ORIGINAL RESEARCH

RETRACTED ARTICLE: Upregulation of FTX Promotes Osteosarcoma Tumorigenesis by Increasing SOX4 Expression via miR-214-5p

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Background: Long-chain non-coding RNA (Lng, NA) plays a Kenner in the biological processes of tumors. LncRNA-FTX has been the invasion of tumors. However, its function and mechanism in osteosarcoma have not been study

Methods: qRT-PCR was measured the expression wels of FTX and miR-214-5p in osteosarcoma. The protein levels of aRY-noted HMG but transcription factor 4 (SOX4) were detected by Western Blot. Cholecystokine (CCK-8) assay, cell colony formation and Transwell assay, Annexin V/ 1C/PI assay were a lyzed the effects of FTX and miR-214-5p on cell proliferation, cell invasion and apoptosis. The relationship between FTX, miR-214-5p and SOX4 was an lyzed by bioinformatics analysis and Luciferase. The tumor changes in mice were detected by vivo experiments in nude mice.

Results: The explosite blevels of 1.1.4 were increased in osteosarcoma tissues and cell lines and negatively concluded the expression levels of miR-214-5p. FTX could modulate the expression of miR-216-5p in osteosarcoma cell lines. sh-FTX inhibited the growth and meta-asis of osteosarcoma. FTX could regulate the growth of osteosarcoma through miR-216-5p. The spockdow of miR-214-5p reversed the inhibitory effect of sh-FTX on osteosarcoma cell proliferation and growth in mice. Furthermore, FTX regulated the expression of SOX4 charcting as a sponge of miR-214-5p in osteosarcoma.

Conclusio FTX could promote proliferation, invasion and inhibited apoptosis by regulatx miR-214-5p/SOX4 axis in osteosarcoma, suggesting that FTX might be a potential target for teosarcoma treatment.

Keywords: FTX, miR-214-5p, SOX4, osteosarcoma, proliferation, apoptosis

Introduction

Osteosarcoma (OS) is the most common primary malignant tumor in children and adolescents.^{1,2} The age of onset of osteosarcoma is 15–25 years, and the incidence rate is 4.5/1 million. The annual new osteosarcoma patients in the United States are about 900 cases, the degree of malignancy is high.³ At present, the principle of treatment of osteosarcoma is surgery combined with neoadjuvant radiotherapy and chemotherapy.⁴ The overall prognosis is still relatively poor.⁵ The important reason for its poor prognosis is that the development of osteosarcoma is a very complicated biological process. Therefore, in order to improve the therapeutic effect of osteosarcoma, it is necessary to research the molecular mechanism to provide more effective clinical.

The long-chain non-coding RNA (lncRNA) is defined as a transcript that does not encode a protein of more than 200 nucleotides in length.^{6,7} LncRNA can affect

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the transcription and translation of the encoded gene through various mechanisms, such as chromosomal remodeling, transcription, and protein inhibition.⁸ LncRNA is abnormally expressed in human cancers and can be used as biomarkers for the diagnosis and prognosis of different tumors, including gastric cancer, liver cancer and colon cancer.^{9,10} It is confirmed that various lncRNAs are abnormally expressed in osteosarcoma.¹¹ These oncogenic or tumor suppressor lncRNA regulates OS pathogenesis and serves as an independent prognostic biomarker or multi-drug resistance in osteosarcoma cells (MDR).¹² LncRNA-FTX is a recently discovered lncRNA, which has been found to be abnormally expressed in a variety of malignancies.¹³ However, the mechanism of lncRNA-FTX in OS has not been studied.

LncRNA can act not only on proteins or mRNA but also on mi RNA.14 miRNAs are approximately 22 nt in length. miRNA plays a part in the regulation of physiological processes, such as cell biological development, lipid metabolism and hormone secretion, as well as the occurrence and development of diabetes, viral infection and tumor.^{15,16} Studies have found that miRNAs are involved in the progression of tumors as oncogenes or tumor sur pressor genes.¹⁷ The miRNAs not only enriches the unde standing of gene regulatory networks in theory, but also has great potential in clinical applications. minute alays a critical role in the development of osteosa oma cells.^{18,19} MiR-214-5p has been confirmed to be abnow mally expressed in various tumor tis res, suggering that it plays a part in tumorigenesis, builts action in on osarcoma is still unclear.²⁰

gh-mobility group box) gene The SOX (Sry-like family subcomponent. A to , and SOX protein is an nator the promotes embryoimportant transcriptional nic development and umory pest and development.^{21,22} SOX4 belows to the group on the SOX gene family, and its encoded p. tei is locanzed in the nucleus. SOX4 is an important member of the SOX transcription factor family, and interference where its expression inhibits tumor cell proliferation and promotes apoptosis.²³ SOX4 plays a critical role in the development of colorectal cancer. The high expression of SOX4 can inhibit the proliferation and invasion of colorectal cancer cells.²⁴ Therefore, it was speculated that lncRNA-FTX regulated the progression of osteosarcoma by modulating the miR-214-5p/SOX4 axis. The main purpose of this study was to explore the mechanism of lncRNA-FTX regulation of osteosarcoma.

Materials and Methods Tissue Sample

From 2010 to 2012, osteosarcoma and adjacent normal tissues were collected at the Affiliated Hospital of Guangdong Medical University for surgical resection. The information of patients (including gender, ages, stages, etc.) was provided in Supplementary data. Exclusion criteria: patients with primary malignant tumor in other parts, deformity of bone and important organs, dysfunction of heart, lung, liver and kidney, diseases of blood system, and stroke of card bral vessels; patients who have received antigamor treatment before admission. All specimens were dia, osed as ostopsarcoma by clinical, imaging, and bi cological seminations and the patient did not underge any properative eatment. The patient's clinical information was collected. This study was approved by the Research Eth s Committee of the Affiliated Heroita of Guangde Medical University. All patients signed a writer consent form.

Ce Culture

Hun normal c eoblasts HFOB1.19 cells and osteosarcoma c 1 line KHOS, MG63, U2OS, HOS and Saos-2 constained from the Central Culture Collection of the Canese Academy of Sciences (Shanghai, China). HFOB 1.19 cells were cultured in F12 medium containing 10% BS. The HOS cell line was maintained in Eagle's MEM medium, and the remaining cell lines were cultured in RPMI-1640 medium containing 10% FBS.

HE Staining

The osteosarcoma tissue removed during the operation was dissected along the largest section of the tumor. It was fixed by 10% formaldehyde, dehydrated by routine method, embedded by paraffin, and sectioned continuously with a thickness of 3 μ M. After HE staining, gradient ethanol dehydration and xylene transparent post sealing were carried out.

Vector Construction and Transfection

For shRNA-mediated FTX silencing, miR-214-5p mimics (5'-GGCCTGGCTGGACAGAGTTG-3') miR-214-5p inhibitor (5'-ACAGCAGGCACAGACAGGCAG-3') and negative control (5'-CCCCCCCCCCC-3') were synthesized by Shanghai Gene Pharmaceutical Co., Ltd. (Shanghai, China). Lentivirus or plasmid transfection was performed using Lipofectamine 3000.

Total RNA in cells was extracted using TRIzol reagent (Boyao, Shanghai, China). After the reverse transcription reaction, qRT-PCR was performed using a ViiATM 7 realtime PCR system (Life Technologies, Grand Island, NY). The expression levels of lnc-FTX and miR-214-5p were calculated by the $2^{-\Delta\Delta CT}$ method. The expression level of lnc-FTX was normalized to GADPH, while the level of miR-214-5p was normalized to U6. qRT-PCR methods were performed with the literature.²⁵ The primer sequences were as follows:

FTX forward: 5'-CAAAGCTGGTCCTGTGCCTG-3', reverse 5'-ATTGAGTGTGGCATCACCTCC-3'. miR-214-5p forward: 5'-GGCCTGGCTGGACAGA-3', reverse: 5'-GTCACATGACAACCCAGCCT-3'. U6 forward: 5'-CTCGCTTCGGCAGCACATATATT-3', reverse: 5'-ACGCTTCACGAATTTGCGTGGC-3'. GAPDH: forward: 5'-GGGCTGCTTTTAACTCTGGT-3', reverse: 5'-GCAGGTTTTTCTAGACGG-3'.

Luciferase Reporter Gene Assay

The miR-214-5p mimic containing the wild-type or mutant FTX or SOX4 fragment-specific sequence or the miRcontrol and pMIR-reporter luciferase vectors wer cotransfected with Lipofectamine 3000 (Invitrogen). A ter 48 h of transfection, luciferase activity was in sured of a dual luciferase assay system (Prometra).

Cell Viability Assay

Cells were seeded in 96-way places at a denses of 5000 cells per well; 100 μ L cl CCK8 solution (Liji, Shanghai, China) was added. At 48 h after translation, the absorbance at 450 nm was measured by microplate reader (Potenov, Beijing, China).

Trans vell In cusion Detection

Matrigel as aluted with RPMI-1640 medium, 50 μ L was spread to the option of the Transwell chamber. Then the Transwell chamber was placed in a 24-well plate, and it was incubated overnight to form a gel. After that, cell migration experiments were performed.

Colony Formation

Cells were plated in 6-well plates and incubated in dmem containing 10% fetal bovine serum. Two weeks later, the cells were fixed in methanol for 30 mins and stained with 1% crystal violet dye.

Western Blot

The protein concentration was quantified using the BCA Protein As-say Kit. It was incubated with anti-SOX4 antibody (1:1000, Shifeng, Shanghai, China) and anti-GAPDH antibody (1:1000, Shifeng, Shanghai, China) overnight. Then, it was incubated for 1 h with anti-rabbit secondary antibody (1:1000, Avivi, Beijing, China). Western blot analysis was performed with reference.²⁶

Apoptosis Assay

The cells were plated in a 6-well state at colensity of 5 x 10^5 cells/well, and cells were harve ed and count when the cells were grown to logarithmic grown phase. After centrifugation of the cells, the cells were suspended, with 165 µL of Annexin V-FITC binding solution; 5 µT of Annexia V-FITC and 10 µL of propidium incide storing solution were added. After incubated in the ork for 10–2 min to was placed in an ice bath.

raft More Model

tale athymic BALB/c nude mice were purchased from the lational Experimental Animal Center (Beijing, China). lv-shloor lv-sh- NHG1 transfected MG63 cells (1×10^6) were subcure ously injected into 8-week-old nude mice (n = 5). For volume was measured weekly. After 4 weeks, the mice were euthanized. All animal experiments were conducted at the Affiliated Hospital of Guangdong Medical University Animal Experiment Center and followed the Guide to Nursing and Use of Laboratory Animals (Bethesda National Institute of Health, Maryland, USA). All animal protocols were approved by the Affiliated Hospital of Guangdong Medical University Animal Protection and Use Committee.

Immunostaining

Immunohistochemical staining was performed by the mouse anti-human Ki-67 monoclonal antibody and immunohistochemistry kit instructions. The color was developed with diaminobenzidine (DAB) coloring solution, counterstained with hematoxylin, and sealed with neutral resin. The cell staining was observed under a light microscope. Ki-67 criteria in 2 fields were counted under an optical microscope. The positive was brownish yellow.

RNA Immunoprecipitation (RIP)

RNA immunoprecipitation experiments were performed using Magna RIP RNA binding protein IPKit (Xiheng, Shanghai, China) and Ago2 antibody (2897; Cell Signaling, Danvers, MA, USA). Finally, purified RNA in the pellet was used to analyze FTX expression.

Statistical Methods

The monitoring data were analyzed by SPSS19.0 statistical software. The results of data analysis were shown as mean \pm standard deviation (mean \pm SD). Multigroup data analysis was founded on one-way ANOVA. LSD test was used for subsequent analysis. P < 0.05 meant the difference was significant.

Results

FTX Was Upregulated in Osteosarcoma Tissues and Cell Lines and Promoted OS Cell Proliferation

The expression of FTX in paracancerous with osteosarcoma was first detected by qPCR. As a result, as shown in Figure 1A, compared with normal tissues, the expression level of FTX in cancer tissues was significantly increased (P < 0.001). The level of FTX expression in osteosarcoma cell lines was also significantly increased compared with that in HFOB 1.19 cells (P < 0.05) (Figure 1B). The bestperforming MG63 and Saos2 cells were selected for the next experiment. As shown in Figure 1C, HE staining showed that there were irregular tumor cells and osteobla like matrix in osteosarcoma. And immunohistochemistry showed that there were more positive cells where to d in tumor group. Next, the biological function of F X in proliferation and invasion of OS cellewas 72 zed. As shown in Figure 1D, compared y a sh-NC up, the expression level of FTX in the FT group was gnificantly decreased (P<0.05) in the MG63 d Saos2 cells. Moreover, the proliferation rate of MG63 and Saos2 cells was significantly reduced in the sh-FTX group (P < 0.05, experiments showed that the Figure 1E and F). Invas. sh-FTX was significanly inh. ited ell invasion (P < 0.05) (Figure 1C In add on, flow ytometry results showed that the sh- V could significantly induce apoptosis (P<0.05) (Figure H). As shown in Figure 1I, compared with sh-NC group, the protein expression level of ki67 was significantly reduced, and the protein expression level of c-casp3 and c-PARP was significantly increased.

miR-214-5p Was Regulated by FTX in Osteosarcoma Cells

As shown in Figure 2A, the expression levels of miR-214-5p were significantly reduced in osteosarcoma cell lines compared with HFOB 1.19 cells (P<0.05). Furthermore, FTX was

significantly negatively correlated with miR-214-5p in osteosarcoma tissues (Figure 2B). We predicted by the online prediction tool Starbase v2.0 and miR-214-5p was identified as a potential target for FTX (Figure 2C). And the expression level of miR-214-5p was significantly increased in the miR-214-5p mimic group compared with the control group (P<0.05), indicating successful transfection (Figure 2D). The luciferase reporter gene assay showed that luciferase activity was significantly decreased in MG63 and Saos2 cells co-transfected with miR-214-5p mimic and FTX-WT (P < 0.05). There was no significant characteristic of the second sec in the luciferase activity of FTX-MUT (Figure 2F) The R experiment further confirmed that SNHG15 w found in the Ago2 precipitate (Figure 2F). Further ore, contared with the shNC group, the expression level of miR-214-5, w significantly up-regulated in the sh-N X group (P < 0.05, Figure 2G).

The Effects of FTX Whe Mediated by miR-214-5p in Esteosarcoma Cells

whether FTX affected the growth of osteosarcoma Next gh miR-214 p was analyzed. As shown in Figure 3A, thro the C pression level of miR-214-5p in the miR-214-5p inhibivas ei aficantly reduced (P < 0.05) in the MG63 and tor grou 2 cells compared with the NC group, indicating that the isfection was successful. The results showed that the sh-FTX was inhibited cell significantly proliferation mpared with the Ctrl group (P<0.05), while the shFTX +miR-214-5p inhibitor reversed the effect of sh-FTX on cell proliferation (P < 0.05, Figure 3B and C). In addition, the sh-FTX was significantly inhibited cell invasion (P < 0.05), while the shFTX+miR-214-5p inhibitor reversed the effect of sh-FTX on cell invasion (P < 0.05) (Figure 3D). Moreover, sh-FTX was significantly induced apoptosis (P < 0.05), and shFTX+miR-214-5p inhibitor reversed the effect of sh-FTX on apoptosis (P <0.05, Figure 3E). And as shown in Figure 3F, compared with the Ctrl group, the sh-FTX was significantly inhibited the protein expression level of ki67, and increased the protein expression level of c-casp3 and c-PARP (P < 0.05), while the shFTX+miR-214-5p inhibitor reversed the effect of sh-FTX on the protein expression level of ki67, c-casp3 and c-PARP.

SOX4 was a Direct miR-214-5p Target

Based on these results, it was aimed to analyze the major target genes of miR-214-5p. We predicted by the online prediction tool Starbase v2.0 and SOX4 was identified as a potential target for miR-214-5p (Figure 4A). Luciferase



Figure I FTX we trised in osteosarcoma tissues and cell lines and promoted OS cell proliferation, invasion and inhibition of apoptosis. (A) Relative expression levels of FTX in osteosarcoma usue (n = 24). (B) FTX mRNA expression levels in osteosarcoma cell lines. (C) HE staining and immunohistochemistry in the normal and tumor groups. (D) FTX mRNA-expression level. (E) CCK8 assay. (F) Transwell experiment. (G) Colony formation experiments. (H) Flow cytometry measured apoptosis. (I) The expression levels of ki67,c-casp3 and c-PARP in the normal and tumor groups. *p <0.05, n = 3.

activity was significantly decreased in MG63 and Saos2 cells co-transfected with miR-214-5p mimic and SOX4-WT (P < 0.05). There was no significant change in luciferase activity of SOX4-MUT (Figure 4B). As shown in Figure 4C, compared with the control group, the expression level of SOX4 in the miR-214-5p mimic group was significantly reduced (P < 0.05), while the expression level of SOX4 was significantly increased in the miR-214-5p inhibitor group (P <0.05). And the expression level of SOX4 after sh-FTX treatment was significantly reduced (P < 0.05) than that of the shNC group (Figure 4D). These results demonstrated that SOX4 was a direct target of miR-214-5p.



Figure 2 miR-214-5p was the type of FTX (A) miR-214-5p mRNA expression in osteosarcoma cell lines. (B) Relationship between FTX and miR-214-5p expression in osteosarcoma tissues. (C) FN 2'-UTP datative target sequence for miR-214-5p. (D) miR-214-5p mRNA expression levels in miR-214-5p mock-transfected MG63 and Saos2 cells. (E) Detection of the ase activities luciferase reporter assay. (F) Anti-Argonaute 2 (Ago2) RNA immunoprecipitation (RIP) assays were used in cells to determine FTX and X RNA expression levels in GG3 and Saos2 cells. (G) miR-214-5p mRNA expression levels in GG3 and Saos2 cells. (C) The datative target sequence (IP) complex. Anti-immunoglobulin G (IgG) was used as the control. (G) miR-214-5p mRNA expression levels in GG3 and Saos2 cells. (F) assays were used in cells to determine FTX and X RNA expression levels in GG3 and Saos2 cells. (F) assays used as the control. (G) miR-214-5p mRNA expression levels in GG3 and Saos2 cells. (F) assays used as the control. (G) miR-214-5p mRNA expression levels in GG3 and Saos2 cells. (F) assays used as the control. (G) miR-214-5p mRNA expression levels in GG3 and Saos2 cells. (F) assays used as the control. (G) miR-214-5p mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 cells. (F) mRNA expression levels in GG3 and Saos2 c

SOX4 Attentiated the Effects of miR-214-5p Mimic Inhibitor in Osteosarcoma Cells Next, whether SOX4 involved in FTX/miR-214-5pmediated osteosarcoma cell proliferation and invasion was analyzed. As shown in Figure 5A, compared with the vector group, the expression level of SOX4 in the pcDNA-SOX4 group was significantly increased (P < 0.01). In addition, as shown in Figure 5B–E, miR-214-5p mimic was inhibited cell proliferation, invasion and induced apoptosis (P < 0.05), while miR-214-5p

mimic+pcDNASOX4 co-transfection partially reversed miR-214-5p mimic-induced cell proliferation, invasion and apoptosis (P < 0.01).

Downregulation of FTX Restrained Tumor Growth Through Targeting miR-214-5p in vivo

Finally, whether FTX regulated the development of osteosarcoma through regulating miR-214-5p in vivo was investigated. As shown in Figure 6A–C, compared with the Ctrl



Figure 3 FTX except phologeneous to be in oster arcoma cells by modulating miR-214-5p. (**A**) miR-214-5p mRNA expression levels in MG63 and Saos2 cells transfected with miR-214-5p influence. (**B**) CCK8 as (\mathbf{C}) canswell determination. (**D**) Colony formation experiments. (**E**) Flow cytometry to detect apoptosis. (**F**) The expression levels of ki67, c-cruss and c-P. P in the non-mainted tumor groups.*p <0.05, **p <0.01, n = 3.

group, the tuber volume and weight of the sh-FTX group were significantly reduced (P < 0.01). The shFTX+miR-214-5p inhibitor co-transfection could reverse the effect of sh-FTX on tumor weight and volume (P < 0.01). And as shown in Figure 6D, Ki-67 staining results showed that the number of positive cells in shFTX group was decreased, while cotransfection of shFTX with miR-214-5p inhibitor significantly reversed the effect of shFTX on the number of positive cells. Compared with the Ctrl group, the Ki-67. In addition, as shown in Figure 6E, the expression level of SOX4 protein in sh-FTX group was significantly decreased (P <0.01), and the co-transfection of shFTX+miR-214-5p inhibitor reversed the effect of sh-FTX on SOX4 protein expression (P <0.01).

Discussion

Osteosarcoma (OS) is a malignant primary tumor with early distant metastasis and high local recurrence.²⁷ The incidence of OS in men is relatively high.²⁸ With the development of medical level, the treatment of osteosarcoma is also further diversified, such as chemotherapy,



Figure 4 SOX4 was the ext miR-214-5p target. (A) The putative target sequence of miR-214-5p on the SOX4 3'-UTR. (B) Detection of luciferase activity by luciferase reporter assay. (C) miR 14-5p mimic and miR-214-5p inhibitors transfected with SOX4 protein expression levels in MG63 and Saos2 cells. (D) SOX

surgery, bone reconstruction and other treatment options. At present, the treatment of osteosarcoma is mainly based on neoadjuvant chemotherapy combined with surgical treatment, but the current clinical prognosis rate of treatment has not improved.²⁹ The postoperative survival status of patients with osteosarcoma still faces enormous challenges. The main reason for the poor clinical prognosis is

that the current treatment methods cannot inhibit the distant metastasis and drug recurrence of osteosarcoma.³⁰ The main reason of osteosarcoma is still unclear. The rapid development of molecular biology provides new techniques for further investigation of the pathogenesis of osteosarcoma to explore the pathogenesis of osteosarcoma.



Figure 5 SOX4 reduced the role of miR-214-5p mimics in osteosarcoma cele (A) 5 4 m. expression levels in MG63 and Saos2 cells transfected with pcDNASOX4. (B) CCK8 assay. (C) Transwell determination. (D) Colony for the experiment (E) Flow cytometry to detect apoptosis. *p <0.05, **p <0.01, n = 3.

In the study of these new me cular nanisms, the RNA (role of long-chain non-co RNA) has attracted widespread attention. To te, there is increasing evidence that lncRNA involved in pathophysiological processes of muculoskeletal system-related diseases.³¹ It plays a pivotal it in the metastasis of musculoskeletalrelated disease ³² In the process of disease, lncRNA plays e biologic process of osteosarcoma.²⁸ a pivotal le in 285194 acts as a p53-regulated lncRNA, It is found that *k* a key role in the development of which p. osteosarcoma. It has also been found that TUG1 plays a pivotal role in osteosarcoma as an important carcinogenic lncRNA. Up-regulation of TUG1 may indicate a poor prognosis, which can promote metastasis.³⁴ FTX is a recently discovered lncRNA that has been found to be abnormally expressed in a variety of cancers. In a previous study, the expression levels of Inc-FTX were increased in female livers than in male livers and were significantly reduced in HCC tissues compared with normal liver tissues. Lnc-FTX inhibits HCC cell growth and metastasis

both in vitro and in vivo. Mechanistically, lnc-FTX represses Wnt/ β -catenin signaling activity by competitively sponging miR-374a and inhibits HCC cell epithelial-mesenchymal transition and invasion.³⁵ There is currently no research on FTX in osteosarcoma. Studies have found that FTX levels are significantly elevated in osteosarcoma. And FTX knockdown can inhibit cell proliferation and invasion, and induce apoptosis. The tumor volume, weight and SOX4 protein expression levels of the mice in the sh-FTX group are significantly reduced. Therefore, FTX can achieve the development of osteosarcoma by inhibiting its expression.

LncRNA regulates protein translation and cellular activity by modulating mi RNA.³⁶ miRNAs are closely related to human tumors, some can promote tumor differentiation and occurrence, and some can inhibit tumorigenesis.³⁷ Therefore, studying the mechanism and function of miRNA has become a hot spot. Studies have confirmed that the biological behavior of miRNA affecting tumor cells, mainly affecting its proliferation, migration,



Figure 6 Downregulation of FTX restrained tumor powth through argeting miR-214-5p in vivo. (A) Tumor volumes were measured every week, and growth curves are shown. (B) Tumor growth was inhibited in the shown mouse group constrained with the control. (C) Tumor weight (g). (D) Ki-67 staining. (E) The expression levels of SOX4 in tumor tissues. *p < 0.05, **p < 0.01, n = 3.

and aroptosis.³⁸ The role of invasion and adhesion miRNA in osteosart na h sparked great interest among researcher³⁹ Stud, have s¹ wn that miR-29a is coma tissues, and it is under-express 1 in h nan os ulular level that down-regulation of demonstrated at the miR-29a can heat apoptosis, and miR-29a regulates cell apoptosis by tanting Bcl-2.40 MiR-125b is underexpressed in osteosarcoma, but miR-125b can promote the proliferation and migration of osteosarcoma by regulating TAG, resulting in tumor formation in vivo.⁴¹ MiR-214-5p is a miRNA with tumor growth inhibition found in recent years. For example, it has been found that miR-214-5p exerts a tumor suppressor effect in breast cancer.⁴² MiR-214-5p was screened as a target gene for FTX. MiR-214-5p was expressed at a lower level in osteosarcoma cell lines. MiR-214-5p mimic was inhibited cell

proliferation, invasion and induced apoptosis. In addition, FTX was negatively correlated with miR-214-5p, and miR-214-5p expression was up-regulated after sh-FTX transfection. shFTX+miR-214-5p inhibitors could reverse the effects of sh-FTX on cell proliferation, invasion and apoptosis. In vivo experiments showed that co-transfection of shFTX+miR-214-5p inhibitor could n reverse the effect of sh-FTX on tumor weight and volume in mice. These results indicated that FTX may promote osteosarcoma growth by miR-214-5p.

miRNA participates in the process of tumor formation and progression during tumor formation as a regulatory factor.¹⁵ Recent studies have shown that the SOX4 gene is involved in the development of many tumors as a transcription factor in vivo.⁴³ Overexpression of SOX4 is found in tumor tissues such as prostate cancer, melanoma, liver cancer, and acute leukemia.44 Overexpressed SOX4 may enhance the invasion and migration of tumor cells. This transformation process has been shown to be associated with tumor invasion and metastasis. Studies have found that SOX4 can redirect TGF-β-mediated SMAD3-transcriptional output in a context-dependent manner to promote tumorigenesis.⁴⁵ In this study, it was found that SOX4 was a target gene of miR-214-5p. The expression level of SOX4 was decreased in the miR-214-5p mimic group. The expression level of SOX4 was decreased after sh-FTX treatment. The co-transfection of miR-214-5p mimic+pcDNASOX4 could reverse miR-214-5p mimic-induced cell proliferation, invasion and apoptosis. In vivo experiments showed that the protein expression level of SOX4 in sh-FTX group was significantly reduced, and the co-transfection of shFTX +miR-214-5p inhibitor could reverse the effect of sh-FTX on the protein expression of SOX4. FTX could promote proliferation and invasion by modulating the miR-214-5p/ SOX4 axis in osteosarcoma.

Conclusion

FTX promoted proliferation and invasion by regulating miR-214-5p/SOX4 axis in osteosarcoma, suggesting that FTX might be a potential oncogene of osteosarcom

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Disclosure

The author op t no c flict interest.

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