

## Original Article

# Long non-coding RNA Ftx promotes osteosarcoma progression via the epithelial to mesenchymal transition mechanism and is associated with poor prognosis in patients with osteosarcoma

Bo Li<sup>1</sup>, Peng Ren<sup>2</sup>, Zhiyong Wang<sup>3</sup>

<sup>1</sup>Heart Center, Central Hospital of Zibo, Zibo, P. R. China; <sup>2</sup>Department of Orthopedics, Second Hospital of Shandong University, Jinan, Shandong, P. R. China; <sup>3</sup>Department of Emergency Surgery, Qilu Hospital of Shandong University, Jinan, Shandong, P. R. China

Received February 6, 2018; Accepted April 20, 2018; Epub September 1, 2018; Published September 15, 2018

**Abstract:** Objective: Long non-coding RNA Ftx (lncRNA Ftx) is involved in a variety of cancers. However, the association between lncRNA Ftx and osteosarcoma is still unclear. In this study, we investigated the correlation between lncRNA Ftx and osteosarcoma, and the regulative effect of Ftx on the migration and invasion of osteosarcoma cells, as well as its molecular mechanism. Methods: Expression levels of lncRNA Ftx in osteosarcoma tissues and adjacent non-tumor corresponding tissues (ANCTs) were detected using quantitative real-time PCR (qRT-PCR). Differences in patient survival were determined by the Kaplan-Meier method and a log-rank test. The Cox regression analysis was used for univariate and multivariate analyses of prognostic values. Human osteosarcoma cell lines Saos2 and HOS were transfected with the pcDNA-Ftx constructs. The scratch wound healing assay and Transwell assay were used to assess cell migration and invasion capability, respectively. Western blot analysis was conducted to investigate the expression of mesenchymal and epithelial markers. Results: The results showed that the lncRNA Ftx group was higher in osteosarcoma tissues compared with the ANCTs group. Expression of lncRNA Ftx was correlated with the clinical stage and distant metastasis ( $P < 0.05$ ). The overall survival rate was lower in the high lncRNA Ftx group than in the low lncRNA Ftx group (log-rank test,  $P < 0.05$ ). Multivariate analysis revealed that in osteosarcoma patients, higher lncRNA MEG3, advanced clinical stage, and distant metastasis were all independent predictors of overall survival. Cell research showed that transfection of lncRNA Ftx significantly promoted the migration and invasion ability of osteosarcoma cells. In addition, E-cadherin was decreased, while N-cadherin and Snail-1 were increased, at both the protein and mRNA levels. Pre-treatment with Snail-1 siRNA abrogated the promotion effect of Ftx on the migration and invasion of osteosarcoma cells. Conclusions: Increased expression of lncRNA Ftx could not only be a biomarker for progression and prognosis of osteosarcoma, but also could regulate the development of osteosarcoma via the epithelial to mesenchymal transition (EMT) mechanism.

**Keywords:** lncRNA Ftx, osteosarcoma, epithelial to mesenchymal transition, Snail-1

## Introduction

Osteosarcoma (OS), which is characterized by the direct formation of immature bone or osteoid tissue, is one of the most common tumors in children and adolescents. OS accounts for only about 0.5% of all types of cancer [1] and 20% of all primary bone cancers, but it is the second highest cause of cancer-related deaths in children [2]. OS predominantly occurs in the long tubular bones with highly aggressive and early distant metastasis. Approximately 10%-25% of patients have lung metastasis, which is

thought to be the most prominent reason for OS-caused deaths [3]. Although the 5-year survival rate of OS patients has significantly improved to approximately 60-70% [4], only 11%-30% of patients with metastatic OS can survive [5]. Moreover, for a proportion of OS patients, even though they have accepted curative resection of the primary tumor, they still respond poorly to chemotherapy and have a high risk of local relapse [6]. Therefore, it is urgent for us to uncover the key molecules and underlying mechanism which could predict and regulate metastasis and prognosis of OS.

**Table 1.** The correlations between long non-coding RNA Ftx expression and clinicopathological factors of osteosarcoma patients

Factors	Cases	Relative Ftx expression		P value
		High expression	Low expression	
Age (years)				0.625
<25	54	24	30	
≥25	30	15	15	
Sex				0.739
Male	49	22	27	
Female	35	17	18	
Tumor diameter				0.161
<8 cm	37	14	23	
≥8 cm	47	25	22	
Anatomic location				0.403
Tibia/femur	52	26	26	
Elsewhere	32	13	19	
Clinical stage				0.010
I/II	47	16	31	
III	37	23	14	
Distant metastasis				0.020
Absent	58	22	36	
Present	26	17	9	

Long non-coding RNAs (lncRNAs) are evolutionarily conserved, non-protein-coding RNAs that are longer than 200 nucleotides in length. Studies have indicated that lncRNAs are involved in diverse physiological processes including cell growth and apoptosis, as well as cancer progression and metastasis via transcriptional regulatory mechanisms [7, 8]. Previous studies have demonstrated that Ftx is an independent prognostic factor for colorectal cancer and significantly promotes the growth, migration, and invasion of colorectal cancer cells [9]. Ftx is also seen as a critical factor in the proliferation and invasion of glioma cells by regulating miR-342-3p and AEG-1 [10]. Knockdown of lncFtx inhibits renal cell carcinoma cells' proliferation, migration, and invasion [11]. Ftx-derived miR-545 promotes cell proliferation by targeting RIG-I and activating PI3K/Akt signaling in hepatocellular carcinoma [12]. However, in another study, Ftx was reported to inhibit hepatocellular carcinoma proliferation and metastasis by binding MCM2 and miR-374a [13].

Thus, according to the above investigations, Ftx is a newly identified gene that is associated with cancer growth and metastasis. However,

the correlation between Ftx and osteosarcoma, as well as the effects and molecular mechanisms on tumorigenesis and the progression of osteosarcoma, have not been extensively explored. In the present study, we first elucidated expression of Ftx in the serum of osteosarcoma patients and evaluated the prognostic values of Ftx on osteosarcoma. Then, we investigated the biological function of Ftx on osteosarcoma cells in vitro, as well as the possible mechanism involved in this process.

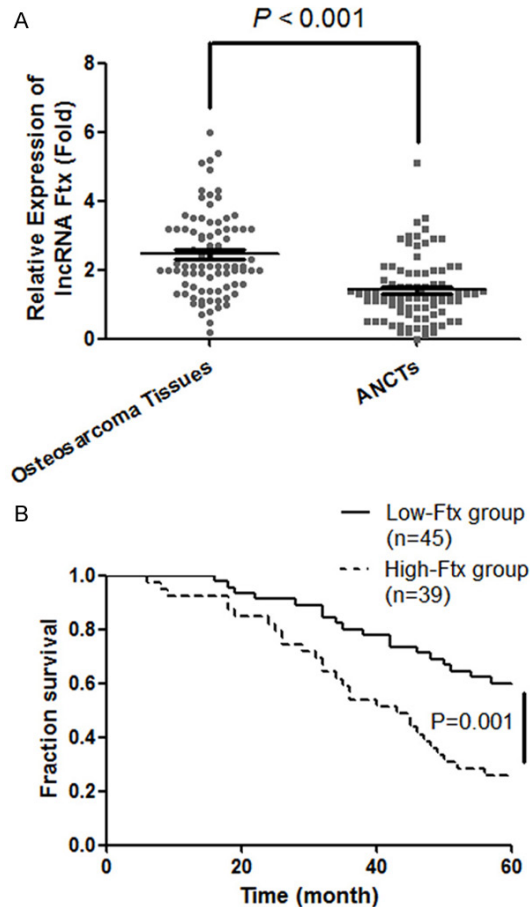
## Patients and methods

### Patient samples

The present study was approved by the Research Ethics Committee of Qilu Hospital. Written informed consent was obtained from all of the patients. Eighty-four patients (49 males and 35 females aged 5 to 63 years) with osteosarcoma who received surgical resection in the Department of Orthopedics, Qilu Hospital were recruited between August 2007 and July 2010. Eighty-four osteosarcoma tissues and paired adjacent non-tumor corresponding tissues (ANCTs) were collected and freshly frozen in liquid nitrogen. Part of the surgically resected specimens, as well as ANCTs, were fixed with formalin, embedded in paraffin, cut into 5-μm sections and stained with hematoxylin and eosin for the determination of tumor grade and stage by two pathologists. The remnant specimens were still stored in liquid nitrogen until the extraction of RNA. No patients had received radiotherapy or chemotherapy before surgery. The clinicopathological information of the patients is summarized in **Table 1**.

### Cell culture and transfection

Human osteosarcoma cell lines Saos2 and HOS were obtained from the American Type Cell Culture Collection (ATCC) and were grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 μg/ml streptomycin and 100 U/ml penicillin. The Ftx sequences were synthesized into the pcDNA3.1 vector by Invitrogen (Shanghai, China). Saos2 and HOS osteosarcoma cells were cultured in six-well plates and transfected with the pcDNA-Ftx constructs (Cell-Ftx group), as well as the pcDNA 3.1 empty vector (Cell-EP group) and normal



**Figure 1.** Expression of lncRNA Ftx was up-regulated in osteosarcoma and was correlated with the prognosis of osteosarcoma patients. A. The relative expression of lncRNA Ftx was detected by real time-PCR in 84 osteosarcoma tissues and paired ANCTs. The changes of Ftx were shown as the relative folds of 18S in the clinical data. B. Kaplan-Meier plots showed overall survival according to the level of lncRNA Ftx in osteosarcoma patients. The data represent the mean  $\pm$  SD of three independent experiments.  $P < 0.001$ .

saline of the same volume (Cell-Con group) as control groups. For transfection, complexes of the vectors and Lipofectamine 2000 (Invitrogen, Shanghai, China) were directly mixed with cells in six-well cell culture plates for 6 h at a density of  $2 \times 10^5$  cells per well.

#### RNA isolation and quantitative real-time PCR

Total RNA was extracted from tissues and cell lines using TranZol Up (Trans, China) according to the manufacturer's instructions. One  $\mu$ g mRNA was reversely transcribed using a First Strand cDNA Synthesis kit (Fermentas, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Then,

the total cDNA was amplified using the FastStart Universal SYBR Green Master (ROX) mix (Roche Diagnostics, Basel, Switzerland) in the Light Cycler real-time PCR detection system (Roche Diagnostics) for 40 cycles at 95°C for 5 sec, 60°C for 30 sec, and 70°C for 10 sec, with 18S as the internal control. The primer sequences for qPCR analyses were as follows: 18S, forward primer: 5'-CTAGTTGGTGGAGCGATTG-3', reverse primer: 5'-GCTGAACGCCACTTGTCC-3' [14]; Ftx, forward primer 5'-TAT GCC ACC TAG CCT TTC TAC A-3' and reverse primer 5'-ATC TCT TCA AAA GCG GCA TAA T-3' [12]. The changes of the relative expression of Ftx were calculated and normalized to 18S using the  $2^{-\Delta\Delta C_t}$  method in the cell research, while the changes of Ftx were shown as the relative folds of 18S in the clinical data.

#### Migration assay

Cell migration was studied using a scratch wound healing assay. The Saos2 and HOS cells were cultured in 12-well plates ( $1 \times 10^5$ /well, Corning, NY, USA). After the cells were transfected with the plasmid for 48 h straight scratches of the same width were made in monolayers of the cultured cells with a pipette tip. After incubation with the reagents for various times, the wound healings were observed along the scrape line [15].

#### Invasion assay

The invasive ability of the Saos2 and HOS cells was detected with modified Boyden chambers with 8- $\mu$ m pore filter inserts (Corning Inc.) coated with Matrigel (50  $\mu$ g/well; BD Biosciences, San Jose, CA, USA). Briefly, the upper chamber contained cells in DMED plus 1% FBS, while the lower chamber contained DMEM plus 10% FBS. After the cells were transfected with the plasmid for 48 h, cells ( $1 \times 10^5$ /well) were re-suspended in the upper chamber. After 24 h incubation at 37°C in 5% CO<sub>2</sub>, the cells on the lower surface were fixed with methanol for 30 min and stained with hexamethyl pararosaniline [16]. Five randomly captured fields of each sample were selected to evaluate the average number of invasive cells.

#### Statistical analysis

SPSS 18.0 software was used for statistical analysis. In the comparison of the 84 pairs of clinical samples of osteosarcoma and ANCTs,

**Table 2.** Univariate and multivariate Cox regression of prognostic factors for overall survival in osteosarcoma

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Age (years)						
<25 vs. ≥25	0.746	0.399-1.394	0.358			
Sex						
Male vs. Female	1.304	0.736-2.313	0.363			
Tumor diameter						
<8 cm vs. ≥8 cm	0.765	0.427-1.371	0.368			
Anatomic location						
Tibia/femur vs. Elsewhere	1.347	0.755-2.405	0.313			
Clinical stage						
I/II vs. III	2.964	1.647-5.333	<0.001	2.506	1.356-4.630	0.003
Distant metastasis						
Absent vs. Present	3.715	2.078-6.643	<0.001	2.428	1.297-4.546	0.006
Ftx						
Low vs. High	2.636	1.458-4.767	0.001	2.162	1.158-4.037	0.016

the paired t-test was used. Differences of the clinicopathological features between the high-Ftx group and the low-Ftx group were evaluated using the Student's t-test and  $\chi^2$  test. Survival analysis was done by using the Kaplan-Meier method and log-rank test. The Cox regression was performed to evaluate the prognostic values of clinicopathological features and lncRNA Ftx. The cell data were presented as means  $\pm$  standard deviation (SD) based at least three repeats of three independent experiments.  $P < 0.05$  indicated a significant difference.

## Results

### *LncRNA Ftx is up-regulated in osteosarcoma patients*

In order to assess the role of lncRNA Ftx in osteosarcoma, a qRT-PCR was first used to detect the expression of lncRNA Ftx in the 84 pairs of clinical samples of osteosarcoma and ANCTs. The results showed that lncRNA Ftx expression was significantly increased in osteosarcoma tissues compared with ANCTs ( $t = 10.881$ ,  $P < 0.001$ , **Figure 1A**).

### *LncRNA Ftx correlated with clinicopathological features*

The median value of lncRNA Ftx in all osteosarcoma tissues was 2.1, which was used as a cut-off value. The patients were divided into two groups, the high lncRNA Ftx expression group ( $\geq 2.2$ ;  $n = 39$ ) and low lncRNA Ftx expression

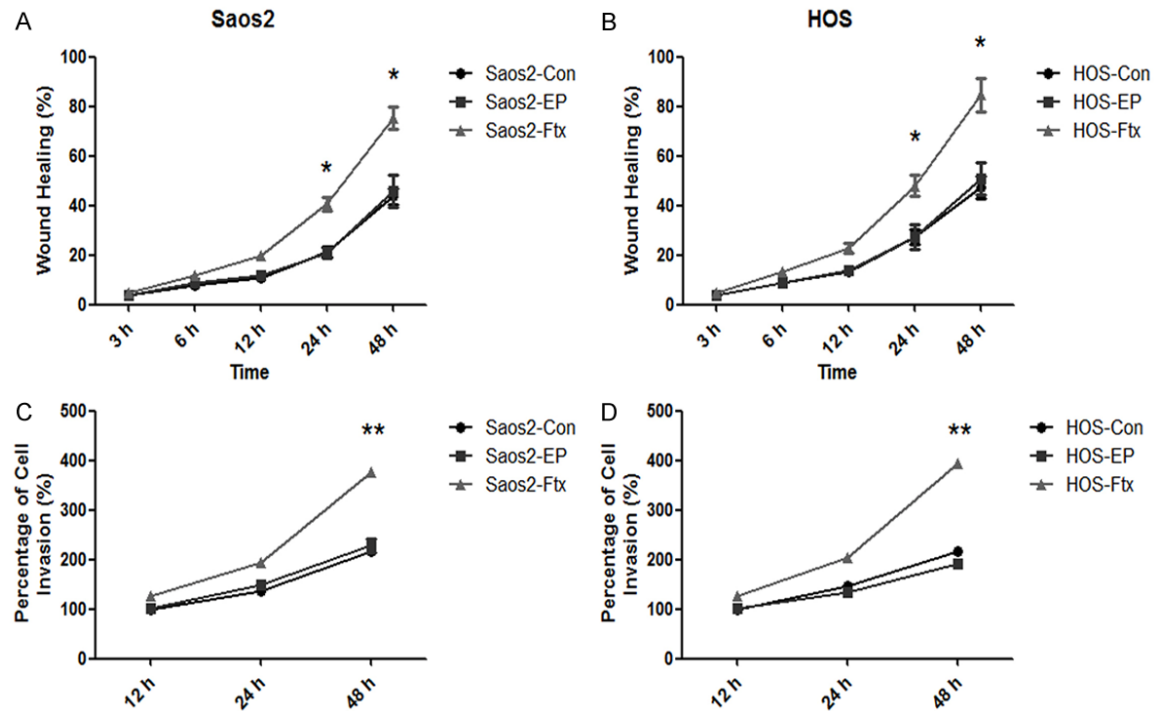
group ( $< 2.2$ ;  $n = 45$ ). The correlations between clinicopathological features and lncRNA Ftx expression in patients with osteosarcoma were summarized in **Table 1**. A high expression of lncRNA Ftx was observed to be closely correlated with clinical stage (I/II vs. III,  $P = 0.010$ ) and distant metastasis (absence vs. presence,  $P = 0.020$ ). We did not find a significant correlation of lncRNA Ftx with other clinicopathological features, such as age ( $< 25$  vs.  $\geq 25$ ,  $P = 0.625$ ), gender (male vs. female,  $P = 0.739$ ), tumor diameter ( $< 8$  vs.  $\geq 8$  cm,  $P = 0.161$ ), or anatomic location (tibia/femur vs. elsewhere,  $P = 0.403$ ).

### *Correlation between lncRNA Ftx expression and prognosis of osteosarcoma patients*

The clinical follow-up time of patients ranged from 3 to 60 months. Overall survival was defined as the interval from the date of diagnosis to pancreatic cancer-related death. Overall survival curves were plotted according to the expression level of lncRNA Ftx by the Kaplan-Meier method. As shown in **Figure 1B**, the Kaplan-Meier survival analysis indicated that the overall survival rate of the high lncRNA Ftx group was significantly lower than that of the low lncRNA Ftx group (log-rank test,  $P = 0.001$ ).

### *Prognostic values of lncRNA Ftx expression in OS*

Univariate and multivariate analysis were used to identify the impact of lncRNA Ftx expression and other clinicopathological features on the



**Figure 2.** LncRNA Ftx induced the migration and invasion of Saos2 and HOS cells. The effects of lncRNA Ftx on the migration invasion of Saos2 and HOS cells were assessed by the wound healing assay and Transwell assay respectively. Saos2 and HOS cells were transfected with pcDNA-Ftx, pcDNA-empty vectors and normal saline respectively. A, B. The healing over the scratch of the lncRNA Ftx group was dramatically increased compared with the empty vectors and control groups, respectively in the Saos2 and HOS cells. C, D. Cells invading to the underside surface of the Transwell filter were stained with hexamethyl pararosaniline, and the invasive cells dramatically increased compared with empty vectors and control groups, respectively in Saos2 and HOS cells. \* $P < 0.05$ , and \*\* $P < 0.01$  compared with the control group. The data shown are means  $\pm$  SD from three independent experiments in duplicate.

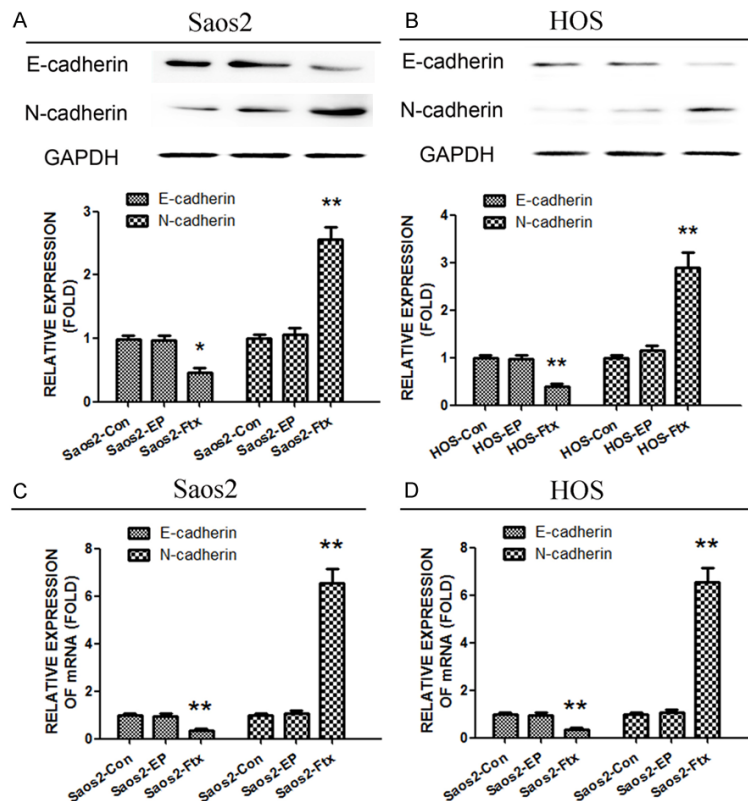
prognosis of osteosarcoma patients. As shown in **Table 2**, univariate analysis showed that lncRNA Ftx expression (HR, 2.636; 95% CI, 1.458-4.767;  $P = 0.001$ ), clinical stage (HR, 2.964; 95% CI, 1.647-5.333;  $P < 0.001$ ) and distant metastasis (HR, 3.715; 95% CI, 2.078-6.643;  $P < 0.001$ ) were significantly correlated with overall survival of osteosarcoma patients. Furthermore, a multivariate Cox regression analysis also showed that lncRNA Ftx expression (HR, 2.162; 95% CI, 1.158-4.037;  $P = 0.016$ ) was an independent predictor for osteosarcoma, as well as the clinical stage (HR, 2.506; 95% CI, 1.356-4.630;  $P = 0.003$ ) and distant metastasis (HR, 2.428; 95% CI, 1.297-4.546;  $P = 0.006$ ).

#### Over-expression of lncRNA Ftx promotes migration and invasion of osteosarcoma cells

To investigate the biological function of lncRNA Ftx in the development and progression of osteosarcoma, Saos2 and HOS cells were trans-

fected with pcDNA-Ftx, pcDNA-empty vectors and normal saline. The wound healing assay and Transwell assay were used to evaluate the migration and invasion of osteosarcoma cells respectively. Osteosarcoma cells were cultured for another 3 h, 6 h, 12 h, 24 h and 48 h after transfection with pcDNA. As shown in **Figure 2A** and **2B**, the results of the wound healing assay demonstrated that healings over the scratches were gradually increased with extended culture time in all of the three groups. But the Ftx over-expression groups had the greatest increase and exhibited significant differences from 24 h after transfection of pcDNAs both in Saos2 and HOS cells. Also, as shown in **Figure 2C** and **2D**, the invading cells that transferred from the upper surfaces to the lower surfaces in the Transwell assay also exhibited a similar tendency after transfection of Ftx in the Saos2 and HOS cells. The results strongly suggested that over-expression of lncRNA Ftx promoted migration and invasion of osteosarcoma cells.





**Figure 3.** LncRNA Ftx decreased the expression of E-cadherin and increased the expression of N-cadherin. Saos2 and HOS osteosarcoma cells were transfected with pcDNA-Ftx, pcDNA-empty vectors and normal saline respectively. A, B. A Western blot assay was used to determine the changes of E-cadherin and N-cadherin protein expressions. C, D. Real time-PCR was also used to detect the mRNA expressions of E-cadherin and N-cadherin. \* $P < 0.05$ , and \*\* $P < 0.01$  compared with the control group. Data shown are means  $\pm$  SD from three independent experiments in duplicate.

#### Effects of over-expression of LncRNA Ftx on EMT marker expression in osteosarcoma cells

The Epithelial to mesenchymal transition is widely seen as one of the most important mechanisms that mediates the acquisition of the invasiveness of cancer cells and the metastasis of the tumors. The characteristic of EMT is the loss of the epithelial marker E-cadherin and gain of the mesenchymal marker N-cadherin [17]. As a result, we examined the expressions of EMT markers in osteosarcoma cells with Western blot and real-time PCR after transfection with pcDNA for 24 h. As shown in **Figure 3A** and **3B**, Ftx over-expression significantly decreased the expression of the epithelial marker E-cadherin and enhanced the expression of the mesenchymal marker N-cadherin both in Saos2 and HOS cells. Then, a real-time PCR was performed and Ftx over-expression also significantly exhibited the same tendency

as that at the protein level (**Figure 3C** and **3D**). The above results suggest that Ftx over-expression could change the expressions of EMT markers and might promote the metastasis of the tumors through this mechanism.

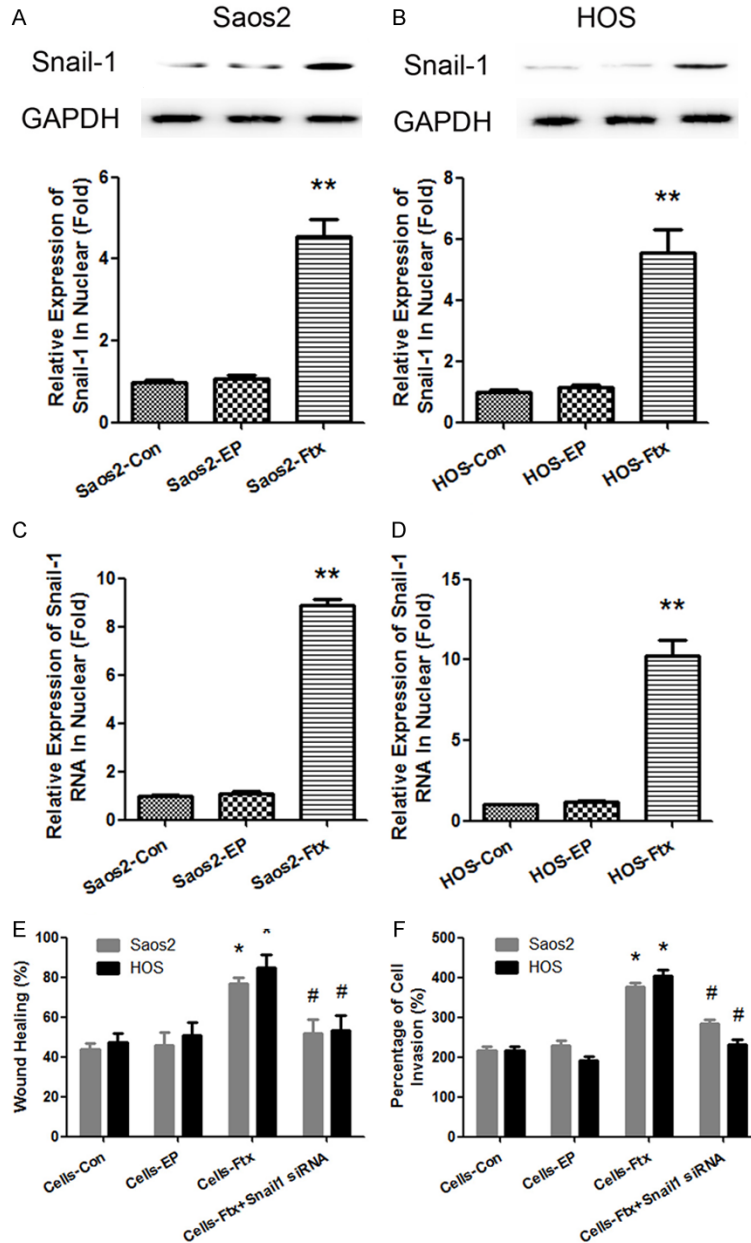
#### Ftx affected migration and invasion of osteosarcoma cells via the Snail pathway

The transcription factor Snail is the most extensively studied and well-acknowledged pathway that triggers the procedure of the epithelial to mesenchymal transition [18]. Therefore, it is crucial to determine the effects of LncRNA Ftx on the expression of Snail and whether it plays a role in the migration and invasion of osteosarcoma cells. As shown in **Figure 4A** and **4B**, over-expression of LncRNA Ftx significantly promoted the expression of Snail-1 both in Saos2 and HOS cells. Then, a real-time PCR was also done, and the results again demonstrated that the over-expression of LncRNA Ftx significantly promoted the expression of Snail-

1 RNA in Saos2 and HOS cells (**Figure 4C** and **4D**). Finally, in order to ascertain whether Snail-1 mediated Ftx-induced migration and invasion, the cells were pre-treated with siRNA of Snail-1 for 2 h before the transfection of the pcDNAs. As shown in **Figure 4F** and **4G**, pre-treatment with the siRNA of Snail-1 significantly abrogated the promotion effect of Ftx over-expression on the migration and invasion of the 2 cells. The above results strongly indicate that Snail-1 is the key regulator by which Ftx promotes osteosarcoma cells' migration and invasion, as well as the irreplaceable position of EMT in the procedure.

#### Discussion

Due to a defect in the encoding protein, long non-coding RNAs (lncRNAs) were long considered to have no function. However, increasing evidence has shown their various roles in bio-



**Figure 4.** LncRNA Ftx increased the expression of Snail-1, and Snail-1 mediated the regulation of EMT markers. Saos2 and HOS osteosarcoma cells were transfected with pcDNA-Ftx, pcDNA-empty vectors and normal saline respectively. A, B. A Western blot assay was used to determine the changes of Snail1 protein expression. C, D. A real time-PCR assay was used to determine the changes of Snail-1 mRNA expression. E. Healing over the scratch showed that Snail-1 siRNA significantly reversed the effects of lncRNA Ftx on the migration ability, respectively in Saos2 and HOS cells. F. A Transwell assay also showed that Snail-1 siRNA significantly reversed the effects of lncRNA Ftx on the invasion ability, respectively in Saos2 and HOS cells. \*P<0.05 versus control group; #P<0.05 versus the Ftx over-expression group. Data shown are means  $\pm$  SD from three independent experiments in duplicate.

logical processes, such as the regulation of gene transcription, as well as the posttranscriptional regulation of RNA splicing [19]. Recently, multiple lncRNAs were found to correlate with

the progression of osteosarcoma and the regulation of tumor genesis, development and prognosis [5, 20]. Therefore, identifying specific lncRNAs and discovering the underlying mechanism involved in tumor genesis may provide promising therapeutic targets for osteosarcoma.

The Ftx transcript is a conserved functional lncRNA located in the X-inactivation center (Xic), which is thought to positively regulate the expression of Xist. In colorectal cancer, Ftx was reported to be significantly up-regulated compared with adjacent normal tissues and was correlated with the differentiation grade, lymph vascular invasion, and clinical stage [9]. LncRNA Ftx harbors 2 clusters of microRNAs in its introns, the miR-374b/421 cluster and the miR-545/374a cluster. Zhao's study revealed that miR-545/374a was up-regulated in hepatocellular carcinoma (HCC) tissue and correlated with the histological grade and metastasis [21]. Liu's study also confirmed that lncRNA Ftx and Ftx-derived miR-545 were up-regulated in both HCC and associated with the poor prognosis of HCC patients. They further discovered the novel pathway by which lncRNA Ftx/miR-545/RIG-I promotes HCC development by activating PI3K/Akt signaling. In glioma and renal cell carcinoma, Ftx was also seen as a critical factor for proliferation and invasion [10, 11]. Although there's still an opposing view of the effect of Ftx in HCC, for example, Liu's study

demonstrated that Ftx inhibited HCC proliferation and metastasis by binding MCM2 and miR-374a [13], most of the present studies showed its ability to promote tumor growth and metas-

tasis. So far, however, there is still no report of lncRNA Ftx on osteosarcoma.

In this study, we identified lncRNA Ftx as a promoter of osteosarcoma. We first proved that Ftx expression was significantly increased in osteosarcoma tissues compared with ANCTs in the 84 pairs of clinical samples. Further study indicated that the high expression of Ftx is correlated with the clinical stage and distant metastasis, with no significant correlations with other clinicopathological features, such as age, gender, tumor diameter, or anatomic location, indicating that Ftx may be a prognostic biomarker for osteosarcoma. After 60 months follow-up, overall survival curves indicated that the high Ftx group had a remarkably lower overall survival rate than the low Ftx group. Multivariate regression analysis indicated that lncRNA Ftx expression could be seen as an independent prognostic factor for overall survival of osteosarcoma patients and increased expression of lncRNA Ftx might play a key role in the progression of osteosarcoma.

EMT is characterized by loss of epithelial markers, such as E-cadherin, and in turn, the acquisition of mesenchymal markers, such as N-cadherin. Tumor-associated EMT has been widely acknowledged as an important mechanism that promotes osteosarcoma progression and metastasis [22]. As previously reported, EMT was associated with higher tumor recurrence, poorer prognosis and decreased survival rates [23]. Many lncRNAs were reported to be involved in the regulation of EMT, and thereby affecting tumor development and metastasis. Up-regulation of lncRNA PlncRNA-1 promotes proliferation and induces the epithelial-mesenchymal transition in prostate cancer [24]. lncRNA CCAT2 is associated with tumor metastasis by regulating the Snail-2-mediated epithelial-mesenchymal transition [25]. Furthermore, lncRNA Ftx was also reported to inhibit HCC cell epithelial-mesenchymal transition and invasion [13]. The data in the present study showed the role of lncRNA Ftx which significantly decreased and increased expression of E-cadherin and N-cadherin respectively, which strongly suggested to us that visfatin is an inducer of EMT in osteosarcoma cells.

Snail-1 has been considered one of the most important EMT process regulators, which mediates the loss of E-cadherin and the acquisition of N-cadherin. It was reported that Snail-1 was over-expressed in osteosarcoma [26], and its

overexpression promoted the proliferation, migration and invasion of cancers [27, 28]. The present study also confirmed the promoting effect of Snail1 on the migration and invasion of osteosarcoma. Also, the results in this research strongly verified that Snail-1 was the key regulator by which Ftx promoted osteosarcoma cells' migration and invasion.

Taken together, the present study first demonstrated that lncRNA Ftx is markedly up-regulated in osteosarcoma, and its expression is significantly correlated with the development of tumors as well as the prognosis of the patients. Up-regulation of lncRNA Ftx could significantly enhance the migration and invasion of osteosarcoma cells. Additionally, our study also explored the underlying mechanism, and demonstrated that lncRNA Ftx plays its role via promoting the epithelial-mesenchymal transition.

## Acknowledgements

This study was supported by the Key Science and Technology Program of Shandong Province (grant no. 2015GSF118054), the National Natural Science Foundation of China (grant no. 81700321) and Science Foundation of Shandong Province (grant nos. ZR2014HP005 and ZR2017QH003).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Zhiyong Wang, Department of Emergency Surgery, Qilu Hospital of Shandong University, 107 Wen Hua Xi Road, Jinan 250012, Shandong, P. R. China. Tel: +86 1568904-5686; E-mail: qlyywy@163.com

## References

- [1] Zheng H, Min J. Role of long noncoding RNA HOTAIR in the growth and apoptosis of osteosarcoma cell MG-63. *BioMed Res Int* 2016; 2016: 5757641.
- [2] Rothermundt C, Seddon BM, Dileo P, Strauss SJ, Coleman J, Briggs TW, Haile SR, Whelan JS. Follow-up practices for high-grade extremity osteosarcoma. *BMC Cancer* 2016; 16: 301.
- [3] Miller BJ, Cram P, Lynch CF, Buckwalter JA. Risk factors for metastatic disease at presentation with osteosarcoma: an analysis of the SEER database. *J Bone Joint Surg Am* 2013; 95: e89.
- [4] Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the surveillance, epidemiology



- gy, and end results program. *Cancer* 2009; 115: 1531-1543.
- [5] Chen R, Wang G, Zheng Y, Hua Y, Cai Z. Long non-coding RNAs in osteosarcoma. *Oncotarget* 2017; 8: 20462-20475.
- [6] Broadhead ML, Clark JC, Myers DE, Dass CR, Choong PF. The molecular pathogenesis of osteosarcoma: a review. *Sarcoma* 2011; 2011: 959248.
- [7] Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. *Cancer Discov* 2011; 1: 391-407.
- [8] Zhao Y, Guo Q, Chen J, Hu J, Wang S, Sun Y. Role of long non-coding RNA HULC in cell proliferation, apoptosis and tumor metastasis of gastric cancer: a clinical and in vitro investigation. *Oncol Rep* 2014; 31: 358-364.
- [9] Guo XB, Hua Z, Li C, Peng LP, Wang JS, Wang B, Zhi QM. Biological significance of long non-coding RNA Ftx expression in human colorectal cancer. *Int J Clin Exp Med* 2015; 8: 15591-15600.
- [10] Zhang W, Bi Y, Li J, Peng F, Li H, Li C, Wang L, Ren F, Xie C, Wang P, Liang W, Wang Z, Zhu D. Long noncoding RNA Ftx is upregulated in gliomas and promotes proliferation and invasion of glioma cells by negatively regulating miR-342-3p. *Lab Invest* 2017; 97: 447-457.
- [11] He X, Sun F, Guo F, Wang K, Gao Y, Feng Y, Song B, Li W, Li Y. Knockdown of long noncoding RNA Ftx inhibits proliferation, migration, and invasion in renal cell carcinoma cells. *Oncol Res* 2017; 25: 157-166.
- [12] Liu Z, Dou C, Yao B, Xu M, Ding L, Wang Y, Jia Y, Li Q, Zhang H, Tu K, Song T, Liu Q. Ftx non coding RNA-derived miR-545 promotes cell proliferation by targeting RIG-I in hepatocellular carcinoma. *Oncotarget* 2016; 7: 25350-25365.
- [13] Liu F, Yuan JH, Huang JF, Yang F, Wang TT, Ma JZ, Zhang L, Zhou CC, Wang F, Yu J, Zhou WP, Sun SH. Long noncoding RNA Ftx inhibits hepatocellular carcinoma proliferation and metastasis by binding MCM2 and miR-374a. *Oncogene* 2016; 35: 5422-5434.
- [14] Li B, Zhao Y, Liu H, Meng B, Wang J, Qi T, Zhang H, Li T, Zhao P, Sun H, Xu J, Song H, Dong Z, An F. Visfatin destabilizes atherosclerotic plaques in apolipoprotein e-deficient mice. *PLoS One* 2016; 11: e0148273.
- [15] Cheng G, Liu C, Sun X, Zhang L, Liu L, Ouyang J, Li B. Visfatin promotes osteosarcoma cell migration and invasion via induction of epithelial-mesenchymal transition. *Oncol Rep* 2015; 34: 987-994.
- [16] Liu T, Liu P, Ding F, Yu N, Li S, Wang S, Zhang X, Sun X, Chen Y, Wang F, Zhao Y, Li B. Ampelopsin reduces the migration and invasion of ovarian cancer cells via inhibition of epithelial-to-mesenchymal transition. *Oncol Rep* 2015; 33: 861-867.
- [17] Rosano L, Cianfrocca R, Spinella F, Di Castro V, Nicotra MR, Lucidi A, Ferrandina G, Natali PG, Bagnato A. Acquisition of chemoresistance and EMT phenotype is linked with activation of the endothelin A receptor pathway in ovarian carcinoma cells. *Clin Cancer Res* 2011; 17: 2350-2360.
- [18] Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer* 2007; 7: 415-428.
- [19] Zhang H, Chen Z, Wang X, Huang Z, He Z, Chen Y. Long non-coding RNA: a new player in cancer. *J Hematol Oncol* 2013; 6: 37.
- [20] Wang K, Jiang W, Cheng C, Li Y, Tu M. Pathological and therapeutic aspects of long noncoding RNAs in osteosarcoma. *Anticancer Agents Med Chem* 2017; [Epub ahead of print].
- [21] Zhao Q, Li T, Qi J, Liu J, Qin C. The miR-545/374a cluster encoded in the Ftx lncRNA is overexpressed in HBV-related hepatocellular carcinoma and promotes tumorigenesis and tumor progression. *PLoS One* 2014; 9: e109782.
- [22] Yang G, Yuan J, Li K. EMT transcription factors: implication in osteosarcoma. *Med Oncol* 2013; 30: 697.
- [23] Cichon MA, Radisky DC. ROS-induced epithelial-mesenchymal transition in mammary epithelial cells is mediated by NF- $\kappa$ B-dependent activation of Snail. *Oncotarget* 2014; 5: 2827-2838.
- [24] Jin Y, Cui Z, Li X, Jin X, Peng J. Upregulation of long non-coding RNA PlncRNA-1 promotes proliferation and induces epithelial-mesenchymal transition in prostate cancer. *Oncotarget* 2017; 8: 26090-26099.
- [25] Xu Y, Wang B, Zhang F, Wang A, Du X, Hu P, Zhu Y, Fang Z. Long non-coding RNA CCAT2 is associated with poor prognosis in hepatocellular carcinoma and promotes tumor metastasis by regulating Snail2-mediated epithelial-mesenchymal transition. *Onco Targets Ther* 2017; 10: 1191-1198.
- [26] Yang H, Zhang Y, Zhou Z, Jiang X, Shen A. Snail-1 regulates VDR signaling and inhibits 1,25(OH)-D(3) action in osteosarcoma. *Eur J Pharmacol* 2011; 670: 341-346.
- [27] Larriba MJ, Ordonez-Moran P, Chicote I, Martín-Fernández G, Puig I, Muñoz A, Palmer HG. Vitamin D receptor deficiency enhances Wnt/ $\beta$ -catenin signaling and tumor burden in colon cancer. *PLoS One* 2011; 6: e23524.
- [28] Stubbins RE, Hakeem A, Nunez NP. Using components of the vitamin D pathway to prevent and treat colon cancer. *Nutr Rev* 2012; 70: 721-729.