2-AS1 distinctly inhibited the proliferation of osteosarcoma cells ($p<0.05$). Western blotting results demonstrated that the knockdown of FOXD2-AS1 remarkably upregulated the expression of pro-apoptotic protein Bax and repressed that of anti-apoptotic protein Bcl-2 in drug-resistant human osteosarcoma cells ($p<0.05$). Moreover, the knockdown of FOXD2-AS1 significantly weakened the migration and invasion abilities of drug-resistant human osteosarcoma cells ($p<0.05$). Finally, it was found that the expression level of miR-143 was distinctly elevated in drug-resistant human osteosarcoma cells after knockdown of FOXD2-AS1 ($p<0.05$).

CONCLUSIONS: LncRNA FOXD2-AS1 knockdown inhibits the resistance of human osteosarcoma cells to cisplatin, promotes their apoptosis and weakens their invasion and migration abilities. The possible underlying mechanism may be related to the inhibition of miR-143 expression by lncRNA FOXD2-AS1 in drug-resistant cell lines.

Key Words:
LncRNA FOXD2-AS1, Osteosarcoma, Drug resistance, MiR-143.

Introduction

Primary osteosarcoma is one of the most common bone tumors mainly occurring in the long bone. It is characterized by extreme proneness to metastasis, especially metastasis to the lung. Current studies have shown that osteosarcoma is prevalent in youngsters[1]. Despite prompt chemotherapy and extensive tumor removal, recurrence still occurs in 30-50% of patients who initially suffer from localized osteosarcoma. Meanwhile, distant tumor metastasis occurs in 20-30% of

Corresponding Author: Xiaohe Wang, BM; e-mail: 975111530@qq.com

new cases[2]. Although significant advances have been achieved in surgical resection, adjuvant chemotherapy and radiotherapy and other treatment strategies in recent years, the prognosis of osteosarcoma patients is far from satisfactory[3]. The resistance of tumor cells to chemotherapeutic drugs is an important cause of failure for osteosarcoma treatment[4]. Therefore, further identifying the effector molecules and signaling pathways that modulate tumor resistance is vital for searching for crucial targets for the treatment of osteosarcoma.

In recent years, the roles of lncRNAs in human diseases have attracted much attention[5,6]. LncRNAs act as "star molecules" that can interact with RNAs, DNAs or proteins. Meanwhile, they promote or inhibit the expression of protein-coding genes to modulate several important life activities in mammals, such as cell proliferation, differentiation, apoptosis, development, and metabolism[7-9]. Of note, the expression level of lncRNA BRAF-activated non-coding RNA (BANCR) is considerably upregulated in osteosarcoma tissues and cells. Meanwhile, such an increase is significantly correlated with large tumor volume, positiveness for distant metastasis, and clinically late stage. Additionally, anti-sense lncRNA FOXC2-adjacent opposite strand RNA 1 (AS1) increases the protein expression of FOXC2 to potentiate the resistance of osteosarcoma cells to doxorubicin[10]. However, the exact role of lncRNA FOXD2-AS1 in the drug resistance of osteosarcoma cells and its mechanism therein have not been fully elucidated.

In the present study, human osteosarcoma U2-OS cell line was used as the research object. The expression of lncRNA FOXD2-AS1 in non-drug-resistant and drug-resistant osteosarcoma cells was first detected. Meanwhile, its expression in drug-resistant U2-OS cell line was restrained to further explore its role in the resistance of human osteosarcoma cell line to cisplatin. All our findings might help to provide a certain reference for the forthcoming prevention of drug resistance of osteosarcoma patients clinically.

Materials and Methods

Materials and Cells

Human osteosarcoma U2-OS cell line was purchased from the Shanghai Cell Bank, Type Culture Collection Committee, Chinese Academy of Sciences (Shanghai, China). The cells were stimulated by cisplatin at an increasing series of concentrations for 2 consecutive months for inducing resistance. Fetal bovine serum (FBS) was provided by Gibco (Rockville, MD, USA), and Roswell Park Memorial Institute-1640 (RPMI)-1640 medium was bought from Gibco (Rockville, MD, USA).

Culture of Resistant Human Osteosarcoma Cells

Drug-resistant human osteosarcoma cells were routinely cultured in RPMI-1640 medium supplemented with 10% FBS and a certain volume of double antibodies in a thermostatic incubator with 5% CO_2 at 37°C. To maintain the drug resistance, human osteosarcoma cells were stimulated by 5 μmol/L cisplatin monthly. Cells in the logarithmic growth phase were used for the following experiments.

Cell Transfection

Osteosarcoma cells were first inoculated into 6-well plates and cultured in a thermostatic incubator for 36 h. Upon reaching the density of 70-80%, the cells were transfected with lncRNA FOXD2-AS1 non-sense sequence (NS) and small interfering (si)-FOXD2-AS1 according to the instructions of Lipofectamine™ RNAi MAX transfection reagent (Invitrogen, Carlsbad, CA, USA). After transfection, the cells were cultured in a thermostatic incubator at 37°C.

Detection of Osteosarcoma Cell Proliferation and Resistance to Cisplatin Via Cell Counting Kit (CCK)-8 Assay

Each group of cells in the logarithmic growth phase was seeded into 96-well plates, stimulated by different concentrations of cisplatin (0, 1, 5, 10, and 15 μmol) and cultured in a thermostatic incubator at 37°C for 48 h. Color development solution was prepared using RPMI-1640 medium and CCK-8 solution (10:1; Dojindo Molecular Technologies, Kumamoto, Japan) in the dark. Briefly, 100 μL of the color development solution was added into 96-well plates, followed by incubation for 2 h at 37°C. Absorbance (A) at 450 nm was measured using an ultraviolet spectrophotometer. Half maximal inhibitory concentration (IC50) in each group of cells was finally calculated using GraphPad Prism 6.0 (La Jolla, CA, USA).

Detection of LncRNA FOXD2-AS1 Via Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNAs were first extracted from osteosarcoma cells in each group using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The concentration and purity of RNAs were measured using the ultraviolet spectrophotometer. A_{260}/A_{280}=1.8-2.0 indicated that the concentration and purity of extracted RNAs were eligible. Subsequently, extracted RNAs were reversely transcribed into complementary deoxyribose nucleic acids (cDNAs), and the cDNAs were stored in a refrigerator at -80°C for use. RT-PCR was finally performed in the system composed of 2.5 μL of 10× Buffer, 1 μL of cDNAs, 0.5 μL of both 20 μmol/ L forward primers and reverse primers, 10 μL of LightCycler® 480 SYBR Green I Master (2×) and 5.5 μL of ddH_2O (note: the RT-PCR amplification was conducted in the same system) (Table I).

5-Ethynyl-2′-Deoxyuridine (EdU) Staining

48 h after lncRNA FOXD2-AS1 was knocked down using siRNA transfection in human osteosarcoma cells, the cells were stained according to the instructions of the Click-iT EdU staining kit (Invitrogen, Carlsbad, CA, USA). Upon completion of staining, the cells were photographed under a fluorescence microscope. 3 fields of view were randomly selected for each sample. Finally, EdU-positive cells were counted and quantified.

Wound-Healing Assay

Cells in the logarithmic phase were first seeded into 96-well plates at a density of 5×10^4 cells/well. Then, scratches were made using a pipette tip at the center of plates. Scratched cells were washed by phosphate buffered saline (PBS), while the remaining cells were cultured in replaced serum-free medium. The migration of cells was finally photographed and recorded under a high-magnification microscope at 0 and 48 h, respectively.

Transwell Assay

Transwell chambers (with a membrane pore size of 8 μm) were first coated with Matrigel diluted at 1:8 (Corning, Corning, NY, USA). After incubation in an incubator at 37°C for 2 h, the solidified Matrigel was harvested. Two cell lines were separately diluted into single-cell suspensions using serum-free medium and inoculated into the upper transwell chamber at a density of 5×10^4 cells/100 μL. Meanwhile, complete RPMI-1640 medium containing 10% fetal bovine serum was added into the lower transwell chamber. After culture for 48 h, the cells were immobilized using 5% glutaraldehyde, permeabilized, and stained with 0.1% crystal violet. Finally, invasive cells were photographed under a microscope.

Detection of Apoptosis-Associated Proteins Via Western Blotting

Cells were first rinsed with PBS for three times to remove the fluid. Subsequently, 1,000 μL of cell lysate was added, and thoroughly shaken for 10 min. Cells at the dish bottom were scraped fully using a brush and placed in prepared Eppendorf (EP; Hamburg, Germany) tubes. Subsequently, collected cells were lysed using an ultrasonic cell crusher for no more than 15 s in total (1-2 s/time). After letting stand for 15 min, the cells were centrifuged at 12,000 rpm for 0.5 h, and the supernatant was collected into EP tubes. Protein concentration was measured by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA) and ultraviolet spectroscopy. All proteins were diluted to the constant concentration, aliquoted and preserved in a refrigerator at -80°C for use. Afterwards, protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Then, the membranes were incubated with primary antibodies at 4°C overnight. On the next day, the membranes were incubated with the goat anti-rabbit secondary antibody for 1 h. Immuno-reactive bands were finally scanned by an Odyssey scanner and

Table I. Primer sequences of all indicators in RT-PCR.

Target gene		Primer sequence
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Forward	5'-GACATGCCGCCTGGAGAAAC-3'
	Reverse	5'-AGCCCAGGATGCCCTTTAGT-3'
LncRNA FOXD2-AS1	Forward	5'-AGCTGATGGGCTGATGCTAGCTGA-3'
	Reverse	5'-ACGTGATGCTAGTCGGGAACTCCA-3'

quantified. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal protein reference.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Measurement data were presented using mean ± standard deviation. *t*-test was used to compare the differences between two groups. $p<0.05$ was considered statistically significant.

Results

Expression of LncRNA FOXD2-AS1 in Each Group of Cells

Cells in the logarithmic growth phase were collected from both Control group and Cisplatin-RES group, and total RNAs were extracted. The content of lncRNA FOXD2-AS1 therein was then determined. According to RT-PCR results (Figure 1), the expression level of lncRNA FOXD2-AS1 in cells in Cisplatin-RES group rose significantly to about 6.29 times higher than that in Control group ($p<0.05$). This indicates that lncRNA FOXD2-AS1 may be associated with decreased sensitivity of osteosarcoma cells to cisplatin.

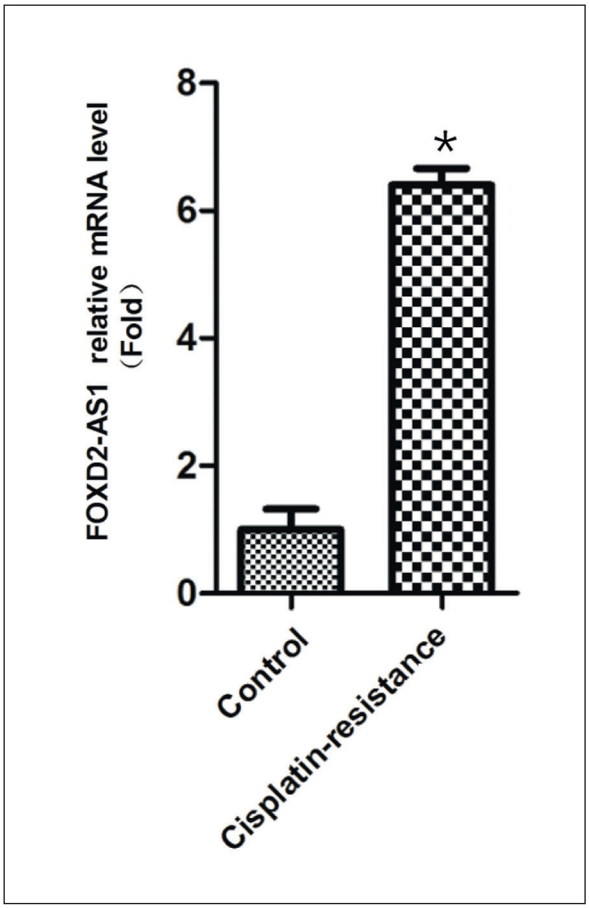

Figure 1. Expression of lncRNA FOXD2-AS1 in each group of cells: Control group: non-drug-resistant human osteosarcoma cells, and Cisplatin-RES group: drug-resistant human osteosarcoma cells. *$p<0.05$: There was a statistically significant difference compared with Control group.

Effect of LncRNA FOXD2-AS1 on the Drug Resistance of Osteosarcoma Cells

At 48 h after lncRNA FOXD2-AS1 knockdown, IC50 in each group of cells was determined *via* CCK-8 assay. Based on the results (Figure 2), IC50 was 1.99 μmol/L in Cisplatin-RES group, 2.01 μmol/L in Cisplatin-RES + NS group, and 0.63 μmol/L in Cisplatin-RES + si-FOXD2-AS1 group, respectively. The above results suggest that inhibiting lncRNA FOXD2-AS1 can significantly enhance the sensitivity of osteosarcoma cells to cisplatin ($p<0.05$).

Effect of LncRNA FOXD2-AS1 Knockdown on the Proliferation of Drug-Resistant Osteosarcoma Cells

According to the EdU staining results (Figure 3), EdU-positive cells in resistant human osteosarcoma cells evidently decreased at 48 h after the knockdown of lncRNA FOXD2-AS1 ($p<0.05$), implying that lncRNA FOXD2-AS1 knockdown represses the proliferation of drug-resistant human osteosarcoma cells.

Effect of LncRNA FOXD2-AS1 Knockdown on the Invasion of Drug-Resistant Osteosarcoma Cells

The effect of lncRNA FOXD2-AS1 on the invasion of drug-resistant human osteosarcoma cells was explored *via* transwell assay. The results showed that the invasion ability of drug-resistant human osteosarcoma cells was distinctly weakened at 48 h after the knockdown of lncRNA FOXD2-AS1 (Figure 4) ($p<0.05$).

Effect of LncRNA FOXD2-AS1 Knockdown on the Migration of Drug-Resistant Osteosarcoma Cells

Wound-healing assay results indicated that the migration ability of drug-resistant human osteosarcoma cells was remarkably weakened at 48 h after the inhibition of lncRNA FOXD2-

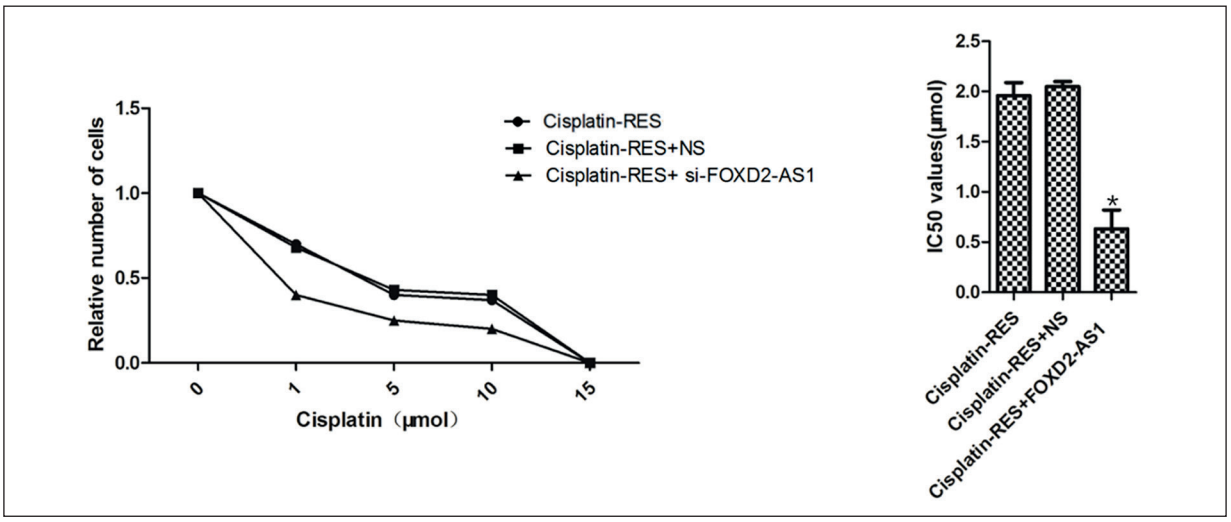

Figure 2. Effect of lncRNA FOXD2-AS1 knockdown on the resistance of osteosarcoma: Cisplatin-RES group: drug-resistant human osteosarcoma cells, Cisplatin-RES + NS group: drug-resistant human osteosarcoma cells + non-sense sequence, Cisplatin-RES + si-FOXD2-AS1 group: drug-resistant human osteosarcoma cells + FOXD2-AS1 knockdown. *p<0.05: There was a statistically significant difference compared with Cisplatin-RES group.

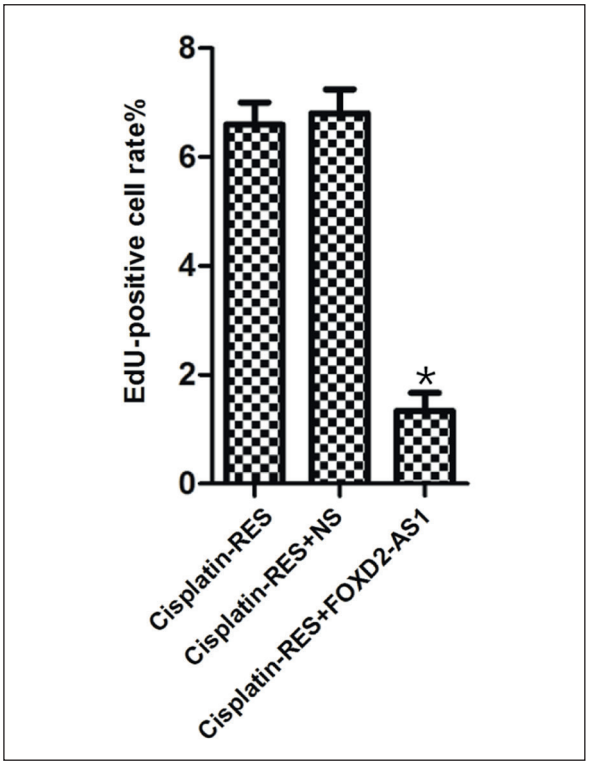

Figure 3. Effect of lncRNA FOXD2-AS1 knockdown on the proliferation of osteosarcoma cells: Cisplatin-RES group: drug-resistant human osteosarcoma cells, Cisplatin-RES + NS group: drug-resistant human osteosarcoma cells + non-sense sequence, Cisplatin-RES + si-FOXD2-AS1 group: drug-resistant human osteosarcoma cells + FOXD2-AS1 knockdown. *p<0.05: There was a statistically significant difference compared with Cisplatin-RES group.

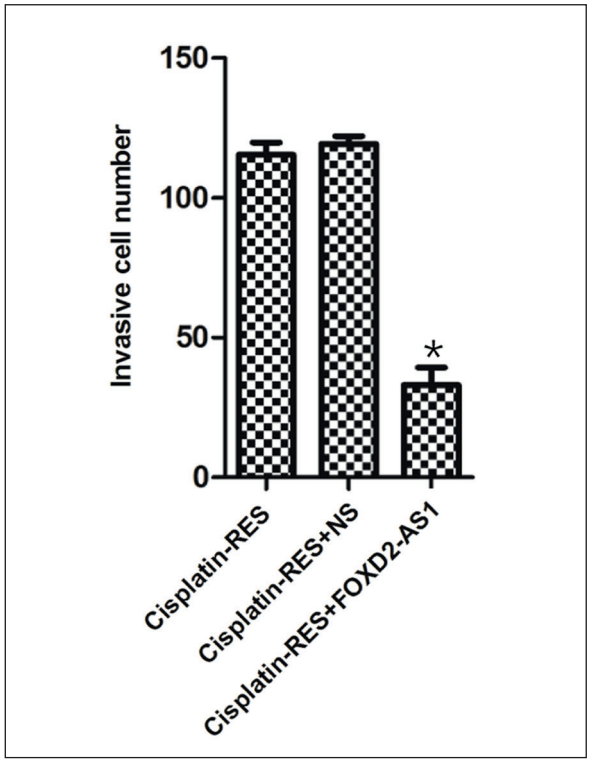

Figure 4. Effect of lncRNA FOXD2-AS1 knockdown on the invasion of osteosarcoma cells: Cisplatin-RES group: drug-resistant human osteosarcoma cells, Cisplatin-RES + NS group: drug-resistant human osteosarcoma cells + non-sense sequence, and Cisplatin-RES + si-FOXD2-AS1 group: drug-resistant human osteosarcoma cells + FOXD2-AS1 knockdown. *p<0.05: There was a statistically significant difference compared with Cisplatin-RES group.

AS1 (p<0.05) (Figure 5). This suggests that lncRNA FOXD2-AS1 has a potential promoting effect on the drug resistance of osteosarcoma cells.

Effect of LncRNA FOXD2-AS1 Knockdown on the Apoptosis of Drug-Resistant Osteosarcoma Cells

The changes in the expressions of apoptosis-associated proteins in each group of cells were detected *via* Western blotting. It was found that Cisplatin-RES + si-FOXD2-AS1 group had an evidently higher level of pro-apoptotic protein Bax and a markedly lower level of anti-apoptotic protein Bcl-2 than Cisplatin-RES group (p<0.05) (Figure 6).

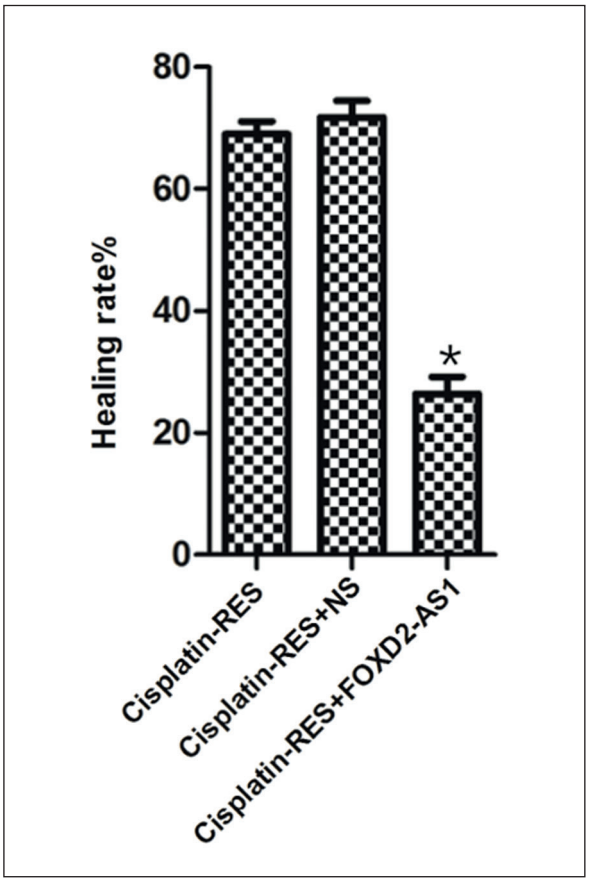

Figure 5. Effect of lncRNA FOXD2-AS1 knockdown on the migration of osteosarcoma cells: Cisplatin-RES group: drug-resistant human osteosarcoma cells, Cisplatin-RES + NS group: drug-resistant human osteosarcoma cells + nonsense sequence, and Cisplatin-RES + si-FOXD2-AS1 group: drug-resistant human osteosarcoma cells + FOXD2-AS1 knockdown. *p<0.05: There was a statistically significant difference compared with Cisplatin-RES group.

Expression Level of MiR-143 in Each Group of Drug-Resistant Osteosarcoma Cells

Previous studies have demonstrated that miR-143 is an effect target of lncRNA FOXD2-AS1 and can inhibit the drug resistance of tumor cells. Hence, the expression level of miR-143 in each group was determined *via* RT-PCR. According to the results (Figure 7), cisplatin-resistant osteosarcoma cells with lncRNA FOXD2-AS1 knockdown had a distinctly higher expression level of miR-143 (p<0.05).

Discussion

Osteosarcoma, a common malignancy of bones and joints, has an extremely high morbidity rate in children and young people. Its annual morbidity rate worldwide is about 4/1,000,000[11]. With the development of treatment means, such as surgery, radiotherapy and adjuvant therapies, 5-year survival rate of non-metastatic osteosarcoma patients is about 60-70%. However, the survival is still extremely short in metastatic or recurrent osteosarcoma patients[12]. Currently, adjuvant chemotherapeutic drugs, such as cisplatin, doxorubicin, and methotrexate are used for the first-line therapy of osteosarcoma. However, they may cause a series of clinical problems, including organ toxicity and chemoresistance[13]. At present, there is no mature and efficacious osteosarcoma biomarker providing references for chemotherapy. Meanwhile, dose-limiting toxicity further restricts the application of these drugs. Therefore, it is urgent to search for novel treatment targets and approaches for osteosarcoma.

LncRNAs are a kind of long-strand RNA molecules with about over 200 nt in length. Although they cannot encode proteins, they can directly or indirectly modulate the expression of target genes at the transcriptional/(post-transcriptional) level, epigenetic modification level and other levels, ultimately affecting the development and progression of diseases[14,15]. Su et al[16] have suggested that lncRNA FOXD2-AS1 promotes the progression and recurrence of bladder cancer through the Akt/E2F1 positive feedback loop. LncRNA FOXD2-AS1 overexpression drives the development of gastric cancer through epigenetic silence of EZH2 and LSD1. Meanwhile, the expression level of lncRNA FOXD2-AS1 is positively correlated with poor prognosis of gastric cancer patients[17]. In the present study, it was

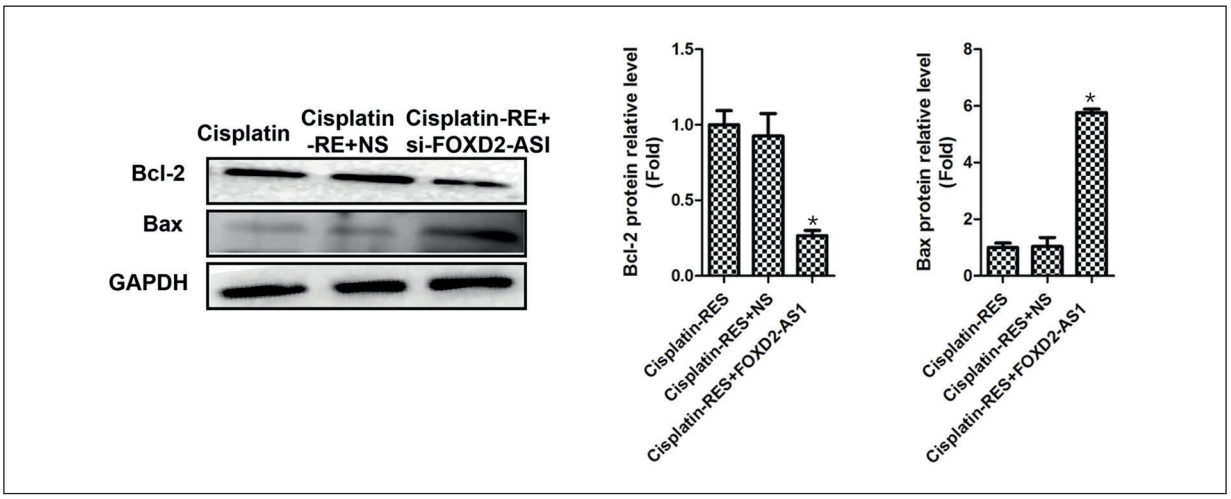

Figure 6. Effect of lncRNA FOXD2-AS1 knockdown on the apoptosis of drug-resistant osteosarcoma cells: Cisplatin-RES group: drug-resistant human osteosarcoma cells, Cisplatin-RES + NS group: drug-resistant human osteosarcoma cells + non-sense sequence, and Cisplatin-RES + si-FOXD2-AS1 group: drug-resistant human osteosarcoma cells + FOXD2-AS1 knockdown. *p<0.05: There was a statistically significant difference compared with Cisplatin-RES group.

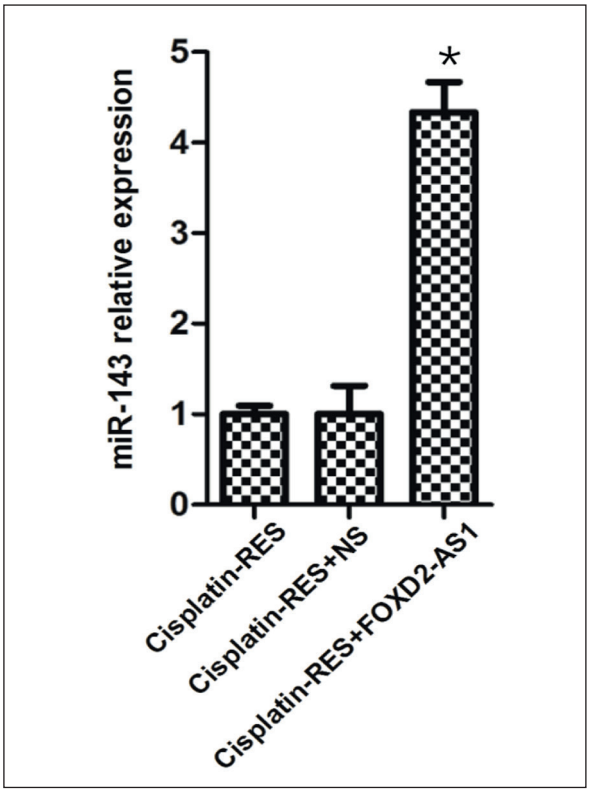

Figure 7. Effect of lncRNA FOXD2-AS1 knockdown on miR-143 expression in osteosarcoma cells: Cisplatin-RES group: drug-resistant human osteosarcoma cells, Cisplatin-RES + NS group: drug-resistant human osteosarcoma cells + non-sense sequence, and Cisplatin-RES + si-FOXD2-AS1 group: drug-resistant human osteosarcoma cells + FOXD2-AS1 knockdown. *p<0.05: There was a statistically significant difference compared with Cisplatin-RES group.

found that the expression of lncRNA FOXD2-AS1 increased significantly in drug-resistant osteosarcoma cells.

MiRNAs, a class of 19-22 nt-long small RNAs, can cleave mRNAs or inhibit protein translation to negatively modulate the expression of target genes[18]. It has been estimated that miRNAs can regulate more than 30% of proteins in humans, thereby playing an important role in the progression of various tumors[19]. As an important miRNA family member, miR-145 has been observed to exert a vital anti-tumor effect. For example, miR-143 can interfere with the ERK signaling pathway to repress the proliferation and metastasis of prostate cancer cells[20]. Besides, miR-143 targets hexokinase 2 to inhibit tumor cell glycolysis, ultimately repressing cancers[21]. Numerous studies have corroborated the cross-regulations between lncRNAs and miRNAs. MiRNAs can trigger the decay of lncRNAs, and lncRNAs can serve as the sponge or bait of miRNAs, compete with miRNAs for the interaction with mRNAs and be degraded into miRNAs. LncRNA FOXD2-AS1 promotes the development of malignancies through many targets by complex mechanisms[6]. Consistently, LncRNA FOXD2-AS1 represses the miR-363-5p/S100A1 pathway in a targeted manner to aggravate nasopharyngeal carcinoma[22]. Besides, it sponges miR-143 to weaken the sensitivity of bladder cancer cells to the chemotherapy drug gemcitabine[23]. Furthermore, LncRNAs are competing endogenous RNAs (ceRNAs) contain-

ing miRNA response elements (MERs), which can inhibit the regulation of miRNAs on downstream genes by competitively binding miRNA sites[24]. According to the findings in the present study, after inhibition of lncRNA FOXD2-AS1, the expression level of miR-143 increased significantly in drug-resistant human osteosarcoma cells. Meanwhile, the proliferation, invasion and migration abilities of cells were notably weakened, and the apoptosis level markedly rose. It can be inferred that lncRNA FOXD2-AS1 may sponge miR-143 to weaken the sensitivity of osteosarcoma cells to cisplatin as well. However, the specific mechanism remains to be further elucidated.

Conclusions

In short, the present study revealed for the first time that inhibition of miR-143 by lncRNA FOXD2-AS1 may be one of the mechanisms in the resistance of osteosarcoma cells to cisplatin. LncRNA FOXD2-AS1 probably serves as a potential target for potentiating the sensitivity of osteosarcoma patients to cisplatin in future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding Acknowledgements

The Natural Science Foundation Project of Anhui Province (1708085 MH215).

References

1) Qu Q, Chu X, Wang P. MicroRNA-195-5p suppresses osteosarcoma cell proliferation and invasion by suppressing naked cuticle homolog 1. Cell Biol Int 2017; 41: 287-295.
2) Liu D, Zhang C, Li X, Zhang H, Pang Q, Wan A. MicroRNA-567 inhibits cell proliferation, migration and invasion by targeting FGF5 in osteosarcoma. EXCLI J 2018; 17: 102-112.
3) Huang S, Jin A. ZIC2 promotes viability and invasion of human osteosarcoma cells by suppressing SHIP2 expression and activating PI3K/AKT pathways. J Cell Biochem 2018; 119: 2248-2257.
4) Chang J, Li Y, Wang X, Hu S, Wang H, Shi Q, Wang Y, Yang Y. Polyphyllin I suppresses human osteosarcoma growth by inactivation of Wnt/beta-catenin pathway in vitro and in vivo. Sci Rep 2017; 7: 7605.
5) Zhang HJ, Wei QF, Wang SJ, Zhang HJ, Zhang XY, Geng Q, Cui YH, Wang XH. LncRNA HOTAIR alleviates rheumatoid arthritis by targeting miR-138 and inactivating NF-kappaB pathway. Int Immunopharmacol 2017; 50: 283-290.
6) Zhang M, Wu WB, Wang ZW, Wang XH. lncRNA NEAT1 is closely related with progression of breast cancer via promoting proliferation and EMT. Eur Rev Med Pharmacol Sci 2017; 21: 1020-1026.
7) Loewen G, Jayawickramarajah J, Zhuo Y, Shan B. Functions of lncRNA HOTAIR in lung cancer. J Hematol Oncol 2014; 7: 90.
8) Liu Y, Zhao M. LnCaNet: pan-cancer co-expression network for human lncRNA and cancer genes. Bioinformatics 2016; 32: 1595-1597.
9) Zheng S, Jiang F, Ge D, Tang J, Chen H, Yang J, Yao Y, Yan J, Qiu J, Yin Z, Ni Y, Zhao L, Chen X, Li H, Yang L. LncRNA SNHG3/miRNA-151a-3p/RAB22A axis regulates invasion and migration of osteosarcoma. Biomed Pharmacother 2019; 112: 108695.
10) Zhang CL, Zhu KP, Ma XL. Antisense lncRNA FOXC2-AS1 promotes doxorubicin resistance in osteosarcoma by increasing the expression of FOXC2. Cancer Lett 2017; 396: 66-75.
11) Meng Q, Tang B, Qiu B. Growth inhibition of Saos-2 osteosarcoma cells by lactucopicrin is mediated via inhibition of cell migration and invasion, sub-G1 cell cycle disruption, apoptosis induction and Raf signalling pathway. J BUON 2019; 24: 2136-2140.
12) Winkler K, Bielack SS, Delling G, Jurgens H, Kotz R, Salzer-Kuntschik M. Treatment of osteosarcoma: experience of the Cooperative Osteosarcoma Study Group (COSS). Cancer Treat Res 1993; 62: 269-277.
13) Scully SP, Ghert MA, Zurakowski D, Thompson RC, Gebhardt MC. Pathologic fracture in osteosarcoma: prognostic importance and treatment implications. J Bone Joint Surg Am 2002; 84: 49-57.
14) Li J, Meng H, Bai Y, Wang K. Regulation of lncRNA and its role in cancer metastasis. Oncol Res 2016; 23: 205-217.
15) Chen D, Sun Q, Cheng X, Zhang L, Song W, Zhou D, Lin J, Wang W. Genome-wide analysis of long noncoding RNA (lncRNA) expression in colorectal cancer tissues from patients with liver metastasis. Cancer Med 2016; 5: 1629-1639.
16) Su F, He W, Chen C, Liu M, Liu H, Xue F, Bi J, Xu D, Zhao Y, Huang J, Lin T, Jiang C. The long non-coding RNA FOXD2-AS1 promotes bladder cancer progression and recurrence through a positive feedback loop with Akt and E2F1. Cell Death Dis 2018; 9: 233.
17) Xu TP, Wang WY, Ma P, Shuai Y, Zhao K, Wang YF, Li W, Xia R, Chen WM, Zhang EB, Shu YQ. Upregulation of the long noncoding RNA FOXD2-AS1 promotes carcinogenesis by epigenetical-

ly silencing EphB3 through EZH2 and LSD1 and predicts poor prognosis in gastric cancer. Oncogene 2018; 37: 5020-5036.

18) Gregory RI, Shiekhattar R. MicroRNA biogenesis and cancer. Cancer Res 2005; 65: 3509-3512.

19) Clape C, Fritz V, Henriquet C, Apparailly F, Fernandez PL, Iborra F, Avances C, Villalba M, Culine S, Fajas L. MiR-143 interferes with ERK5 signaling, and abrogates prostate cancer progression in mice. PLoS One 2009; 4: e7542.

20) Wang Y, Cao L, Wang Q, Huang J, Xu S. LncRNA FOXD2-AS1 induces chondrocyte proliferation through sponging miR-27a-3p in osteoarthritis. Artif Cells Nanomed Biotechnol 2019; 47: 1241-1247.

21) Fang R, Xiao T, Fang Z, Sun Y, Li F, Gao Y, Feng Y, Li L, Wang Y, Liu X, Chen H, Liu XY, Ji H. MicroRNA-143 (miR-143) regulates cancer glycolysis via targeting hexokinase 2 gene. J Biol Chem 2012; 287: 23227-23235.

22) Chen G, Sun W, Hua X, Zeng W, Yang L. Long non-coding RNA FOXD2-AS1 aggravates nasopharyngeal carcinoma carcinogenesis by modulating miR-363-5p/S100A1 pathway. Gene 2018; 645: 76-84.

23) An Q, Zhou L, Xu N. Long noncoding RNA FOXD2-AS1 accelerates the gemcitabine-resistance of bladder cancer by sponging miR-143. Biomed Pharmacother 2018; 103: 415-420.

24) Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature 2014; 505: 344-352.