Knockdown of lncRNA FEZF1-AS1 Inhibits metastasis of Osteosarcoma Cells by miR-4456/GALNT10

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Research

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Abstract

Background: Osteosarcoma (OS) is among the malignant tumors with high mortality and low survival, especially in children and adolescents. Research shows that LncRNA FEZ family zinc finger 1 antisense RNA 1 (FEZF1-AS1) enhances osteosarcoma progression. Nevertheless, the function and mechanism of FEZF1-AS1 in metastasis of OS remains unclear.

Methods: We used qRT-PCR to assay for the expression of FEZF1-AS1, miR-4456, and GALNT10 in OS tissue specimens and cell lines. We also investigated the progression of OS through metastasis using the wound healing and Transwell assays. Moreover, we used the dual-luciferase reporter test, RIP assays, and western blot to validate whether FEZF1-AS1 serves as a competing endogenous RNA (ceRNA), modulating the expression of GALNT10 through sponging miR-4456 in OS.

Results: FEZF1-AS1 was up modulated in OS tissues. Silencing FEZF1-AS1 repressed OS cell migration and invasion. microRNA-4456 (miR-4456) was involved in FEZF1-AS1-induced migration and invasion. miR-4456 was down modulated in OS tissue specimens and cell lines. Functionally, the up modulation of miR-4456 reversed the facilitative influence of FEZF1-AS1 on OS cell infiltration and migration. Mechanically, FEZF1-AS1 interacted with miR-4456 in a reciprocal suppressed manner. Moreover, miR-4456 targets GALNT10 via the Luciferase assay. Besides, the up modulation of GALNT10 reversed the migration and invasion inhibited by FEZF1-AS1 knockdown. Silencing of FEZF1-AS1 inhibits OS cell infiltration and migration through miR-4456 /GALNT10 sponging.

Conclusion: Herein, we demonstrated that FEZF1-AS1 is a prospective bio signature of metastasis in OS patients. Mechanistically, we showed that the FEZF1-AS1/miR-4456/GALNT10 axis is a target for novel therapeutic development for OS.

Introduction

OS is the most frequent bone tumor in children and teenagers with high fatality rates [1]. Despite the much effort spent over the decades, the overall survival of OS patients remains dismal [2]. In most instances, the malignancies metastasize to the lung tissues in OS patients [3]. Standard neoadjuvant chemotherapy successfully raises the likelihood of limb rescue, which considerably enhances the 5-year survival rate and quality of life in OS patients [4]. The results of previous studies posit that lymph-node metastasis and distant metastasis of malignancies is a multiplex and multiphase process entailing the participation of genes, proteins, miRNAs, and signaling cascades [5, 6]. Therefore, it is urgent to comprehend the pathogenesis of OS metastasis to enhance the outcomes in patients.

Emerging research evidence posits that noncoding RNAs, including long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs serve significant roles in modulating pathogenesis, detection, and prognosis of OS [7, 8]. LncRNAs function as crucial OS biosignatures and drug targets by serving as competitive endogenous RNAs (ceRNAs) for miRNAs to repress mRNA expression [9]. Increasing evidence demonstrates that FEZF1-AS1acts as an oncogene to enhance the progression of cancers, such as non-

small lung cancer [10], Glioblastoma [11], ovarian cancer [12], and OS [13]. FEZF1-AS1, as a ceRNA modulates miR-144/CXCR4 promotes, OS cell proliferation, Warburg effect, and inhibits OS cell apoptosis [13]. FEZF1-AS1 facilitates OS progression via modulating the miR-4443/NUPR1 cascade [14]. Nevertheless, the role of FEZF1-AS1 in OS metastasis has not been elucidated.

Research evidence shows that miRNAs participate in the pathogenesis of OS by functioning as oncogenes or tumor inhibitors via various cascades [4, 15]. The findings of early studies indicate that the atypical expression of miRNAs is closely linked to OS progress[16]. For instance, miR-451a hinders the growth and metastatic phenotypes of papillary thyroid carcinoma cells through repressing ZEB1[4] and miR-134/ VEGFA suppresses tumorigenesis and progression by mediated IRF1 in OS [15]. These data imply that miRNAs play a critical role in OS. Nevertheless, the function of miR-4456 in OS remains unclear. miR-4456 plays a suppressive role in AKI [17]. Additionally, miR-4456 is down modulated in Hypersexual disorder [18].

Glycosyltransferase, also referred to as glycogene, is a kind of enzyme that moderates the modifications of proteins and lipids [19]. It serves an essential function in normal cell development and other physiological processes [20]. Moreover, it is a crucial biomarker in cancer [21]. For instance, it represses the Hnf4α/ miR-122 cascade in hepatitis B virus-correlated hepatocellular carcinoma and facilitates the activity of the prospective oncogenic GALNT10 protein [22]. Nevertheless, the function and mechanism of miR-4455 and GALNT10 in OS metastasis remains unclear.

Here, we elucidated the expression of FEZF1-AS1 in OS tissues and cell lines, as well as the knockdown of FEZF1-AS1suppressed metastasis. Therefore, we inspected the therapeutic influence of FEZF1-AS1 on OS and the ceRNA modulatory network of FEZF1-AS1/miR-4456/GALNT10.

Materials And Methods

Human tissue samples

We collected 31 pairs of specimens consisting of osteosarcoma and adjacent normal tissues from osteosarcoma patients (from January 2013 to November 2019) at The Tumor Hospital of Harbin Medical University. The Ethics Committee of The Affiliated Hospital of Harbin Medical University approved this study, and we obtained written informed permission from all the patients. Clinicopathological parameters of OS patients were showed in Table1. All tissues were harvested and then kept at -80℃ or snap-frozen in liquid nitrogen immediately pending use.

Cell culture

We bought the normal human osteoblast cell line hFOB1.19 and osteosarcoma cell lines (143B, U2OS, MG63, and HOS) from the American Type Culture Collection (Manassas, VA). We cultured all the cell lines in Dulbecco's modified Eagle's medium (DMEM, (HyClone, Logan, USA) added with 10% fetal bovine

serum (FBS, Gibco, NY, USA), 100 U/mL penicillin, and 100 mg/mL streptomycin (Baomanbio, Shanghai, China) and incubated in an incubator (5% CO_2, 37°C).

Cell transfection

The FEZF1-AS1-siRNA, miR-4456 mimics, miR-4456 inhibitor, GALNT10 plasmids, and their corresponding negative controls (shNC, NC mimics, NC inhibitor, pcDNA3.1) were constructed and provided by RIBIOBIO (Guangzhou, China). We grew the OS cells in the medium until a confluence of approximately 60-70%. After that, we transfected all the plasmids mentioned above into 143B or U2OS using the riboFECT CP Transfection Kit (C10511-1, RIBIOBIO, Guangzhou, China) as per instructions of the manufacturer. After 48h or 72 h following transfection, we attained the OS cells for downstream experiments.

RT-qPCR

To assess the expression of FEZF1-AS1, GLANT10, and miR-4456 in OS tissue or cells, we performed RT-qPCR. The trizol reagent (Cat: 15596026, Invitrogen, Carlsbad, USA) was utilized to extract total RNA as per the protocol of the manufacturer. The TransScript Green One-Step qRT-PCR SuperMix kit (Takara, Kyoto, Japan) was used to assay for the relative gene expression via qPCR. GAPDH or U6 was used as an internal control. The 2−ΔΔ-Ct approach was used to examine the relative level of expression of genes in OS. The sequences of the primers used in this study are shown in Table S1.

Western blot assay

We harvested the treated cells and lysed the using RIPA (Radio Immunoprecipitation Assay, Beyotime, Shanghai, China) buffer. We separated the cell lysates on 12.5% SDS/PAGE (sodium dodecyl sulfate-polyacrylamide), then transferred onto PVDF (polyvinylidene fluoride membrane, Millipore, Billerica, USA) membranes, followed by blocking with 5% non-fat dried milk for 1h at room temperature (RT). After that, we incubated the membranes with primary antibodies at 4℃ against GALNT10, E-cadherin, Vimentin, and GAPDH. Subsequently, we incubated the membranes with the second antibodies separately at RT for 2h, and the proteins detected using an EasyBlot ECL kit (ECL kit, Santa Cruz Biotechnology, Santa Cruz, USA). The antibodies targeting GALNT10, E-cadherin, Vimentin, and GAPDH were all purchased from Abcam Co., Ltd. (Pudong, Shanghai, China). The density of the bands was imaged via enhanced chemiluminescence. GAPDH was employed as an endogenous control.

Bioinformatics Analysis and Luciferase Reporter Assay

We predicted the putative binding sites of miR-4456 and FEZF1-AS1 or GALNT10 via bioinformatics analysis using starbase 2.0 (starbase.sysu.edu.cn). We generated the wild-type vectors (FEZF1-AS1-WT and GALNT10-WT) and mutant-type vectors (FEZF1-AS1-Mut and GALNT10-Mut) using the pGL3 vector (Promega, Madison, WI, USA). In the luciferase reporter test, we used the riboFECT CP Transfection Kit (Catalogue number, manufacturer, country) to co-transfect the U2OS cells with WT or Mut luciferase

reporter vectors and miR-4456 mimic or miR-NC mimic. Following 48h, post-transfection, we conducted the luciferase enzyme activity test in each group using the luciferase reporter assay kit (Catalogue number, Promega, country). The luciferase activity of Renilla was employed as the standard.

RNA Immunoprecipitation (RIP)

We harvested 1×10^7 U2OS and 143B cells with or without miR-4456 mimic and then lysed them in RIP buffer. We performed the RIP test using a Magna RNA immunoprecipitation kit (Millipore, Billerica, MA, USA), as well as magnetic beads, pre-coated with Ago2 or IgG antibody. The levels of FEZF1-AS1 in the complex were assayed via qRT-PCR after the isolation of RNA using the Trizol reagent.

Trans-well assay

Trans-well assays were used to detected OS cell invasion. We used the Trans-well chambers (Corning, NY, USA) to observe OS cell infiltration. We seeded 200μl of OS cell suspension (1×10^5 cells) in the serum-free medium into the upper chamber, which was coated with Matrigel (BD Biosciences, San Diego, CA, USA). After that, we added 800μl of DMEM added with 10% FBS to the lower chamber. Following 48h of incubation, we removed the cells on the top side of the membrane via a swab. We fixed the infiltrative cells on the bottom and stained with 0.1% crystal violet for 15min. The number of invading cells was estimated using a phase-contrast microscope (Olympus, Tokyo, Japan).

Wound healing assays

We seeded the OS cells into 6-well plates and then utilized a sterile 10μl plastic micropipette tip to scratch through the monolayer. After that, we washed the cells thrice using physiological saline, then replaced it with DMEM medium added 0.2% FBS. The cells were scratches for 24h and 48h, then observed the migration of the cells using a microscope (indicate which type of microscope and the manufacturer).

ceRNA modulatory network design and evaluation

The determined prognosis-correlated lncRNAs and miRNAs were utilized to identify lncRNA-miRNA modulatory relationships from the correlations using miRcode (mircode.org) and starBase (starbase.sysu.edu.cn/index). Moreover, we selected the miRNA-mRNA modulatory correlations based on the modulatory information in TargetScan (targetscan.org), miRTarBase (mirtarbase.mbc.nctu.edu.tw) (30), miRanda (microrna.org/microrna), and miRBase (mirbase.org).

Immunohistochemistry

We fixed the tissue samples in 10% formalin and then sliced them into 4μm sections. Subsequently, we incubated the sections at 60ºC for 1h, followed by conventional xylene dewaxing and gradient alcohol dehydration. Then, we blocked the sections blocked using normal goat serum solution at 37°C for 10min, followed by incubation with the primary antibody, anti-GALNT10 for 12h at 4°C. After that, we incubated the sections with the secondary antibody, biotinylated anti-mouse for 10min at RT. The image analysis

software (Nikon, Tokyo, Japan) was utilized to count the number of positive cells. The percentage proportion of the positive cells in each field was computed; the percentage of <10% was regarded as negative, and > 10% was considered as positive.

Statistical analysis

All statistical analyses were conducted independently three to six times in the SPSS 20.0 software (Chicago, IL, USA). Student's t-test and ANOVA were applied in determining the significance of differences. A $P < .05$ signified a marked difference.

Results

FEZF1-AS1 is up modulated in OS

We performed qRT-PCR assays to determine the expression level of FEZF1-AS1 in OS, and the results are shown in Figure 1A. FEZF1-AS1 was up modulated in OS tissue samples. At the same time, the up modulation of FEZF1-AS1 was more frequent in OS tissue samples with lymph node metastasis and distant metastasis (Figure 1B). We additionally examined FEZF1-AS1 levels in normal human osteoblast cell line (hFOB) and osteosarcoma cell lines (143B, U2OS, MG63, and HOS). The levels of FEZF1-AS1 in the OS cell lines were 1.5-3.6-fold higher than in the hFOB cells (Fig. 1C). These data indicate that the upregulation of FEZF1-AS1 in OS causes a dismal prognosis.

FEZF1-AS1-si represses OS cell migration and invasion

We conducted the qRT-PCR test to assess the expression of FEZF1-AS1 in 143B and U2OS cells inserted with FEZF1-AS1-siRNA via transfection (Figure 2A). The wound-healing assay indicated that the metastasis potential was distinctly elevated in the 143B and U2OS cells with the transfection of FEZF1-AS1-siRNA (Figure 2B-C). The transwell assays revealed that FEZF1-AS1-siRNA silencing suppressed remarkably the migration of 143B and U2OS compared with the control (Figure 2D-E). These data show that FEZF1-AS1-siRNA inhibits osteosarcoma cell infiltration and migration. We evaluated the protein levels of EMT biosignatures to gather more evidence. Consequently, E-cadherin was remarkably elevated, whereas Vimentin markedly decreased in the FEZF1-AS1 silenced group. These data indicated that FEZF1-AS1 reduces cell metastasis in OS.

FEZF1-AS1 is a ceRNA of miR-4456

CeRNAs serves a crucial role in the mechanisms of lncRNAs, miRNA, and mRNA in OS [23]. Therefore, we analyzed the miRNA-binding site in FEZF1-AS1 using bioinformatics tools. We found a potential miR-4456 binding site in FEZF1-AS1 (Fig. 3A) and, then, investigated the level of miR-4456 in U2OS cells. The expression of miR-4456 was distinctly reduced in U2OS cells compared with the hFOB cells (Fig. 3B), which was negatively associated with FEZF1-AS1 levels. Depletion of FEZF1-AS1 led to increased levels of miR-4456 (Fig. 3C), whereas over-expression of miR-4456 suppressed FEZF1-AS1 levels (Fig. 3D). We

additionally found that miR-4456 mimics remarkably reduced luciferase activity in FEZF1-AS1-WT compared with the FEZF1-AS1-Mut group (Fig. 3E). Therefore, FEZF1-AS1 is a ceRNA of miR-4456.

FEZF1-AS1 serves as a ceRNA for miR-4456 to enhance GALNT10 expression

miRNAs generally suppress gene functions by targeting the 3'-UTR of their target genes [24]. Therefore, we used several bioinformatics tools to identify miR-4456 targets in OS. Subsequently, the potent oncogene GALNT10 was revealed as a possible target (Fig. 4A). In the tissues obtained from the OS patients, GALNT10 expression was remarkably higher (2.3-fold) than that in the normal tissues (Fig. 4B-C). Furthermore, we found that the expressions of GALNT10 and FEZF1-AS1 were positively associated (Fig.4D). The transfection of miR-4456 mimics decreased GALNT10 mRNA and protein levels (Fig. E, F). A dual-luciferase reporter test was conducted to examine if GALNT10 is a functional target of miR-4456. Our data indicated that only the co-transfection with miR-4456 mimics and the wild-type binding site of GALNT10 (GALNT10-WT) reduced luciferase activity (Fig. 4G). These data reveal that GALNT10 is a direct target of miR-4456.

Since we identified FEZF1-AS1 as a ceRNA of miR-4456, we next examined if FEZF1-AS1 controlled GALNT10 expression. GALNT10 protein levels were repressed by FEZF1-AS1 siRNA transfection (Fig.4H). A dual-luciferase reporter test demonstrated that the depletion of FEZF1-AS1 markedly reduced the luciferase activity in GALNT10-WT (Fig. 4I). Next, we examined the presence of FEZF1-AS1 and miR-4456. U2OS cell extracts were immunoprecipitated against Ago2, and mRNA levels in immunoprecipitants were examined by real-time PCR. Both FEZF1-AS1 and miR-4456 were augmented in Ago2 pellets relative to the IgG control (Fig. 4J). These data demonstrate that FEZF1-AS1 is a ceRNA for miR-4456 in promoting GALNT10 expression.

Over-expression GALNT10 expression reverses the repression of OS metastasis stimulated by FEZF1-AS1 silencing

A GALNT10 expression vector was prepared and co-transfected with FEZF1-AS1 siRNA into U2OS cells to confirm the role of FEZF1-AS1 via GALNT10 in OS cells. Consequently, FEZF1-AS1 siRNA decreased FEZF1-AS1 levels and GALNT10 expression. Co-transfection with a GALNT10 expression vector re-established GALNT10 expression in U2OS cells (Fig. 5A). The metastasis assay showed that restored GALNT10 expression augmented metastasis development (Fig. 5B). Therefore, FEZF1-AS1 regulated OS cell growth via GALNT10.

Discussion

Herein, we elucidated the therapeutic function of FEZF1-AS1 silencing in the progression of OS. We established that the expression of FEZF1-AS1 was augmented in OS tissues as well as cell lines. Knockdown on FEZF1-AS1 was correlated with reduced OS cell infiltration and migration. Besides, we established that the mechanism through which FEZF1-AS1 participates in OS metastasis is mediated by

miR-4456 and GALNT10. Additionally, we identified a novel prospective mechanism of FEZF1-AS1, which could be essential in OS drug design.

Research evidence shows that the ceRNA modulatory network of lncRNA/miRNA/mRNA is significant in the progression, reoccurrence, and therapy of OS [25]. FEZF1-AS1 enhances oncogenesis and malignancy in OS [13, 14]. Nevertheless, the function and mechanism of FEZF1-AS1 in OS metastasis remains unclear. Here, we demonstrated that FEZF1-AS1 is up modulated in OS tissues and cell lines and is associated with metastasis, implying that FEZF1-AS1 is an oncogene in OS consistent with the findings of previous studies (insert citations). We used the loss-of-function assays in this study and demonstrated that silencing FEZF1-AS1 represses OS cell metastasis vial the wound-healing and trans-well tests. Hence, the FEZF1-AS1 knockdown has a therapeutic effect on OS.

In the present study, we focused on unraveling the novel molecular cascade of FEZF1-AS1 in OS progression. The ceRNA modulatory network constitutes the primary mechanism of lncRNA in human tumors [26]. The findings of several studies indicate that FEZF1-AS1 promotes NSCLC and invasion through the up-regulation of NOTCH1 by serving as a sponge of miR-34a [10]. FEZF1-AS1 serves as a ceRNA sponge of miR-144/CXCR4 to facilitate cell proliferation, Warburg Effect, and represses cell death in OS [13]. The prospective interaction of FEZF1-AS1 with miRNA was explored using bioinformatics tools to investigate the potential mechanism of FEZF1-AS1 in OS. Here, we first verified FEZF1-AS1 as a mimic of miR-4456 in OS cells using the luciferase reporter test. Our data showed that miR-4456 is down modulated in OS, revealing that miR-4456 has a tumor-inhibitory function. miR-4456 was inversely correlated with FEZF1-AS1 mRNA expression in OS tissues. The knockdown of FEZF1-AS1 up modulated the expression of miR-4456, while over-expression of miR-4456 inhibited the expression of FEZF1-AS1. Luciferase assay data showed that miR-4456 mimics the interface the 3'UTR of FEZF1-AS1-WT, whereas it cannot regulate FEZF1-AS1-Mut. These data suggest that FEZF1-AS1 moderated OS progression by sponging miR-4456. To explore further the ceRNA modulatory network, we validated GALNT10 as a functional target of miR-4456 via bioinformatics analyses and the luciferase reporter test. We established that the expression of GALNT10 is up modulated in OS. GALNT10 is an oncogene in many cancers [21]. GALNT10 participates in viral invasion or replication, immune cell migration, and viral clearance from tissues [27]. Our data showed GALNT10 was down modulated by miR-4456 but positively associated with FEZF1-AS1 in OS. This indicates that FEZF1-AS1 acts as a ceRNA for miR-4456 to repress GALNT10 levels in OS cells. Additionally, we demonstrated that the over-expression of GALNT10 reverses the metastasis inhibition of OS cells induced by FEZF1-AS1 knockdown, indicating that FEZF1-AS1 promotes OS cell metastasis, at least partially through GALNT10.

In conclusion, here, we elucidated the influence of silencing FEZF1-AS1 on OS metastasis by targeting miR-4456/GALNT10. We first provide the ceRNA modulatory network of FEZF1-AS1/miR-4456/GALNT10 in OS metastasis, revealing FEZF1-AS1 as a novel target of OS metastasis therapy.

Declarations

Ethics approval and consent to participate

The Ethics Committee of The Affiliated Hospital of Harbin Medical University approved this study, and we obtained written informed permission from all the patients.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Not applicable

Authors' contributions

Jing-Long Yan& Zhenxing Si: Conceptualization, Methodology, Software

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Table 1

Table 1 Clinicopathological Parameters of Osteosarcoma Patients (n=31)

Clinical feature	n	*P* value
Age		0.05
≥20yr	15	
≤20yr	16	
Gender		0.05
Man	13	
Woman	18	
Tumor size		0.024
≥5cm	12	
≤5cm	19	
Clinical stage		0.001
IIA-B	11	
IIIA-B	20	
Distant metastasis		0.031
Yes	19	
No	12	

Figures

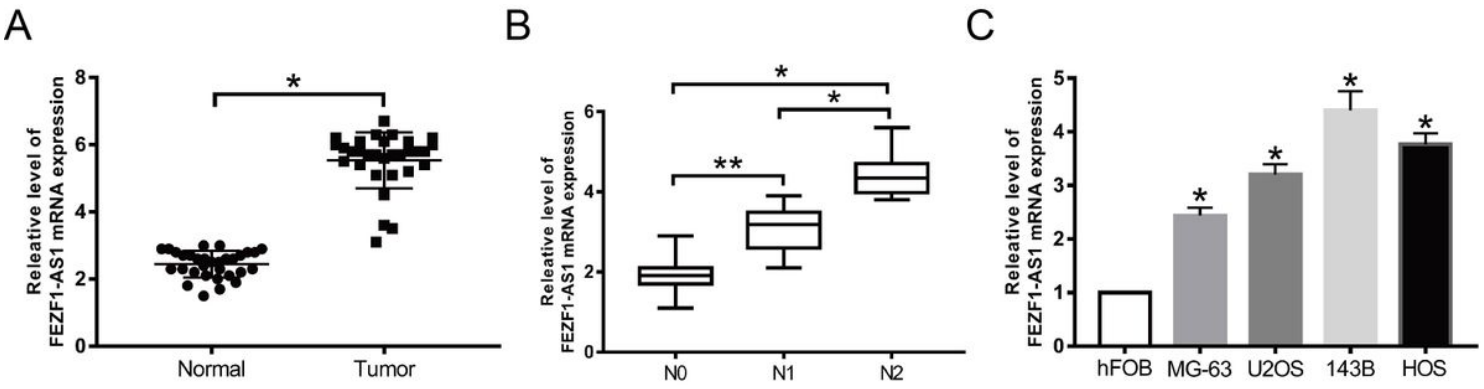

Figure 1

FEZF1-AS1 expression is elevated in OS. (A) The expression of FEZF1-AS1 was assyed in OS tissues and normal samples via qRT-PCR, n=31. (B) The expression of FEZF1-AS1 in OS patients was analyzed with lymph-node metastasis and distant metastasis. (C) The FEZF1-AS1 levels were quantitated in OS cells by qRT-PCR. *P<.05.

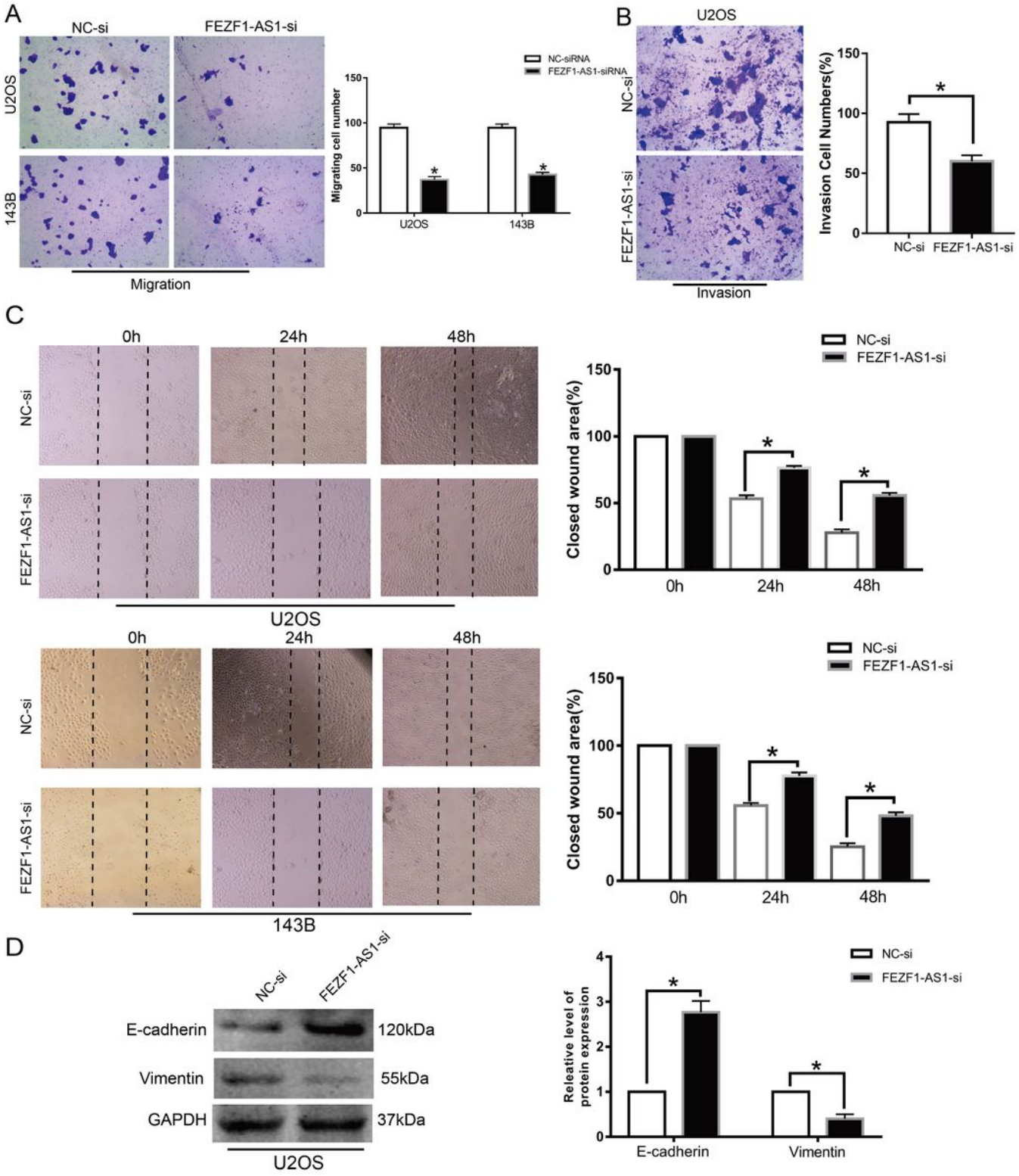

Figure 2

Silencing of FEZF1-AS1 represses OS progression. U2OS cells and 143B cells inserted with FEZF1-AS1-si via transfection. (A) Representative images showing the migration of U2OS and 143B cells. We counted the cells of the corresponding assays in at least four random microscope fields (*100 magnification). Cell migration is indicated as a percentage proportion of the control values. (B) Representative images showing infiltration of U2OS cells. We counted the cells of the corresponding assays in at least four

random microscope fields (*100 magnification). Cell infiltration is indicated as a percentage proportion of the control values. (C) Representative images from wound-healing tests of U2OS and 143B cells (40 magnification). (D) Western blotting evaluations of the expression of EMT-associated markers in U2OS cells. Scale bar, 50mm. n = 3–6; *P<.05 vs. NC-si.

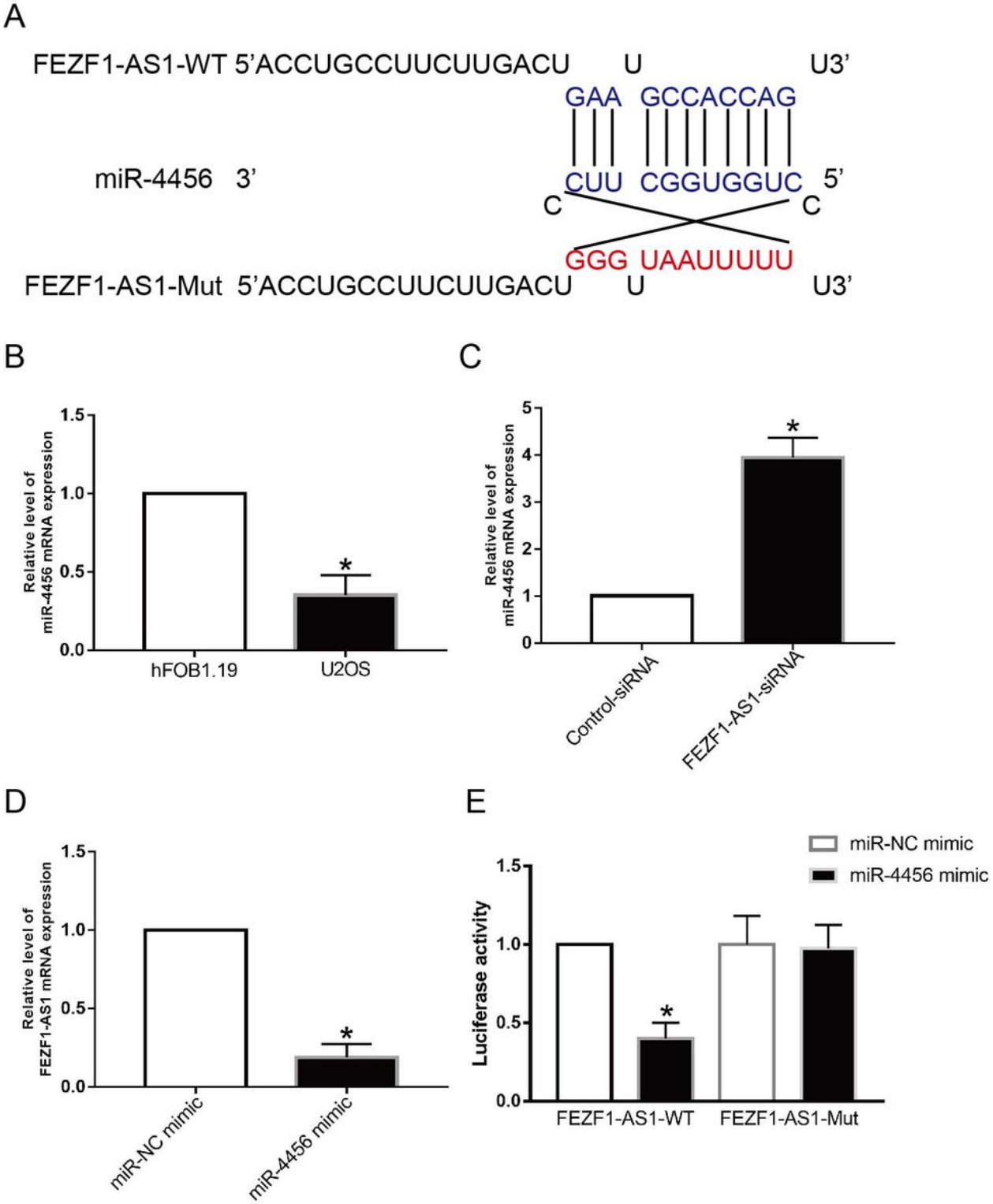

Figure 3

miR-4456 is bound to FEZF1-AS1. (A). The prospective binding sites of FEZF1-AS1 and miR-4456. (B and C) relative level of miR-4456 or FEZF1-AS1 measured in U2OS cells inserted with miR-4456 mimic or miR-NC and FEZF1-AS1-si or NC-si via co-transfection (D) The expression of FEZF1-AS1-after transfection with miR-4456 mimic or miR-NC mimic assayed using qRT-PCR. (E) Luciferase enzyme activity was examined in U2OS cells inserted with miR-4456 or miR-NC and FEZF1-AS1-WT or FEZF1-AS1-MUT, via co-transfection. *P<.05.

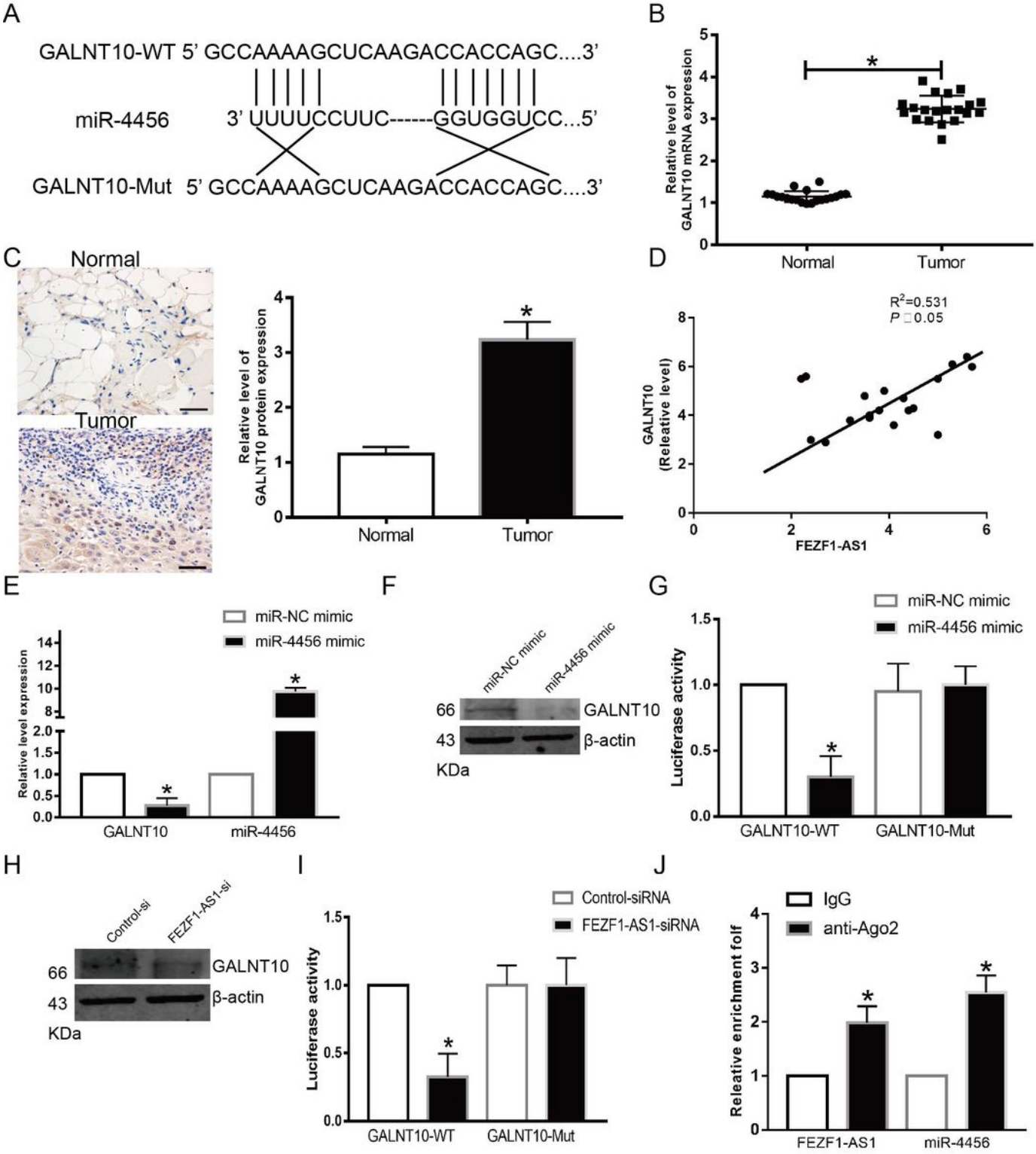

Figure 4

FEZF1-AS1 serves as a ceRNA for miR-4456 to enhance GALNT10 expression. (A) The prospective binding sites of GALNT10 and miR-4456. (B and C) The expression of GALNT10 in OS tissue. (D) Correlation between galnt10 and fezf1-as1 in OS tissues. (E-F) Relative mRNA and protein level of GALNT 10 and miR-4456 after transfection with miR-4456 mimic or miR-NC mimic. (G) Luciferase enzyme activity was assessed in U2OS cells inserted with miR-4456 or miR-NC through co-transfection. (H) The protein level of GALNT10 after transfection with FEZF1-AS1-si. (I) Luciferase enzyme activity was inspected in U2OS cells inserted with FEZF1-AS1-si or Control-si via co-transfection. (J) The enrichment of FEZF1-AS1 was assayed in the two cells transformed via transfection with miR-4456 or miR-NC after Ago2 RIP. *P<.05.

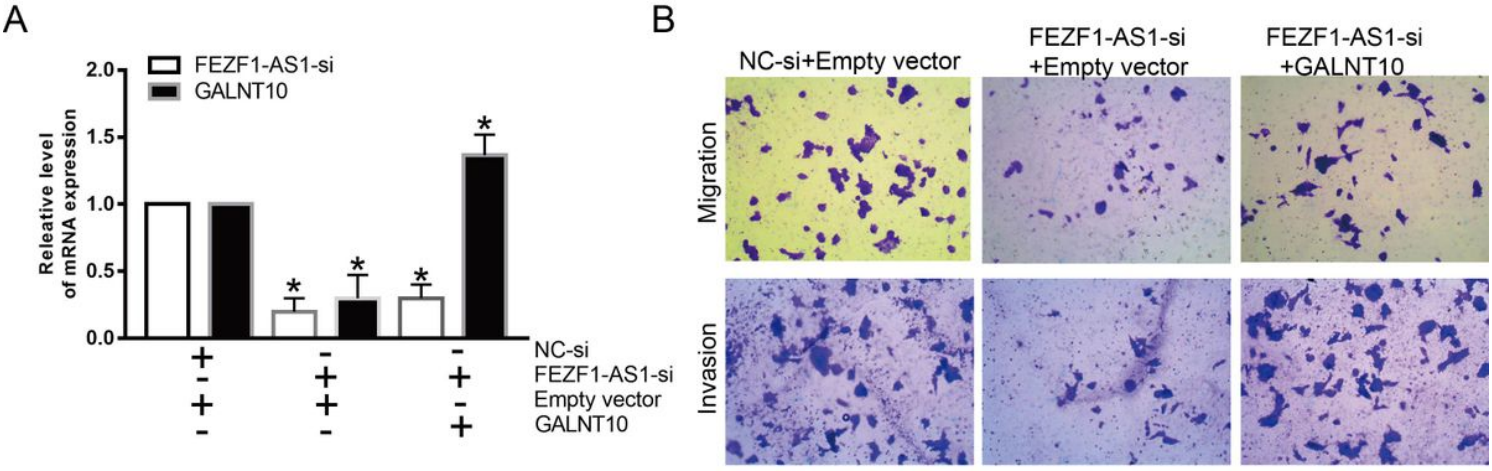

Figure 5

Over-expression of GALNT10 reverses inhibition of OS metastasis stimulated by FEZF1-AS1 silencing U2OS cells co-transfected with indicated reagents and (A) mRNA levels of FEZF1-AS1 and GALNT10 were examined by real-time PCR. (B) Cell infiltration and migration was assessed using the wound-healing assay and Tran swell tests.

Supplementary Files

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- tableS1.docx