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

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

Methods: qRT-PCR was measured the expression levels of FTX and miR-214-5p in osteosarcoma. The protein levels of SRY-related HMG box transcription factor 4 (SOX4) were detected by Western Blot. Cholecystokinin (CCK-8) assay, cell colony formation and Transwell assay, Annexin V-FITC/PI assay were analyzed 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 analyzed by bioinformatics analysis and Luciferase. The tumor changes in mice were detected by vivo experiments in nude mice.

Results: The expression levels of FTX were increased in osteosarcoma tissues and cell lines and negatively correlated with the expression levels of miR-214-5p. FTX could modulate the expression of miR-214-5p in osteosarcoma cell lines. sh-FTX inhibited the growth and metastasis of osteosarcoma. FTX could regulate the growth of osteosarcoma through miR-214-5p. The knockdown 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 by acting as a sponge of miR-214-5p in osteosarcoma.

Conclusion: FTX could promote proliferation, invasion and inhibited apoptosis by regulating miR-214-5p/SOX4 axis in osteosarcoma, suggesting that FTX might be a potential target for osteosarcoma 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
The SOX (Sry-like high-mobility group box) gene family subcomponent is A to H, and SOX protein is an important transcriptional regulator that promotes embryonic development and tumorigenesis and development. SOX4 belongs to the C group of the SOX gene family, and its encoded protein is localized in the nucleus. SOX4 is an important member of the SOX transcription factor family, and interference with its expression inhibits tumor cell proliferation and promotes apoptosis. 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. 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 cardio cerebral vessels; patients who have received anti-tumor treatment before admission. All specimens were diagnosed as osteosarcoma by clinical, imaging, and histological examinations and the patient did not undergo any preoperative treatment. The patient’s clinical information was collected. This study was approved by the Research Ethics Committee of the Affiliated Hospital of Guangdong Medical University. All patients signed a written consent form.

Cell Culture

Human normal osteoblasts HFOB1.19 cells and osteosarcoma cell lines KHOS, MG63, U2OS, HOS and Saos-2 were obtained from the Central Culture Collection of the Chinese Academy of Sciences (Shanghai, China). HFOB 1.19 cells were cultured in F12 medium containing 10% FBS. 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 μM. 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'-GGCCTGGCTGGACAGGTG-3') miR-214-5p inhibitor (5'-ACACGAGGACAGACAGGGC-3') and negative control (5'-CCCCCCCATCCCCCCC-3') were synthesized by Shanghai Gene Pharmaceutical Co., Ltd. (Shanghai, China). Lentivirus or plasmid transfection was performed using Lipofectamine 3000.
Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)
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 real-time PCR system (Life Technologies, Grand Island, NY). The expression levels of lnc-FTX and miR-214-5p were calculated by the ΔΔCT method. The expression level of lnc-FTX was normalized to GAPDH, while the level of miR-214-5p was normalized to U6. qRT-PCR methods were performed with the literature. The primer sequences were as follows:

FTX forward: 5′-CAAAGCTGGTCTGTGCCTG-3′;
reverse: 5′-ATTGAGTGTGGCATCACCTCC-3′.

miR-214-5p forward: 5′-GGCCTGGTGAACAGACA-3′;
reverse: 5′-GTCACTGCAACACCCAGCCT-3′.

U6 forward: 5′-CTCGTTCTGGCAGCAGATATT-3′;
reverse: 5′-ACGCCTACGAATTTGCGTGGC-3′.

GAPDH: forward: 5′-GGCTGGCTGGACAGA-3′;
reverse: 5′-GCAGTTTTTCTAGACGG-3′.

Luciferase Reporter Gene Assay
The miR-214-5p mimic containing the wild-type or mutant FTX or SOX4 fragment-specific sequence or the miR-control and pMIR-reporter luciferase vectors were co-transfected with Lipofectamine 3000 (Invitrogen). After 48 h of transfection, luciferase activity was measured by a dual luciferase assay system (Promega).

Cell Viability Assay
Cells were seeded in 96-well plates at a density of 5000 cells per well; 100 μL of CCK8 solution (Liji, Shanghai, China) was added. At 48 h after transfection, the absorbance at 450 nm was measured by microplate reader (Potenov, Beijing, China).

Transwell Intrusion Detection
Matrigel was diluted with RPMI-1640 medium, 50 μL was spread to the bottom 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 Assay 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.

Apoptosis Assay
The cells were plated in a 6-well plate at a density of 5 x 10^5 cells/well, and cells were harvested and counted when the cells were grown to logarithmic growth phase. After centrifugation of the cells, the cells were suspended with 195 μL of Annexin V-FITC binding solution; 5 μL of Annexin V-FITC and 10 μL of propidium iodide staining solution were added. After incubated in the dark for 10–20 min, it was placed in an ice bath.

Xenograft Mouse Model
Male athymic BALB/c nude mice were purchased from the National Experimental Animal Center (Beijing, China). lv-sh-NC or lv-sh-SNHG1 transfected MG63 cells (1 x 10^6) were subcutaneously injected into 8-week-old nude mice (n = 5). Tumor 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
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 change in the luciferase activity of FTX-MUT (Figure 2E). The RIP experiment further confirmed that SNHG15 was found in the Ago2 precipitate (Figure 2F). Furthermore, compared with the sh-FTX group, the expression level of miR-214-5p was significantly up-regulated in the sh-FTX group (P<0.05, Figure 2G).

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
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 1 FTX was raised in osteosarcoma tissues and cell lines and promoted OS cell proliferation, invasion and inhibition of apoptosis. (A) Relative expression levels of FTX in osteosarcoma tissue (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.
SOX4 Attenuated the Effects of miR-214-5p Mimic Inhibitor in Osteosarcoma Cells

Next, whether SOX4 involved in FTX/miR-214-5p-mediated 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
group, the tumor 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 co-transfection 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. The incidence of OS in men is relatively high. With the development of medical level, the treatment of osteosarcoma is also further diversified, such as chemotherapy,
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. 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. 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 4 SOX4 was a direct 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-214-5p mimic and miR-214-5p inhibitors transfected with SOX4 protein expression levels in MG63 and Saos2 cells. (D) SOX4 protein expression levels in MG63 and Saos2 cells. *p <0.05, **p <0.01, n = 3.
In the study of these new molecular mechanisms, the role of long-chain non-coding RNA (lncRNA) has attracted widespread attention. To date, there is increasing evidence that lncRNA is involved in the pathophysiological processes of musculoskeletal system-related diseases.\textsuperscript{31} It plays a pivotal role in the metastasis of musculoskeletal-related diseases.\textsuperscript{32} In the process of disease, lncRNA plays a pivotal role in the biological process of osteosarcoma.\textsuperscript{28} It is found that lnc285194 acts as a p53-regulated lncRNA, which plays a key role in the development of osteosarcoma.\textsuperscript{33} 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.\textsuperscript{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 lnc-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, Inc-FTX represses Wnt/β-catenin signaling activity by competitively sponging miR-374a and inhibits HCC cell epithelial-mesenchymal transition and invasion.\textsuperscript{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 miRNA.\textsuperscript{36} miRNAs are closely related to human tumors, some can promote tumor differentiation and occurrence, and some can inhibit tumorigenesis.\textsuperscript{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,
invasion and adhesion and apoptosis. The role of miRNA in osteosarcoma has sparked great interest among researchers. Studies have shown that miR-29a is under-expressed in human osteosarcoma tissues, and it is demonstrated at the cellular level that down-regulation of miR-29a can inhibit apoptosis, and miR-29a regulates cell apoptosis by targeting Bcl-2. MiR-125b is under-expressed in osteosarcoma, but miR-125b can promote the proliferation and migration of osteosarcoma by regulating TAG, resulting in tumor formation in vivo. 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. 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 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. Recent studies have shown that the SOX4 gene is involved in the development of many tumors as a transcription factor in vivo. Overexpression of SOX4 is found in tumor tissues such as prostate cancer, melanoma,
liver cancer, and acute leukemia. 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. 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+pcDNA-SOX4 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 osteosarcoma.

Funding
This work was supported by the Medical Research Fund Project of Guangdong Province (No. A2019104), Science and Technology Planning Project of Guangdong Province (No. 2016B090917001 and 2017B090912006), and Science and Technology Project of Zhanjiang city (No. 2018A01036).

Disclosure
The authors report no conflict of interest.

References
1. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature. 1986;323(6089):643–646. doi:10.1038/323643a0
2. Khanna C, Wan X, Bose S, et al. The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. Nat Med. 2004;10 (2):182–186. doi:10.1038/nm892
3. Unni KK, Dahlin DC, McLeod RA, Pritchard DJ. Intraosseous well-differentiated osteosarcoma. Cancer. 1977;40(3):1337–1347. doi:10.1002/1097-0142(197709)40:3<1337::AID-CNCR2820400351>3.0.CO;2-X
4. Yang H, Peng Z, Da Z, et al. MicroRNA-148a acts as a tumor suppressor in osteosarcoma via targeting Rho-associated coiled-coil kinase. Oncol Res. 2017;25(8):1231–1243. doi:10.3727/096504117X7135
5. Liu D, Zhang C, Li X, Zhang H, Wan A. MicroRNA-567 inhibits cell proliferation, migration and invasion by targeting FGFR5 in osteosarcoma. Excli J. 2018;17:102–112. doi:10.17179/excli2017-932
6. Chen W, Jia Y, Zheng Q, Zhu Y. Downregulation of IncRNA OGFPR1 inhibits hepatocellular carcinoma progression by AKT/mTOR and Wnt/β-catenin signaling pathways. Cancer Manag Res. 2018;10:1817–1826. doi:10.2147/CMAR.S164911
7. Du P, Zhao H, Peng R, et al. LncRNA-XIST interacts with miR-29c to modulate the chemoresistance of glioma cell to TMZ through DNA mismatch repair pathway. Biosci Rep. 2017;37(5):BR20170069. doi:10.1042/BSR20170069
8. Hao F, Mou Y, Zhang L, Wang S, Yang Y. LncRNA AFAPI-AS1 is a prognostic biomarker and serves as oncogenic role in retinoblastoma. Biosci Rep. 2018;38(3):BR20180384. doi:10.1042/BSR20180384
9. Zhang J, Li XY, Hu P, Ding YS. IncRNA NORAD contributes to colorectal cancer progression by inhibition of miR-202-5p. Oncol Rep. 2018. doi:10.3727/096504018X15190844870055
10. Gu Z, Hou Z, Zheng L, Wang X, Wu L, Zhang C. LncRNA DICER1-AS1 promotes the proliferation, invasion and autophagy of osteosarcoma cells via miR-30b/ATG5. Biomed Pharmacother. 2018;104:110–118. doi:10.1016/j.biopha.2018.04.193
11. Qu G, Ma Z, Tong W, Yang J. LncRNA WWOX-AS1 inhibits the proliferation, migration and invasion of osteosarcoma cells. Mol Med Rep. 2018;18(1):779–788. doi:10.3892/mmr.2018.9058
12. Wei L, Peng X, Wen-Hui R. Overexpression of IncRNA UCA1 promotes osteosarcoma progression and correlates with poor prognosis. J Bone Oncol. 2016;5(2):80–85. doi:10.1016/j.jbo.2016.05.003
13. Li X, Zhao Q, Qi J, et al. IncRNA Fix promotes aerobic glycolysis and tumor progression through the PPARy pathway in hepatocellular carcinoma. Int J Oncol. 2018;53(2):551–566.
14. Xiao H, Tang K, Liu P, et al. LncRNA MALAT1 functions as a competing endogenous RNA to regulate ZEB2 expression by sponging miR-200s in clear cell kidney carcinoma. Oncotarget. 2015;6(35):38005–38015. doi:10.18632/oncotarget.5357
15. Zhang G, Chen L, Khan AA, Li B, Yan J. miRNA-124-3p/neuropilin-1(NRP-1) axis plays an important role in mediating glioblastoma growth and angiogenesis. Int J Cancer. 2018;143(3):635–644. doi:10.1002/ijc.31329
16. Wu Y, Zhang J, Hong Y, Wang X. Effects of kaglinate injection on serum miRNA-21 in patients with advanced lung cancer. Med Sci Monit. 2018;24:2901–2906.
17. Vishnoi A, Rani S. MiRNA biogenesis and regulation of diseases: an overview. Methods Mol Biol. 2017;1509:1–22.
18. Xin X, Wei W, Li X, Li Y, Zheng G. MicroRNA-495 suppresses osteosarcoma invasion and migration by targeting HSP90AA1. Oncotarget. 2018;9.
19. Shen H, Wang W, Ni B, Zou Q, Wang Z. Exploring the molecular mechanisms of osteosarcoma by the integrated analysis of miRNAs and miRNA microarrays. Int J Mol Med. 2018;42(1):21–30.
20. Pang J, Li Z, Wang G, Li N, Gao Y, Wang S. miR-214-5p targets KLF5 and suppresses proliferation of human hepatocellular carcinoma cells. J Cell Biochem. 2018;120(2):1850–1859.
21. Julian LM, Mcdonald AC, Stanford WL. Direct reprogramming with SOX factors: masters of cell fate. Curr Opin Genet Dev. 2017;46:24–36. doi:10.1016/j.gde.2017.06.005
22. Kröcher O, Widmer M, Elsener M, Rothe D. Adsorption and desorption of SO4 on diesel oxidation catalysts. Ind Eng Chem Res. 2009;48(22):9847–9857. doi:10.1021/ie900882p
23. Xu EE, Sasaki S, Speckmann T, Nian C, Lynn FC. SOX4 facilitates facultative β-cell proliferation through repression of Cdkn1a. Diabetes. 2017;66(8):db161074. doi:10.2337/db16-1074
24. Vishnubalaji R, Hamam R, Yue S, Al-Obede O, Alajez NM. MicroRNA-320 suppresses colorectal cancer by targeting SOX4, FOXM1, and FOXQ1. Oncotarget. 2016;7(24):35789–35802. doi:10.18632/oncotarget.8937
25. Zhang L, Zhang Q, Wang X, Yang X, Li X, Yuan M. Selection of reference genes for qRT-PCR and expression analysis of high-altitude-related genes in grassland caterpillars (Lepidoptera: erebidae: gynaephora) along an altitude gradient. *Ecol Evol*. 2017;7(21):9054–9065. doi:10.1002/ece3.3431

26. Tahrin M, Yang PC. Western blot: technique, theory, and trouble shooting. *N Am J Med Sci*. 2012;4(9):429–434. doi:10.4103/1947-2714.100998

27. Zhang H, Wang G, Ding C, et al. Increased circular RNA URBAP2 acts as a sponge of miR-143 to promote osteosarcoma progression. *Oncotarget*. 2017;8(37):61687–61697. doi:10.18632/oncotarget.18671

28. Liu K, Hou Y, Liu Y, Zheng J. LncRNA SNHG15 contributes to proliferation, invasion and autophagy in osteosarcoma cells by sponging miR-141. *J Biomed Sci*. 2017;24(1):46. doi:10.1186/s12929-017-0353-9

29. Lai Q, Ye C, Gao T, et al. Therapeutic effect of neoadjuvant chemotherapy combined with curettage to treat distal femoral osteosarcoma: a case report. *Medicine*. 2017;96(46):e8672. doi:10.1097/MD.0000000000008672

30. Akahoshi Y, Takeuchi S, Chen SH, et al. The results of surgical treatment combined with intra-arterial infusion of anti-cancer agents in osteosarcoma. *Clin Orthop Relat Res*. 1976;120:103.

31. Guan YX, Zhang ZM, Chen XZ, Zhang Q, Liu SZ, Zhang YL. Lnc-RNA H19 to facilitate bladder cancer metastasis. *Biomarkers* – *Drug Discov Today*. 2018;22(2):424–432. doi:10.1016/j.drdus.2016.10.014

32. Akahoshi Y, Takeuchi S, Chen SH, et al. The results of surgical treatment combined with intra-arterial infusion of anti-cancer agents in osteosarcoma. *Clin Orthop Relat Res*. 1976;120:103.

33. Guan YX, Zhang ZM, Chen XZ, Zhang Q, Liu SZ, Zhang YL. Lnc-RNA H19 to facilitate bladder cancer metastasis. *Biomarkers* – *Drug Discov Today*. 2018;22(2):424–432. doi:10.1016/j.drdus.2016.10.014

34. Zhang Q, Geng PL, Yin P, Wang XL, Jia JP, Yao J. Down-regulation of long non-coding RNA TUG1 inhibits osteosarcoma cell proliferation and promotes apoptosis. *Asian Pac J Cancer Prev*. 2013;14(4):2311–2315. doi:10.7314/APJCP.2013.14.4.2311

35. Liu F, Yuan J-H, Huang J-F, et al. Long noncoding RNA FTX inhibits hepatocellular carcinoma proliferation and metastasis by binding MCM2 and miR-374a. *OncoGene*. 2016;35(41):5422–5434.

36. Ganju A, Khan S, Hafeez BB, et al. miRNA nanotherapeutics for cancer. *Drug Discov Today*. 2017;22(2):424–432. doi:10.1016/j.drdus.2016.10.014

37. Pashaei E, Pashaei E, Ahmady M, Ozen M, Aydin N, Ahmad A. Meta-analysis of miRNA expression profiles for prostate cancer recurrence following radical prostatectomy. *PLoS One*. 2017;12(6): e0179543. doi:10.1371/journal.pone.0179543

38. Wu F, Li J, Guo N, Wang XL, Liao YQ. MiRNA-27a promotes the proliferation and invasion of human gastric cancer MGC803 cells by targeting SFRP1 via Wnt/β-catenin signaling pathway. *Am J Cancer Res*. 2017;7(3):405.

39. Yuan J, Lang J, Liu C, Zhou K, Chen L, Liu Y. The expression and function of miRNA-451 in osteosarcoma. *Med Oncol*. 2015;32(1):324. doi:10.1007/s12323-014-0324-x

40. Guo L, Huang X, Liang P, et al. Role of XIST/miR-29a/LIN28A pathway in denatured dermis and human skin fibroblasts (HSFs) after thermal injury. *J Cell Biochem*. 2018;119(2):1463.

41. Shen Y, Shen Z, Guo L, Zhang Q, Zhu Y. MiR-125b-5p is involved in oxygen and glucose deprivation injury in PC-12 cells via CBS/H2S pathway. *Nitric Oxide*. 2018;78:11–21. doi:10.1016/j.niox.2018.05.004

42. Chen X, Wang YW, Zhu WJ, et al. A four-microRNA signature predicts lymph node metastasis and prognosis in breast cancer. *Human Pathol*. 2018;76:122–132.

43. Rosa E, Hurtado-Puerto A, Falcão, MJC, de Brietzke A, Amerio R. Oral lichen planus and malignant transformation: the role of p16, Ki-67, p53 and p63. *Exp Ther Med*. 2018;15(5):4157–4166.

44. Dong H, Hu J, Wang L, Qi M, Han B. SOX4 is activated by C-MYC in prostate cancer. *Med Oncol*. 2019;36(11). doi:10.1007/s12323-019-1317-6

45. Vervoort SJ, Lourenço AR, Tufegdzic Vidakovic A, et al. SOX4 can redirect TGF-β-mediated SMAD3-transcriptional output in a context-dependent manner to promote tumorigenesis. *Nucleic Acids Res*. 2018;46(18):9578–9590.