Oncology Research, Vol. 26, pp. 1335–1343
Printed in the USA.

0965-0407/18 $90.00 + .00
DOI: https://doi.org/10.3727/096504018X15188367859402
E-ISSN 1555-3906
www.cognizantcommunication.com

Long Noncoding RNA FEZF1-AS1 Promotes Osteosarcoma Progression by Regulating the miR-4443/NUPR1 Axis

Chengwei Zhou,[1] Jianxiang Xu,[1] Jinti Lin, Renjin Lin, Kai Chen, Jianzhong Kong, and Xiaolong Shui

Department of Orthopedics Surgery, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang Province, P.R. China

Long noncoding RNA (lncRNA) FEZF1-AS1 was demonstrated to facilitate cell proliferation and migration in some cancers. However, the functions of FEZF1-AS1 and its molecular mechanism in osteosarcoma remain to be elucidated. In our study, we found that the expression of FEZF1-AS1 was upregulated in osteosarcoma samples and cell lines compared with normal tissues or cells. Besides, we showed that the expression levels of FEZF1-AS1 in osteosarcoma patients were positively correlated with tumor metastasis and TNM stage. Additionally, FEZF1-AS1 knockdown inhibited cell proliferation, migration, and invasion in U2OS and MG63 cells, while upregulation had the opposite effects in vitro. Moreover, FEZF1-AS1 depletion inhibited tumor growth and metastasis in vivo. We found that FEZF1-AS1 sponged miR-4443 to promote NUPR1 expression in U2OS and MG63 cells. Furthermore, knockdown of miR-4443 abrogated FEZF1-AS1 silencing-induced inhibition of cell proliferation, migration, and invasion in osteosarcoma. Finally, we found that restoration of NUPR1 rescued the proliferation, migration, and invasion abilities of FEZF1-AS1-depleted U2OS and MG63 cells. Our study indicated that FEZF1-AS1 could promote osteosarcoma progression by sponging miR-4443 to promote NUPR1 expression. The FEZF1-AS1/miR-4443/NUPR1 axis may act as a novel therapeutic strategy for osteosarcoma treatment.

Key words: FEZF1-AS1; miR-4443; NUPR1; Progression; Osteosarcoma

INTRODUCTION

Osteosarcoma (OS), one of the most aggressive and common malignant bone tumors, often occurs among adolescents[1]. Because of the poor prognosis, OS leads to a high number of deaths every year, worldwide[2]. In recent decades, many methods have been developed for OS treatment, including adjuvant chemotherapy and radiotherapy. However, owing to distant metastases such as lung metastasis, the overall 5-year survival rate of OS patients is still very low[3,4]. Therefore, to develop novel and effective therapeutic strategies for OS treatment, it is necessary to determine the exact molecular mechanism that regulates OS development and progression.

Long noncoding RNAs (lncRNAs) are transcripts of longer than 200 nucleotides and possess little protein-coding potential[5]. Accumulating data have proven that lncRNAs exert important functions in diverse biological processes, such as cell proliferation and apoptosis[6,7]. Emerging evidence shows that there is a close relationship between lncRNAs and human diseases, especially cancers[8,9]. For instance, lncRNA growth arrest specific 5 (GAS5) plays an important role in the development of lung cancer[10]. The molecular mechanisms by which lncRNAs exert physiological functions are diverse. Most lncRNAs can act as competing endogenous RNAs to sponge miRNAs. For example, Li and colleagues reported that lncRNA colon cancer-associated transcript 1 (CCAT1) serves as a competing endogenous RNA (ceRNA) to antagonize the effect of miR-410 in human HCT-116 and HCT-8 cells[11]. In addition, Wu et al. showed that the nuclear-enriched abundant transcript 1 (NEAT1)/mir-98-5p axis regulates the development of non-small cell lung cancer (NSCLC)[12]. Moreover, lncRNAs also could bind to specific proteins to regulate tumor occurrence, such as in liver cancer[13]. The roles of lncRNAs in cancers have attracted great attention and have been explored widely. However, there are still large numbers of lncRNAs whose functions have not yet been defined.

lncRNA FEZ family zinc finger 1–antisense 1 (FEZF1-AS1) was previously reported to facilitate cell proliferation and migration in colorectal carcinoma and gastric cancer[14,15]. He and colleagues indicated that FEZF1-AS1

[1]These authors provided equal contribution to this work and are co-first authors.
Address correspondence to Xiaolong Shui, Department of Orthopedics Surgery, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, No. 109 Xue Yuan West Road, Wenzhou, Zhejiang Province 325027, P.R. China. E-mail: xlshui07@163.com

enhances epithelial–mesenchymal transition (EMT) by the WNT pathway in NSCLC[16]. However, the role of FEZF1-AS1 in OS remains elusive. In this study, we found that FEZF1-AS1 was upregulated in OS tissues and cell lines. The expression of FEZF1-AS1 was positively related to tumor size and metastasis. Knockdown of FEZF1-AS1 significantly inhibited tumor progression in OS in vitro and in vivo. We found that FEZF1-AS1 sponged miR-4443 to upregulate nuclear protein 1 (NUPR1) expression in U2OS and MG63 cells. Furthermore, knockdown of miR-4443 abrogated FEZF1-AS1 silencing-induced inhibition of cell proliferation, migration, and invasion, while restoration of NUPR1 rescued these abilities in U2OS and MG63 cells. Collectively, our study demonstrated that FEZF1-AS1 could promote OS progression by sponging miR-4443 to promote NUPR1 expression. The FEZF1-AS1/miR-4443/NUPR1 axis may act as a novel therapeutic strategy for OS treatment.

Table 1. Correlations Between Clinicopathological Features and the Expression of FEZF1-AS1 in Osteosarcoma (OS) Tissues

	FEZF1-AS1		
Feature	Low	High	*p* Value
All cases	24	34	
Age (years)			0.795
<20	13	17	
≥20	11	17	
Tumor size (cm)			0.006
<5	15	8	
≥5	9	26	
Metastases			0.005
No	14	7	
Yes	10	27	
Clinical stage			0.007
I/II	19	14	
III	5	20	

The median expression level was used as the cutoff. Results were considered statistically significant at $p<0.05$.

MATERIALS AND METHODS

Clinical Specimens and Cell Lines

A total of 58 OS samples and the morphologically nontumor tissues (located >3 cm away from the tumor) were collected at The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, China. All patients provided written informed consent. This study was approved by the Ethics Committee of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. Samples were immediately snap frozen and stored at −80°C. Clinicopathological characteristics were obtained from medical records and are listed in Table 1.

The OS cell lines (U2OS, SAOS2, SW1353, and MG63) and fetal osteoblastic cell line hFOB were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in DMEM supplemented with 10% FBS (Gibco, Grand Island, NY, USA) at 37°C in a humidified incubator with 5% CO_2.

Oligonucleotide and Transfection

The miR-4443 mimics, inhibitors, and corresponding negative controls (NCs) were synthesized by GenePharma (Shanghai, P.R. China) and transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The cDNA encoding FEZF1-AS1 was polymerase chain reaction (PCR) amplified and subcloned into the pcDNA3.1 vector (Invitrogen). The empty pcDNA3.1 vector was used as the control. The sequence of FEZF1-AS-specific siRNA was 5′-GTGGAAAGGAAGAGAGCTT-3′.

Cell Proliferation Assay

Cell proliferation was detected by a Cell Counting Kit (7 Sea Biotech, Shanghai, P.R. China). Cells were grown in 96-well plates (1×10^4 per well) and incubated in 37°C with 5% CO_2 until the cell confluency rate reached 70%. After transfection with plasmid for 48 h, the cells were incubated for a further 24, 48, and 72 h. CCK-8 (10 µl) solution was added into each well. The absorbance at 450 nm was measured with SUNRISE Microplate Reader (Tecan, Switzerland).

Colony Formation Assay

For the colony formation ability assay, OS cells were counted at 24 h posttransfection and seeded into 24-well plates at 400 cells per well. Culture medium was replaced every 3 days. After approximately 14 days, the cells were cleaned with 1× PBS, stained with common crystal violet dye, and counted using an inverted microscope (IX83; Olympus Corporation, Tokyo, Japan).

In Vitro Migration and Invasion Assays

For the Transwell (24-well insert, 8-mm pore size with polycarbonate membrane; Corning Costar, Lowell, MA, USA) migration assays, 700 µl of media supplemented with 20% FBS was added to the lower chamber, and 1×10^5 cells resuspended in serum-free media were added to the upper insert after transfection. Transwell membranes were fixed and stained using crystal violet after the specified time. The cells adhering to the lower surface of the membrane were counted under a light microscope (Olympus). As for the invasion assay, cells were plated onto BD BioCoat Matrigel Invasion Chambers (a pore size of 8 µm; BD Biosciences, San Jose, CA, USA). The other steps were the same as for the migration assay.

Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from cultured cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol, and cDNA was synthesized from total RNA by a PrimerScript RT Reagent Kit (Takara, Japan). miRNA from the total RNA was reverse transcribed using the Prime-Script miRNA cDNA Synthesis Kit (TaKaRa). RT-PCR was performed with the SYBR Green Premix Ex Taq II (Takara) on an Applied Biosystems Step One Plus Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). GAPDH was used as the endogenous control for the detection of mRNA expression levels, while U6 was used as an endogenous control for miRNA expression analysis.

Tumor Xenograft Model

In short, subcutaneous tumor xenografting was performed by injecting the right flank of 4-week-old female nude mice from Vital River Laboratory Animal Technology (Beijing, P.R. China) with 1×10^7 cells, which have been transfected with siControl or siFEZF1-AS1. Eight nude mice were used per group. After 5 weeks, the mice were killed, and the tumor volumes and weights were measured. All animal experiments were performed with the approval of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University.

Dual-Luciferase Reporter Assay

The FEZF1-AS1 sequence containing the putative miR-4443 binding site was cloned and inserted into pmirGLO vector (Promega, Madison, WI, USA). Cells were seeded in 96-well plates and cotransfected with recombinant plasmids or empty pmirGLO vector, and miR-4443 mimic, inhibitor, or NC. Luciferase activity was measured with the Dual Luciferase Reporter Assay system (Promega) 24 h after transfection. Firefly luciferase activity was normalized against *Renilla* luciferase activity.

Statistical Analysis

All statistical analyses were performed using SPSS 20.0 (IBM, SPSS, Chicago, IL, USA) and GraphPad Prism. Student's *t*-test and one-way ANOVA were used to analyze two or multiple groups, respectively, for statistical significance. Pearson correlation coefficient analysis was used to determine the correlations. The overall survival curves were calculated with the Kaplan–Meier method and were analyzed with the log-rank test. A value of $p<0.05$ was considered statistically significant in all cases.

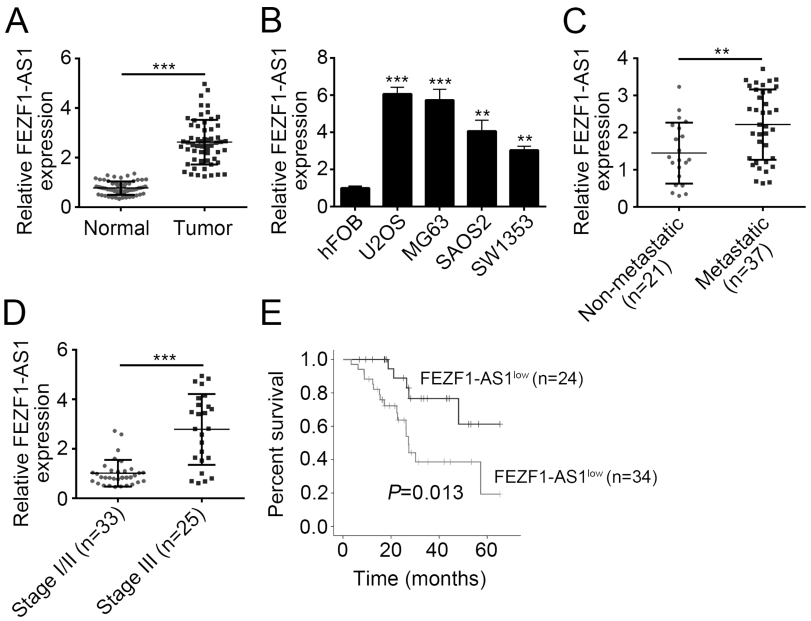

Figure 1. FEZ family zinc finger 1–antisense 1 (FEZF1-AS1) was overexpressed in osteosarcoma (OS) tissues and cell lines. (A) Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) was used to determine the expression of FEZF1-AS1 in OS tissues ($n=58$) and adjacent normal tissues ($n=58$). (B) RT-qPCR analysis showed the expression patterns of FEZF1-AS1 in OS cell lines and hFOB cells. (C) The expression of FEZF1-AS1 in nonmetastatic and metastatic OS tissues was determined by RT-qPCR. (D) The expression of FEZF1-AS1 in stage I/II and stage III OS tissues was analyzed by RT-qPCR. (E) Kaplan–Meier survival curve according to FEZF1-AS1 expression levels. All data are representative of three independent experiments and expressed as mean ± SD. $^{**}p<0.01$ and $^{***}p<0.001$.

RESULTS

FEZF1-AS1 Was Overexpressed in OS Tissues and Cell Lines

To explore the function of FEZF1-AS1 in OS, we performed RT-qPCR analysis to determine the expression patterns of FEZF1-AS1 in 58 pairs of OS and adjacent normal tissues. The results indicated that FEZF1-AS1 was upregulated in OS tissues compared to normal tissues (Fig. 1A). We analyzed the expression of FEZF1-AS1 in OS cell lines by RT-qPCR and found that FEZF1-AS1 was overexpressed in U2OS, MG63, SAOS2, and SW1353 cells (Fig. 1B). Then we analyzed the expression levels of FEZF1-AS1 in the metastatic group and nonmetastatic group. Results showed that FEZF1-AS1 was expressed more highly in the metastatic group (Fig. 1C). Moreover, the expression of FEZF1-AS1 is higher in OS tissues of stage III than stage I/II (Fig. 1D). Then we classified these samples into low or high group according to the mean level of FEZF1-AS1 in OS tissues. Kaplan–Meier analysis indicated that lower FEZF1-AS1 means poorer survival of patients (Fig. 1E) and advanced phenotype of OS (Table 1).

FEZF1-AS1 Depletion Impaired OS Proliferation, Migration, and Invasion In Vitro

To determine the function of FEZF1-AS1 in OS, we knocked down or overexpressed FEZF1-AS1 in U2OS and MG63 cells (Fig. 2A). Then we performed CCK-8 and colony formation assays. Results showed that FEZF1-AS1 knockdown significantly reduced the proliferation

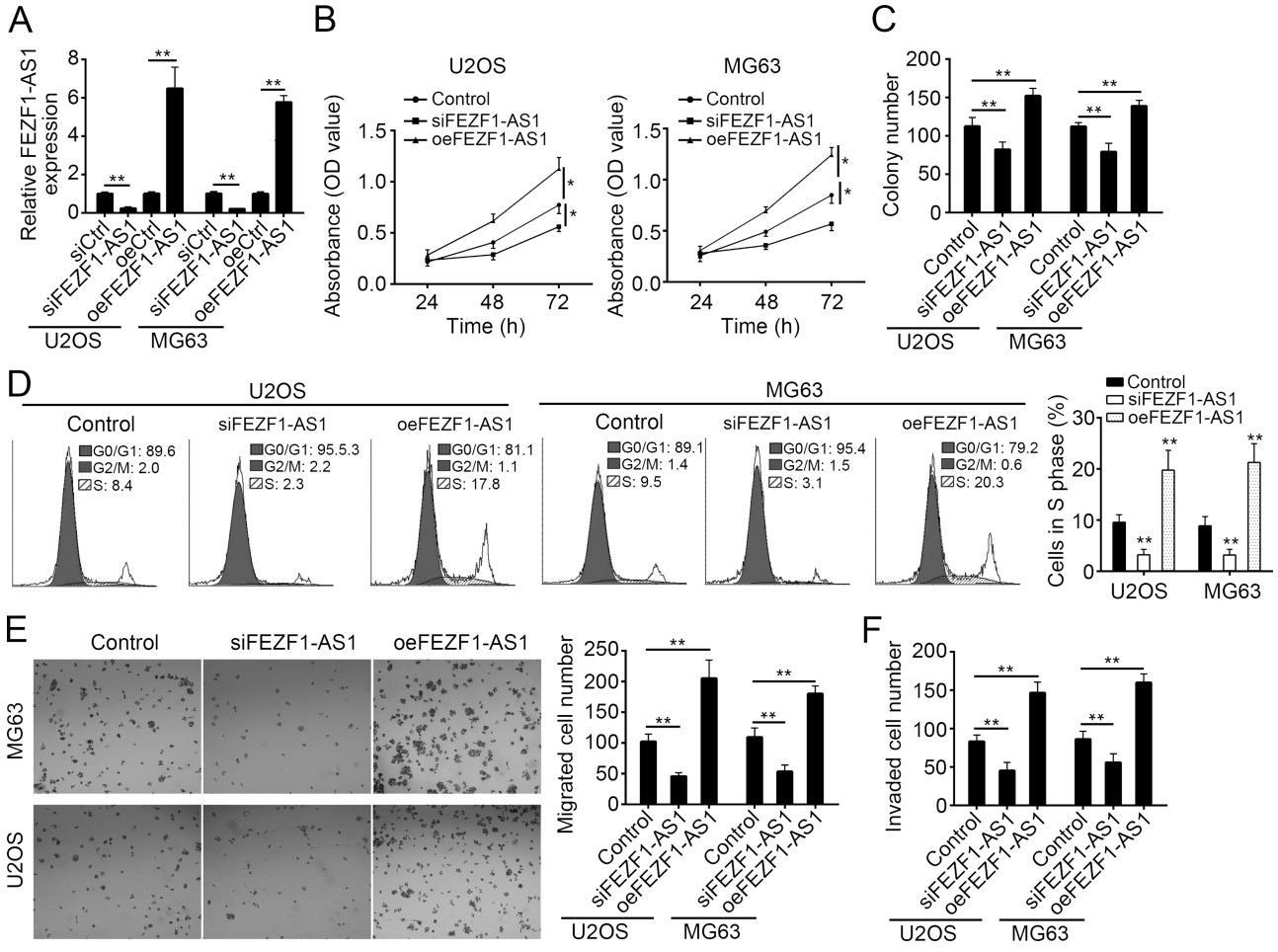

Figure 2. FEZF1-AS1 depletion impaired OS proliferation, migration, and invasion in vitro. (A) The expression of FEZF1-AS1 in U2OS and MG63 cells transfected with control, siFEZF1-AS1, or oeFEZF1-AS1 was determined by RT-qPCR. The effect of FEZF1-AS1 expression on the proliferation of U2OS and MG63 cells was determined by (B) CCK-8 and (C) colony formation assays. (D) Fluorescence-activated cell sorting (FACS) assay showed that FEZF1-AS1 knockdown inhibited cell entry into the S phase, while overexpression promoted entry. (E) Transwell migration and (F) invasion assays showed that FEZF1-AS1 promoted the migration and invasion of U2OS and MG63 cells. All data are representative of three independent experiments and expressed as mean ± SD. $*p<0.05$ and $**p<0.01$.

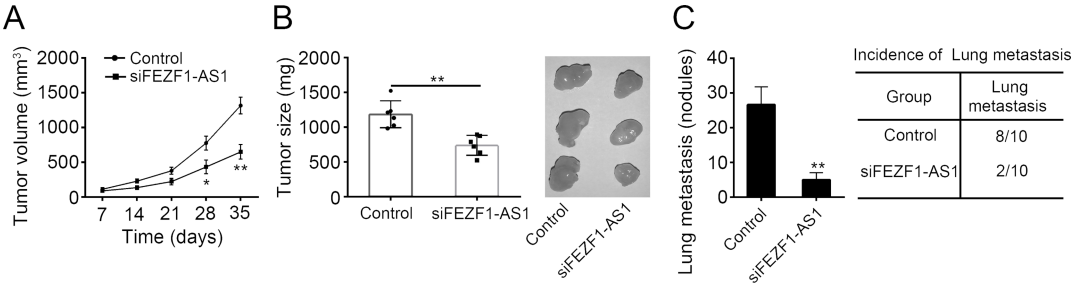

Figure 3. FEZF1-AS1 knockdown inhibited tumor growth and metastasis in vivo. (A) Tumor volumes were measured every 7 days. FEZF1-AS1 knockdown inhibited tumor growth. (B) Tumor sizes were checked at the end of experiments. (C) Lung nodules at week 10 were measured as the numbers of nodules (chi-squared test was used for p value analysis). All data are representative of three independent experiments and expressed as mean ± SD. *$p < 0.05$ and **$p < 0.01$.

and formed colony numbers in U2OS and MG63 cells, while upregulation had the opposite effect (Fig. 2B and C). Moreover, fewer FEZF1-AS1-silenced cells entered into the S phase, while overexpression of FEZF1-AS1 promoted U2OS and MG63 cell entry into cell cycle (Fig. 2D). Next, we checked the effect of FEZF1-AS1 on cell migration and invasion by Transwell assays. We found that FEZF1-AS1 knockdown dramatically inhibited the migration and invasion of U2OS and MG63 cells in vitro, while upregulation had the opposite effects (Fig. 2E and F).

FEZF1-AS1 Knockdown Inhibited Tumor Growth and Metastasis In Vivo

Then we explored the role of FEZF1-AS1 in vivo by an OS xenograft mouse model. Results showed that FEZF1-AS1 depletion significantly delayed tumor growth in vivo and decreased the tumor sizes (Fig. 3A and B). Moreover, to establish a metastatic cancer model in vivo, we injected OS cells into the tail vein of nude mice. Ten weeks after tail vein injection, we analyzed the lung metastasis and found that FEZF1-AS1 knockdown significantly reduced the nodule number in the lung (Fig. 3C).

FEZF1-AS1 Sponged miR-4443 in OS

A previous study showed that lncRNAs were ceRNAs for miRNAs[17]. To further explore the molecular mechanism by which FEZF1-AS1 functions, we made a prediction using an online tool (http://mirdb.org/miRDB/index.html). The result showed that miR-4443 was a potential binding miRNA of FEZF1-AS1. There were two potential binding sites in FEZF1-AS1 (Fig. 4A). However, by dual-luciferase reporter assay, we found that only site #2 could associate with miR-4443 (Fig. 4B). As shown, transfection with miR-4443 mimic significantly inhibited the luciferase activity in MG63 cells transfected with pmirGLO-FEZF1-AS1 containing site #2 (Fig. 4B). In addition, we found that miR-4443 overexpression inhibited the expression of FEZF1-AS1 in U2OS and MG63 cells, while miR-4443 inhibitor increased the expression of FEZF1-AS1 (Fig. 4C). Conversely, overexpression of FEZF1-AS1 decreased the expression of miR-4443 in U2OS and MG63 cells, and FEZF1-AS1 inhibitor increased miR-4443 expression (Fig. 4D). Furthermore, RT-qPCR showed that miR-4443 was downregulated in OS tissues and cell lines compared with normal tissues or cell line (Fig. 4E and F). In addition, we found that the expression of FEZF1-AS1 was reversely correlated with that of miR-4443 in OS tissues (Fig. 4G).

FEZF1-AS1 Regulated Cell Proliferation, Migration, and Invasion by Inhibition of miR-4443

To further determine whether FEZF1-AS1 exerted function in OS by inhibiting miR-4443, we knocked down miR-4443 in FEZF1-AS1-depleted U2OS and MG63 cells (Fig. 5A). Then we performed CCK-8, colony formation, Transwell migration, and invasion assays. Results indicated that FEZF1-AS1 silencing inhibited the proliferation, migration, and invasion of U2OS and MG63 cells, while the addition of miR-4443 inhibition reversed these effects (Fig. 5B–E).

NUPR1 Was a Target Gene of miR-4443, and Restoration of NUPR1 Rescued FEZF1-AS1 Knockdown-Induced Suppression of Cell Proliferation, Migration, and Invasion in OS

To further explore the downstream mechanism mediated by miR-4443, we predicated the potential target mRNA of miR-4443. We found that there is a potential binding site of miR-4443 in the 3′-UTR of NUPR1 mRNA (Fig. 6A). Then we found that the miR-4443 mimic really could inhibit the luciferase activity in MG63 cells transfected with WT 3′-UTR but not mut 3′-UTR (Fig. 6B). Overexpression of miR-4443 inhibited the mRNA and protein levels of NUPR1 in U2OS and MG63 cells, while inhibition of miR-4443 increased NUPR1 expression (Fig. 6C and D). We demonstrated that FEZF1-AS1 sponged miR-4443 in OS. To further determine whether FEZF1-AS1 regulates NUPR1 in OS, we checked the

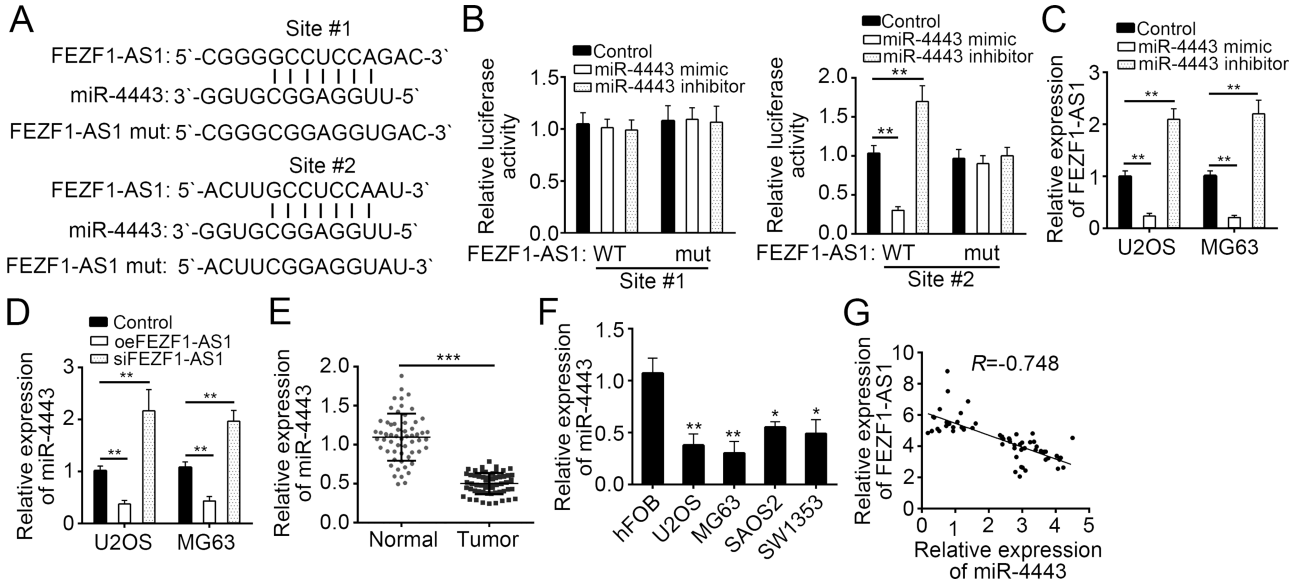

Figure 4. FEZF1-AS1 sponged miR-4443 in OS. (A) The miR-4443 binding sites in FEZF1-AS1 were predicted by bioinformatics analysis. (B) Dual-luciferase reporter assays were conducted with MG63 cells cotransfected with the miR-4443 mimic or inhibitor and FEZF1-AS1-WT or FEZF1-AS1-mut reporter plasmid. (C) RT-qPCR analysis showed that overexpressing miR-4443 significantly decreased the level of FEZF1-AS1 in U2OS and MG63 cells, while inhibiting miR-4443 significantly increased FEZF1-AS1. (D) RT-qPCR results showed that FEZF1-AS1 overexpression inhibited the level of miR-4443 in U2OS and MG63 cells, while FEZF1-AS1 inhibition increased miR-4443. (E) The expression level of miR-4443 in OS tissues was determined by RT-qPCR. (F) The expression patterns of miR-4443 in OS cell lines and hFOB cells were analyzed by RT-qPCR. (G) RT-qPCR showed that the expression of miR-4443 was reversely correlated with that of FEZF1-AS1 in OS tissues. All data are representative of three independent experiments and expressed as mean ± SD. *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

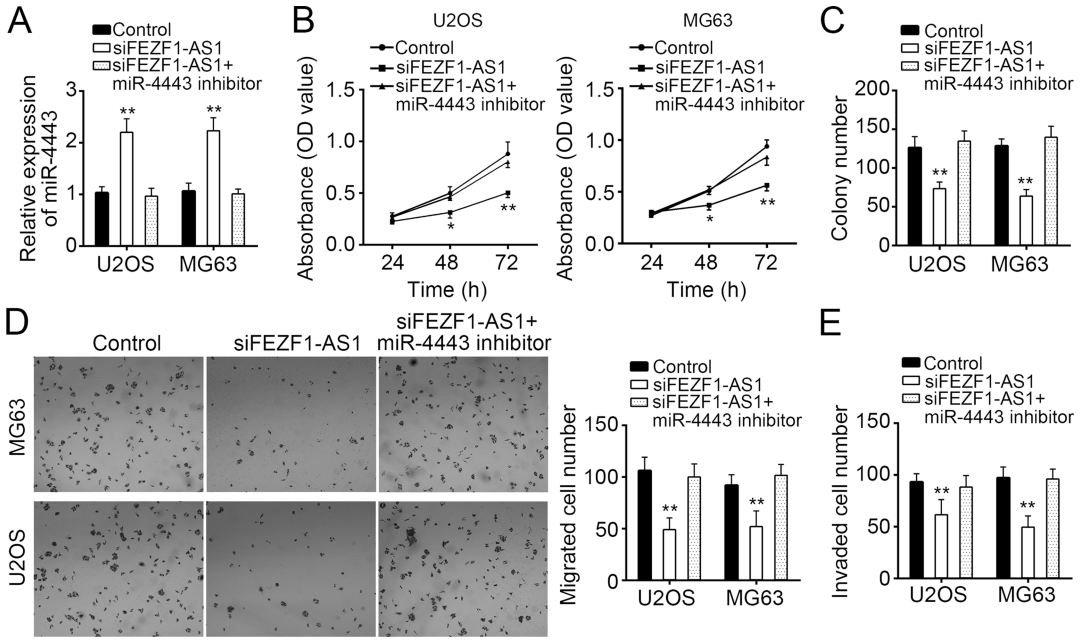

Figure 5. FEZF1-AS1 regulated cell proliferation, migration, and invasion by inhibition of miR-4443. (A) RT-qPCR analysis showed that addition of miR-4443 inhibitors inhibited the expression of miR-4443 in FEZF1-AS1-silenced U2OS and MG63 cells. (B) CCK-8 and (C) colony formation assays showed that FEZF1-AS1 knockdown inhibited U2OS and MG63 cell proliferation, while miR-4443 inhibition reversed it. (D) Transwell migration and (E) invasion assays showed that FEZF1-AS1 knockdown suppressed U2OS and MG63 cell migration and invasion, while miR-4443 inhibition reversed it. All data are representative of three independent experiments and expressed as mean ± SD. *$p<0.05$ and **$p<0.01$.

effect of FEZF1-AS1 on NUPR1 expression. We found that overexpressing FEZF1-AS1 could upregulate the mRNA level of NUPR1 in U2OS and MG63 cells, while inhibiting FEZF1-AS1 decreased NUPR1 expression (Fig. 6E). Consistently, the expression of NUPR1 was also upregulated in OS tissues compared to adjacent normal tissues (Fig. 6F). To further determine the role of NUPR1 on FEZF1-AS1-mediated effects against cell proliferation, migration, and invasion, we restored the expression of NUPR1 in FEZF1-AS1-depleted U2OS and MG63 cells (Fig. 6G). We then performed CCK-8, colony formation, and Transwell assays. Results indicated that restoration of NUPR1 rescued FEZF1-AS1 knockdown-induced suppression of cell proliferation, migration, and invasion in vitro (Fig. 6H–K). Taken together, the above data indicated that FEZF1-AS1 promoted cell proliferation, migration, and invasion via a miR-4443/NUPR1 axis in OS cells.

DISCUSSION

lncRNAs have been reported to play an important role in OS. Several lncRNAs, such as X inactive-specific transcript (XIST) and lnc-small nucleolar RNA host gene 1 (SNHG1), contribute to OS progression[18,19]. Dysregulation

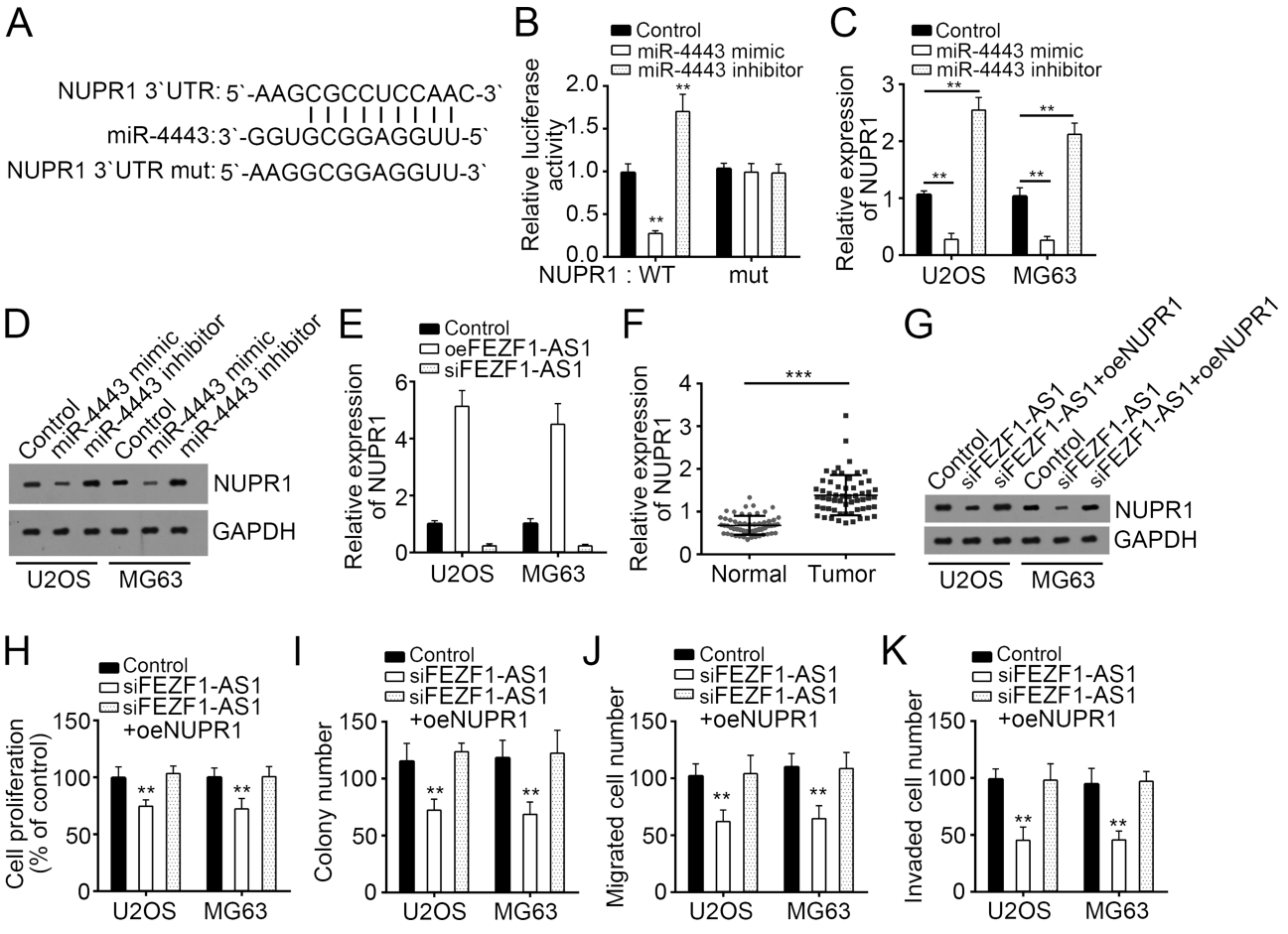

Figure 6. Nuclear protein 1 (NUPR1) was a target gene of miR-4443, and restoration of NUPR1 rescued FEZF1-AS1 knockdown-induced suppression of cell proliferation, migration, and invasion in OS. (A) The miR-4443 binding site in NUPR1 was identified by bioinformatics analysis. (B) Dual-luciferase reporter assays were conducted using MG63 cells cotransfected with miR-4443 mimic or inhibitor and NUPR1-WT or NUPR1-mut reporter plasmid. (C) Relative RT-qPCR and (D) Western blot analysis indicated that overexpressing miR-4443 significantly inhibited the mRNA and protein levels of NUPR1 in U2OS and MG63 cells and vice versa. (E) RT-qPCR showed that FEZF1-AS1 overexpression promoted the mRNA levels of NUPR1 in U2OS and MG63 cells, while FEZF1-AS1 inhibition decreased NUPR1 levels. (F) RT-qPCR analysis showed that NUPR1 expression was upregulated in OS tissues. (G) The protein levels of NUPR1 were restored in FEZF1-AS1-silenced U2OS and MG63 cells. (H) CCK-8 and (I) colony formation assays showed that FEZF1-AS1 knockdown inhibited U2OS and MG63 cell proliferation, while NUPR1 restoration reversed it. (J) Transwell migration and (K) invasion assays indicated that FEZF1-AS1 knockdown inhibited U2OS and MG63 cell migration and invasion, while NUPR1 restoration rescued it. All data are representative of three independent experiments and expressed as mean ± SD. $^{**}p < 0.01$ and $^{***}p < 0.001$.

of lncRNAs is related to abnormal growth, development, and differentiation in various cancers. However, the functions of most lncRNAs still remain to be elucidated. Here we found that lncRNA FEZF1-AS1 was overexpressed in human OS tissues and cell lines. The expression of FEZF1-AS1 is correlated with clinical severity. Moreover, we found that FEZF1-AS1 played essential roles on the proliferation, migration, and invasion of OS cells, which implied that FEZF1-AS1 might be a potential therapeutic target for OS treatment.

Previous studies reported that FEZF1-AS1 promoted tumor progression in various human cancers. For instance, FEZF1-AS1 promotes gastric cancer proliferation by repression of p21 expression[15]. Another study revealed that FEZF1-AS1 was also aberrantly expressed in stomach adenocarcinoma[20]. However, the role of FEZF1-AS1 and its functional molecular mechanism in OS are totally unknown. In our study, we revealed the essential effect of FEZF1-AS1 on OS progression. Through overexpression or knockdown of FEZF1-AS1 in U2OS and MG63 cells, we performed CCK-8, colony formation, and Transwell assays and found that FEZF1-AS1 promoted the proliferation, migration, and invasion of OS cells in vitro. Moreover, we performed xenograft experiments to determine the effect of FEZF1-AS1 on tumor growth and metastasis in vivo. We found that FEZF1-AS1 knockdown remarkably inhibited tumor growth and metastasis. Our study expanded the knowledge of FEZF1-AS1 as a regulator of OS progression.

Accumulating reports showed that lncRNAs could act as ceRNA to associate with miRNAs[21]. To determine the molecular mechanism by which FEZF1-AS1 functions in OS, we made a prediction and demonstrated that FEZF1-AS1 directly bonded to miR-4443 by dual-luciferase reporter assay. The relationship between miRNAs and cancers has been widely studied and elucidated. However, the role of miR-4443 is unknown in cancers. A recent report showed that miR-4443 suppresses the invasiveness of human colon cancer cells[22]. The effect of miR-4443 on cell proliferation and its function in OS need to be determined. In our study, we found that FEZF1-AS1 inhibited the level of miR-4443 that was downregulated in OS tissues compared to adjacent normal tissues. Moreover, by CCK-8 and Transwell assays, we found that inhibition of miR-4443 could rescue the suppression of cell proliferation, migration, and invasion induced by FEZF1-AS1 knockdown in U2OS and MG63 cells. To further determine the downstream mechanism, we searched for the target gene of miR-4443 in OS cells and found that miR-4443 bonded to the 3′-UTR of NUPR1 mRNA. Previous evidence showed that NUPR1 served as an oncogene in various cancers. For instance, knockdown of NUPR1 inhibited the proliferation of glioblastoma cells[23]. In addition, inhibition of NUPR1 decreased human NSCLC growth in vitro and in vivo[24]. However, the function of NUPR1 in OS has not been defined. In our study, we revealed that NUPR1 was downregulated by miR-4443 and upregulated by FEZF1-AS1 in OS cells. We also showed that expression of NUPR1 was upregulated in OS tissues. Moreover, we found that restoration of NUPR1 protein level rescued FEZF1-AS1 knockdown-induced suppression of cell proliferation, migration, and invasion in OS.

In summary, our data demonstrated that lncRNA FEZF1-AS1 promoted OS progression by regulating the miR-4443/NUPR1 axis. The FEZF1-AS1/miR-4443/NUPR1 signal cascade may act as a novel therapeutic strategy for OS treatment.

ACKNOWLEDGMENT: The authors declare no conflicts of interest.

REFERENCES

1. Geller DS, Gorlick R. Osteosarcoma: A review of diagnosis, management, and treatment strategies. Clin Adv Hematol Oncol. 2010;8(10):705–18.
2. Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. Cancer Treat Res. 2009;152:3–13.
3. Meazza C, Luksch R, Daolio P, Podda M, Luzzati A, Gronchi A, Parafioriti A, Gandola L, Collini P, Ferrari A, Casanova M, Terenziani M, Spreafico F, Polastri D, Biassoni V, Schiavello E, Pecori E, Massimino M. Axial skeletal osteosarcoma: A 25-year monoinstitutional experience in children and adolescents. Med Oncol. 2014;31(4):875.
4. Wittig JC, Bickels J, Priebat D, Jelinek J, Kellar-Graney K, Shmookler B, Malawer MM. Osteosarcoma: A multidisciplinary approach to diagnosis and treatment. Am Fam Physician 2002;65(6):1123–32.
5. Mattick JS. The genetic signatures of noncoding RNAs. PLoS Genet. 2009;5(4):e1000459.
6. Liu B, Ye B, Yang L, Zhu X, Huang G, Zhu P, Du Y, Wu J, Qin X, Chen R, Tian Y, Fan Z. Long noncoding RNA lncKdm2b is required for ILC3 maintenance by initiation of Zfp292 expression. Nat Immunol. 2017;18(5):499–508.
7. Kwok ZH, Tay Y. Long noncoding RNAs: lncs between human health and disease. Biochem Soc Trans. 2017;45(3):805–12.
8. Wapinski O, Chang HY. Long noncoding RNAs and human disease. Trends Cell Biol. 2011;21(6):354–61.
9. Anninos P, Chatzimichael A, Adamopoulos A, Kotini A, Tsagas N. A combined study of MEG and pico-Tesla TMS on children with autism disorder. J Integr Neurosci. 2016;15(4):497–513.
10. Li W, Huang K, Wen F, Cui G, Guo H, Zhao S. Genetic variation of lncRNA GAS5 contributes to the development of lung cancer. Oncotarget 2017;8(53):91025–9.
11. Li B, Shi C, Zhao J, Li B. Long noncoding RNA CCAT1 functions as a ceRNA to antagonize the effect of miR-410 on the down-regulation of ITPKB in human HCT-116 and HCT-8 cells. Oncotarget 2017;8(54):92855–63.
12. Wu F, Mo Q, Wan X, Dan J, Hu H. NEAT1/has-mir-98-5p/MAPK6 axis is involved in non-small-cell lung cancer (NSCLC) development. J Cell Biochem. 2017. [Epub ahead of print]

13. Zhu P, Wang Y, Wu J, Huang G, Liu B, Ye B, Du Y, Gao G, Tian Y, He L, Fan Z. LncBRM initiates YAP1 signalling activation to drive self-renewal of liver cancer stem cells. Nat Commun. 2016;7:13608.
14. Chen N, Guo D, Xu Q, Yang M, Wang D, Peng M, Ding Y, Wang S, Zhou J. Long non-coding RNA FEZF1-AS1 facilitates cell proliferation and migration in colorectal carcinoma. Oncotarget 2016;7(10):11271–83.
15. Liu YW, Xia R, Lu K, Xie M, Yang F, Sun M, De W, Wang CL, Ji GZ. LincRNAFEZF1-AS1 represses p21 expression to promote gastric cancer proliferation through LSD1-mediated H3K4me2 demethylation. Mol Cancer 2017;16:39.
16. He R, Zhang FH, Shen N. LncRNA FEZF1-AS1 enhances epithelial-mesenchymal transition (EMT) through suppressing E-cadherin and regulating WNT pathway in non-small cell lung cancer (NSCLC). Biomed Pharmacother. 2017;95:331–8.
17. Sumazin P, Yang X, Chiu HS, Chung WJ, Iyer A, Llobet-Navas D, Rajbhandari P, Bansal M, Guarnieri P, Silva J, Califano A. An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. Cell 2011;147(2):370–81.
18. Zhang R, Xia T. Long non-coding RNA XIST regulates PDCD4 expression by interacting with miR-21-5p and inhibits osteosarcoma cell growth and metastasis. Int J Oncol. 2017;51(5):1460–70.
19. Wang J, Cao L, Wu J, Wang Q. Long non-coding RNA SNHG1 regulates NOB1 expression by sponging miR-326 and promotes tumorigenesis in osteosarcoma. Int J Oncol. 2018;52(1):77–88.
20. Gu JB, Li Y, Fan LQ, Zhao Q, Tan BB, Hua KL, Wu GB. Identification of aberrantly expressed long non-coding RNAs in stomach adenocarcinoma. Oncotarget 2017;8(30): 49201–16.
21. Thomson DW, Dinger ME. Endogenous microRNA sponges: Evidence and controversy. Nat Rev Genet. 2016; 17(5):272–83.
22. Meerson A, Yehuda H. Leptin and insulin up-regulate miR-4443 to suppress NCOA1 and TRAF4, and decrease the invasiveness of human colon cancer cells. BMC Cancer 2016;16:882.
23. Li J, Ren SY, Liu YJ, Lian ZG, Dong B, Yao YQ, Xu YH. Knockdown of NUPR1 inhibits the proliferation of glioblastoma cells via ERK1/2, p38 MAPK and caspase-3. J Neurooncol. 2017;132(1):15–26.
24. Guo XT, Wang W, Hu J, Feng KJ, Pan YM, Zhang LY, Feng YK. Lentivirus-mediated RNAi knockdown of NUPR1 inhibits human nonsmall cell lung cancer growth in vitro and in vivo. Anat Rec. 2012;295(12):2114–21.