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Overexpression of FER1L4 promotes the apoptosis and suppresses epithelial-mesenchymal transition and stemness markers via activating PI3K/AKT signaling pathway in osteosarcoma cells

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ABSTRACT

Novel long non-coding RNA Fer-1-like protein 4 (FER1L4) has been identified as a tumor suppressor in endometrial carcinoma, ovarian cancer, hepatocellular carcinoma, esophageal squamous cell carcinoma. However, the function of FER1L4 in osteosarcoma has not been clear. The aim of the research was to explore the effects of FER1L4 in osteosarcoma. Results showed that FER1L4 was observed to be lowly expressed in osteosarcoma cell lines (US-O2, MG-63 and SaOS-2 cells), especially MG63 cells. Besides, overexpression of FER1L4 remarkably repressed the proliferation, migration and invasion of MG63 cells. FER1L4-induced apoptotic cell death leaded to the activation of caspase-3 and Bax/Bcl2. Moreover, epithelial-mesenchymal transition (EMT) was tremendously suppressed by increased FER1L4, evidences were the increased E-cadherin and reduced vimentin and fibronectin. Blocking FER1L4 expression by sh-FER1L4 treatment increased the expression of SOX9, CD44, ALDH1, Nanog and Oct4, indicating that FER1L4 could effectively decrease cell stemness in osteosarcoma. Furthermore, the protein levels of p-AKT and p-PI3K were remarkably suppressed when FER1L4 was knocked down. In conclusion, the study indicated that FER1L4 acted as a tumor suppressor in osteosarcoma via activating PI3K/AKT pathway may be a new prognostic biomarker and potential therapeutic target for osteosarcoma intervention.

1. Introduction

Osteosarcoma, the most prevalent primary bone tumor, mainly occurs in children and adolescents with a high propensity for local invasion and early systemic metastasis [1]. The incidence of osteosarcoma in people under 20 years of age is estimated at 4 million/year [2]. According to recent researches, around 20% of osteosarcom patients could develop into metastatic osteosarcom, and the 5-year survival rate of patients with osteosarcom remains 60–70%, while only 11%–30% of patients with metastatic OS survive 5 years [3]. Nowadays, surgery combined with chemotherapy has been regarded as one of the most important therapeutic strategy for osteosarcom. However, chemotherapy is commonly seen, resulting in a recurrence of the tumor [4]. Even with the introduction of a variety of therapeutic strategies involving radiotherapy, surgery (wider tumor excision areas), adjuvant chemotherapy, the survival rates were not remarkably improved in patients with osteosarcoma, as the acquired drug resistance in response to chemotherapy is still formidable [5]. Besides, the pathogenesis and clinical progression of osteosarcoma are related to numerous genes and corresponding pathways. Thus, it is important and urgent to explore the molecular mechanism of osteosarcoma as a new therapeutic target.

As reported in latest reports, lncRNAs have considerable potential as diagnostic and prognostic biomarkers and therapeutic targets owing to lncRNAs can affect epithelial-mesenchymal transition (EMT), drug resistance, tumorigenesis and tumor progression in osteosarcoma [6]. Large amount of evidences have indicated that the abnormal expression and function of long non-coding RNAs (lncRNAs) were closely related to the pathogenesis of many cancer types [7]. For example, researchers have demonstrated that lncRNA-HOST2 promoted the metastasis, invasion and proliferation of epithelial ovarian cancer cells via

Abbreviations: lncRNAs, long non-coding RNA; FER1L4, Fer-1-like protein 4; EMT, epithelial-mesenchymal transition; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X

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suppressing the miRNA let-7 level [8]. LncRNA RUSC1-AS-N identified to be increased in hepatocellular carcinoma tissue was proved to be associated with poor prognosis in patients with HCC from GSE54238 and GSE40144 datasets [9]. LncRNA Fer-1-like protein 4 (FER1L4) inhibited the tumorigenesis of osteosarcoma through regulating the level of PTEN via targeting miR-18a-5p [10]. Fer-1-like protein 4 (FER1L4) located on the q23 region of chromosome 10 is reported to be involved in the occurrence and development of multiple tumors, including endometrial carcinoma, hepatocelluar carcinoma, ovarian cancer and osteosarcoma. Besides, further studies have indicated that FER1L4 played an important part in restraining the proliferation, migration and invasion of tumor cells in osteosarcoma, suggesting its role as a tumor suppressor gene [11]. However, until now, little is known the underlying molecular mechanism of FER1L4 in osteosarcoma.

In the represent study, we firstly evaluated the expression levels of FER1L4 in osteosarcoma cell lines. Besides, we observed that FER1L4 was suppressed, and the PI3K/AKT pathway was significantly inactivated in osteosarcoma cells. Consistent with previous studies, we found that FER1L4 served as a tumor inhibitor and exerted its anticancer effects partly through modulating PI3K/AKT signaling.

2. Material and methods

2.1. Cell culture

The human osteosarcoma cell lines (MG63, U-2 OS, SaOS-2) together with normal human osteoblastic cell lines (Hfob1.19) were all purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (China). Hfob1.19 were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) containing 10% FBS, 100 μg/mL streptomycin and 100 IU/mL penicillin (Invitrogen, Carlsbad, CA, USA). MG63, U-2 OS and SaOS-2 were cultivated in McCoy's medium (Sigma Aldrich, MI, USA) complemented with 10% of FBS, 50 U/mL penicillin/streptomycin and 1% GlutaMAX (Thermo Fisher Scientific, Massachusetts, USA). Cell culture was performed in a humidified incubator with 5% CO_2 at 37 °C and passaged every 3–4 days.

2.2. Cell transfection

ShRNA directed against FER1L4 was subcloned into the U6/GFP/Neo plasmid (GenePharma, Shanghai, China). The synthetic plasmids were called sh-FER1L4. The negative control of sh-FER1L4 was U6/GFP/Neo plasmid with a nontargeting sequence, which was called sh-NC. Full-length FER1L4 sequences were ligated into pEX-2, which was called pEX-FER1L4. Empty pEX-2 was served as the negative conctrol of pEX- FER1L4. Transfection reagent Lipofectamine 3000 (Invitrogen Co., CA, USA) was used for the transfection of MG63 cells in accordance with the manufacturer's instructions. G418 (0.5 mg/mL; Sigma) was employed to select stably transfected cells. About 4 weeks' incubation, G418-resistant cell clones were established.

2.3. Quantitative reverse transcription PCR (qRT-PCR)

Total RNA was isolated from cultured cells by using TRIzol reagent (Invitrogen). Then, the extracted RNA reversely transcribed into complementary DNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc., MA, USA) with Oligo (dT18) RT primers in accordance with the manufacturer's protocol. QRT-PCR was employed to measure mRNA expression by SYBR Green Master Mix (Thermo Fisher Scientific Inc.). GAPDH was used as an internal control. The primers used were as follows: FER1L4, 5'-CCGTGTTGAGGTGCTG TTC-3′ (forward) and 5′-GGCAAGTCCACTGTCAGATG-3′ (reverse); ALDH1, 5'-TAGTCCAAAGCACGGCTCTAT-3′ (forward) and 5′- GGTCC TGTATCCAAGCCATCA-3′ (reverse); CD44, 5′-CTGCCGCTTTGCAGGT GTA-3′ (forward) and 5′-CATTGTGGGCAAGGTGCTATT-3′ (reverse); Nanog, 5'- TTTGTGGGCCTGAAGAAAACT-3′ (forward) and 5′-AGGGC TGTCCTGAATAAGCAG-3′ (reverse); SOX9, 5'-AGCGAACGCACATCAA GAC-3′ (forward) and 5′- CTGTAGGCGATCTGTTGGGG-3′ (reverse); Oct4, 5'-CTGCAGTGTGGGTTTCGGGCA-3′ (forward) and 5′-CTTGCTG CAGAAGTGGGTGGAGGAA -3′ (reverse); p85, 5′-GCCTCAGTGGACTT GGATGTGTTC-3′ (Forward) and 5′-GTCTTCGGAGCTTGGTACTTCT TGG-3′ (Reverse). The relative RNA level was detected using the $2^{-\Delta\Delta Ct}$ method.

2.4. Hoechst staining

10^5 transfected MG63 cells were planted into 6-well plate at 37 °C in an atmosphere of 5% CO_2 for 24 h. All cells were washed with PBS for 3 times at 4 °C, 1.0 mL/well stationary liquid was added into each well. Then, cells were stained with a Hoechst 33258 (5 μg/mL) solution at room temperature in the dark for 30 min. After that, the photos were taken under an inverted fluorescent microscope (Olympus, Japan).

2.5. Cell proliferation assay

Cell viability was measured using CCK-8 assay kit (Thermo Fisher Scientifc) according to the manufacturer's protocols. Briefly, the transfected MG63 cells were seeded into 96-well plates at a density of 5×10^3 per well. Subsequently, cells were cultured in 37 °C with 5% CO2 for 12, 24, 36 and 48 h respectively. After that, 10 u L CCK-8 solution was added into every well. After incubating the plates for 4 h at 37 °C, OD values at 450 nm were detected by Fisherbrand™ accuSkan™ GO UV/Vis Microplate Spectrophotometer.

2.6. Colony formation assay

MG63 cells were seeded into a 6-well plate at a concentration of 10^3 per well and incubated in serum-free culture at 37 °C in 5% CO_2 for 3 days. Then, cells were washed with PBS, fixed with 4% paraformaldehyde (Qianchen Biotechnology, Shanghai, China) and then stained with 0.05% crystal violet (Aladdin, Shanghai, China) at room temperature for 15 min. Cell numbers were counted by Image J software.

2.7. Wound healing assays

Cell migration was measured using wound-healing assays. In brief, transfected cells were planted into 6-well plates at a concentration of 5×10^4 per well. After incubation for 24 h, these cultures were then scratched with a sterile plastic micropipette tip to create an artificial wound. After that, cells were incubated with serum-free medium for another 24 h. Photos were taken at 0 and 24 h after the scratch using an X71 inverted microscope (Olympus, Tokyo, Japan).

2.8. Western-blot assay

Protein samples were extracted from sorted cells using RIPA buffer (Beyotime Biotechnology, Nanjing, China) according to the manufacturer's protocols. The concentration of protein was measured by a BCA Protein Assay kit (Beyotime Biotechnology). Total proteins were separated using SDS-PAGE and transferred to PVDF membranes (Millipore, MA, USA). After that, the membranes were blocked with blocking buffer containing 5% nonfat milk for 1 h and incubated at 4 °C overnight with primary antibodies: B-cell lymphoma-2 (Bcl-2), Bcl-2-associated X (Bax), caspase-3, caspase-9, E-cadherin, fibronectin, Nanog, p-AKT, AKT, p-PI3K, PI3K and GAPDH (Abcam, Cambridge, UK). Then, the membranes were incubated with a species-matched HRP-conjugated secondary antibody (Abcam) for 1 h at room temperature. Finally, the membranes were washed 3 times with TBST. Immunoblot bands were detected using an enhanced chemiluminescence (Beyotime Biotechnology).

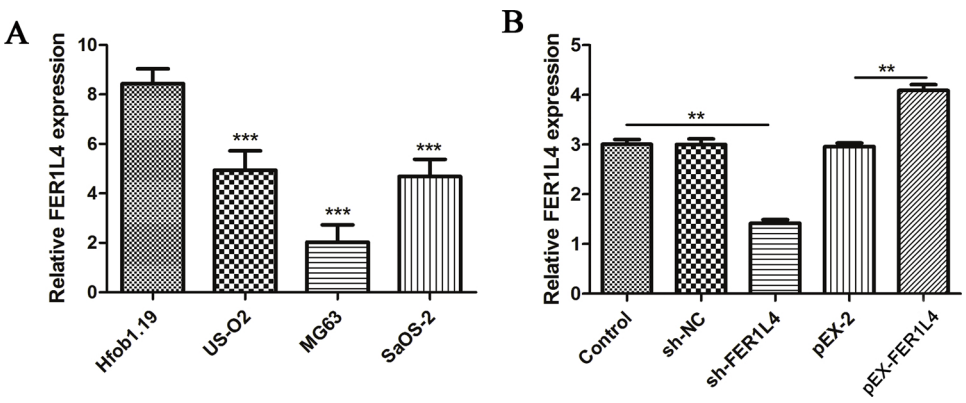

Fig. 1. FER1L4 is aberrantly expressed in osteosarcoma cell lines. (A) The relative expressions of FER1L4 was detected by qRT-PCR assay in Hfob1.19, US-O2, MG63 and SaOS cells respectively. (B) QRT-PCR assay was performed the measure the level of FER1L4 in MG63 cells transfected with sh-NC, sh-FER1L4, pEX-2 and pEX-FER1L4 respectively. ($^{**}P < 0.01$).

2.9. Statistical analysis

Results were from at least three independent experiments and expressed as mean ± SD. Statistical analysis was measured using SPSS 19 software. Significant differences were determined by one-way analysis of variance (ANOVA). $P < 0.05$ was considered as significant difference.

3. Results

3.1. FER1L4 is aberrantly expressed in osteosarcoma cell lines

Expression of lncRNA FER1L4 in Hfob1.19 cell and osteosarcoma cell lines (US-O2, MG-63 and SaOS-2 cells) was measured using qRT-PCR. Results showed that FER1L4 level was remarkably decreased in osteosarcoma cell lines compared with Hfob1.19 cells (Fig. 1A), indicating that downregulation of FER1L4 is likely involved in the pathogenesis of osteosarcoma. Among the 3 cell lines, MG63 cell suffered the biggest drop in the level of FER1L4. Thus, the subsequent experiment was performed on MG63 cells. In order to evaluate whether FER1L4 played an important role in MG63 cell development, we employed specific shRNAs against FER1L4 (shFER1L4) and pEX-2 (pEX-FER1L4) respectively to affect the expression of FER1L4 in MG63 cells. QRT-PCR assay revealed that the mRNA level of FER1L4 was remarkably decreased in sh-FER1L4 group and increased in pEX-FER1L4 group compared with other control groups (Fig. 1B). Data demonstrated that FER1L4 was successfully overexpressed or knocked down in stably transfected MG63 cells.

3.2. Silence of FER1L4 promoted MG63 cells proliferation and suppressed cells apoptosis

Alteration of cell viability, migration, and apoptosis in MG63 cells were all detected after aberrant expression of MG63 cells. CCK-8 assay and colony conformation assay indicated that MG63 transfected with sh-FER1L4 dramatically promoted the cell proliferation compared with cells in the control, sh-NC and pEX-2 groups. However, cells transfected with pEX-FER1L4 showed the weakest cell viability (Figure 2AB). The suppressed expression of FER1L4 visibly promoted migration in MG63 cells according to the wound healing assay (Fig. 2C). Moreover, cell apoptosis was measured using Hoechst staining. As illustrated in Fig. 2D, over-expression of FER1L4 remarkably facilitated cell apoptosis by elevating the expression of Bax/Bcl-2 and cleaved-caspase3. However, these alteration was totally opposite in cells transfected with sh-FER1L4 (Fig. 2E). These findings suggested that increased FER1L4 could effectively inhibite cell viability and migration and promote cell apoptosis in MG63 cells.

3.3. Overexpression of FER1L4 attenuated the EMT and stemness of MG63 cells

In order to evaluate whether knockdown of FER1L4 could suppress the EMT of MG63 cells, western-blot was applied to detect the expression of E-cadherin, fibronectin and vimentin proteins in transfected MG63 cells. The results suggested no remarkable differences in the level of these proteins among the control, sh-NC and pEX-2 groups. However, compared with these groups, in the pEX-FER1L4 group, fibronectin and vimentin levels were dramatically suppressed, but the E-cadherin level was elevated (Fig. 3A). Besides that, the level of stemness markers (ALDH1, Nanog, Oct4, SOX9, CD44) were remarkably inhibited in cells transfected with pEX-PER1L4 and significantly increased in cells transfected with sh-FER1L4. Therefore, these data indicated that elevated FER1L4 could decrease EMT and stemness of MG63 cells.

3.4. FER1L4 knockdown inactivated the PI3K/AKT pathway

PI3K/AKT signaling pathway is known to be closely related to the development and progression of various cancer types. Thus, in order to further clarify the potential mechanism of FER1L4 on cell proliferation, migration and invasion, we focused on PI3K/AKT signaling pathway. Western-blot was used to detect the effect of FER1L4 abnormal expression on PI3K/AKT pathways. As illustrated in Fig. 4A, MG63 cells transfected with sh-FER1L4 exhibited the lowest expression of p-AKT/AKT and p-PI3K/PI3K than cells in other groups. As expected, cells transfected with pEX-PER1L4 showed the highest expression of pEX-PER1L4. Besides, the mRNA expression of p85 was remarkably increased in pEX-FER1L4 group compared with other groups. In conclusion, these results suggested that upregulation of PER1L4 may inhibit the occurrence and progression of osteosarcoma cancer by activating PI3K/AKT signaling pathway activity.

4. Discussion

Osteosarcoma whose prognosis remains poor despite great progression has been made in early diagnosis and combination treatment ranked among the leading causes of cancer mortality in children and adolescence population [12]. Numerous advances have been made in the occurrence and progression of osteosarcoma. However, the specific mechanisms of osteosarcoma still remain to be largely unclear [13]. It is necessary for us to find a useful biomarker to predict the prognosis of osteosarcoma. According to recent researches, more and more evidences have showed that lncRNAs will be emerging regulators of cancer biological functions that can act as a biomarker for potential cancer diagnosis, prognosis, and targeted treatment [14]. For example, lncRNA SNHG1 increases the level of ROCK1 and induced cell proliferation, migration and invasion via targeting miRNA1013p in osteosarcoma [15]. Therefore, the aim of the current research was to clarify how lncRNA FER1L4 regulated the metastasis of osteosarcoma, and found

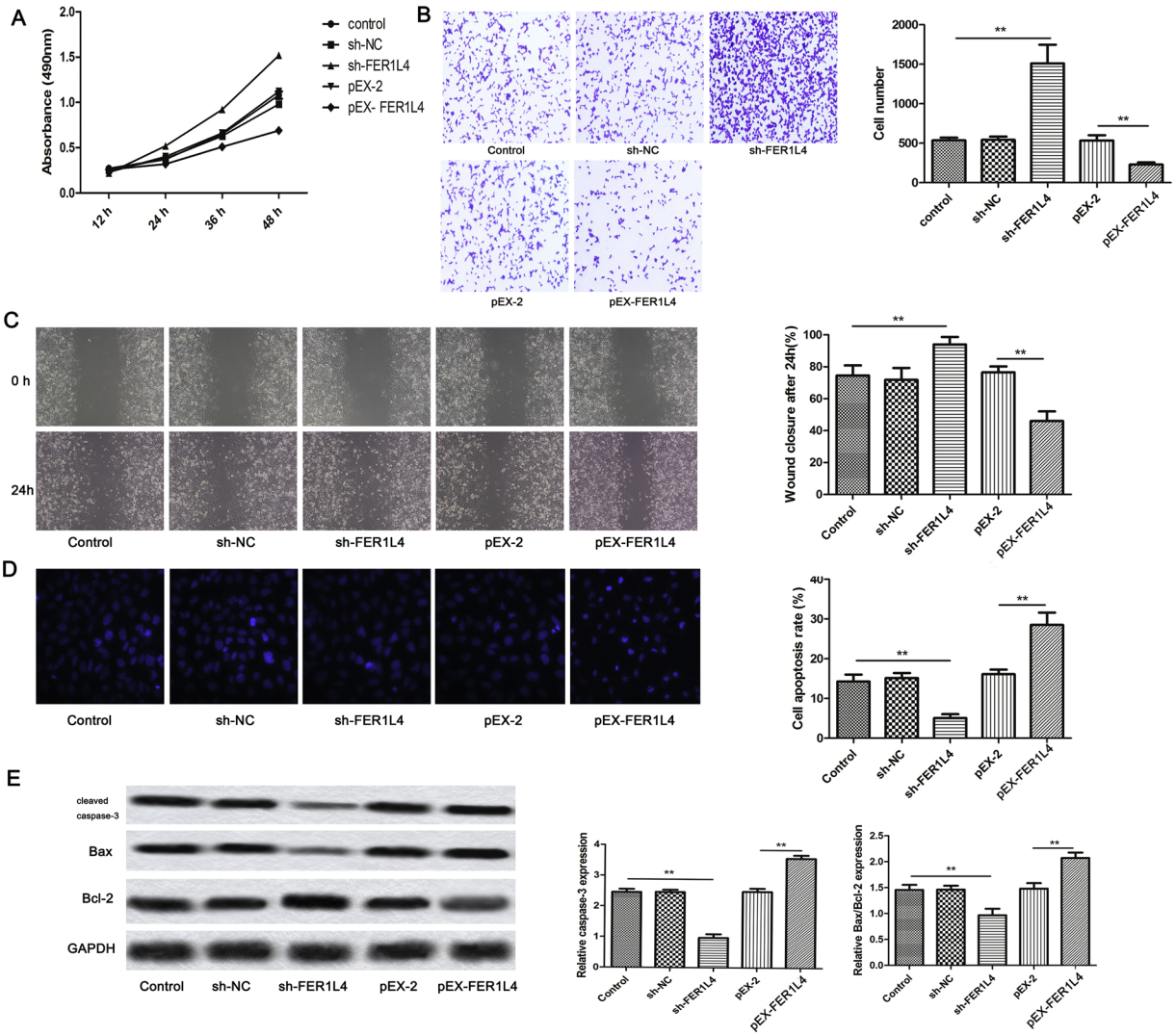

Fig. 2. Silence of FER1L4 promoted MG63 cells proliferation and suppressed cells apoptosis. CCK-8 (A) and colony formation assay (B) was applied to evaluate the cell viability in transfected MG63 cells at 12 h, 24 h, 36 h and 48 h. (C) Cell migration was measured using wound healing assays. (D) Cell apoptosis was analyzed using hocst staining. (E) Protein expressions of caspase-3, Bax and Bcl-2 were measured by western-blot. ($^{**}P < 0.01$).

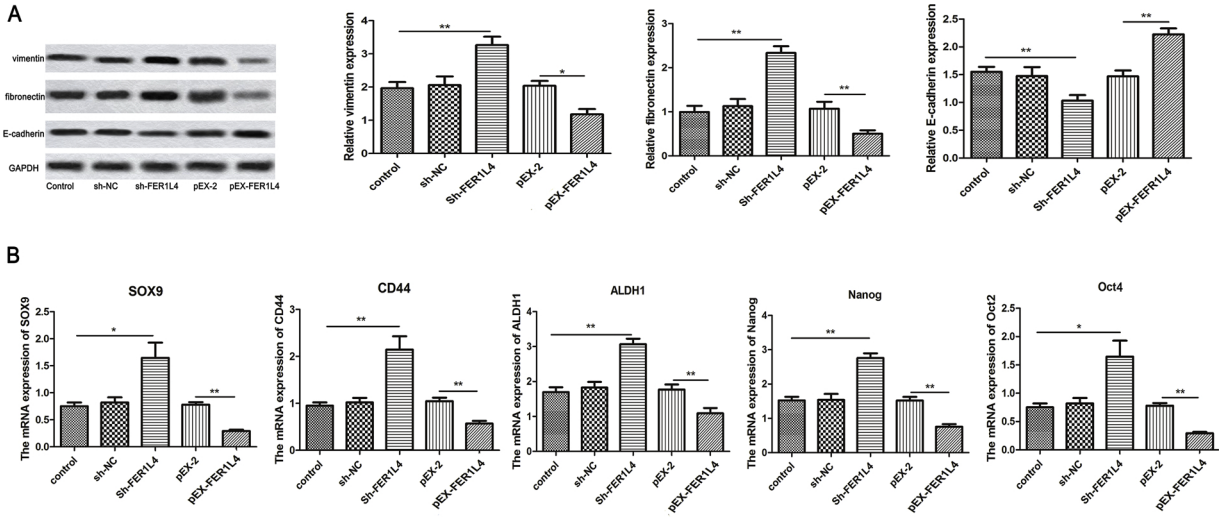

Fig. 3. Overexpression of FER1L4 attenuated the EMT and stemness of MG63 cells. (A) The levels of MET related proteins (E-cadherin, fibronectin and vimentin) were assessed using western-blot. (B) QRT-PCR was used to detect the level of stemness markers (SOX9, CD44, ALDH1, Nanog and Oct4). ($^{*}P < 0.05$, $^{**}P < 0.01$).

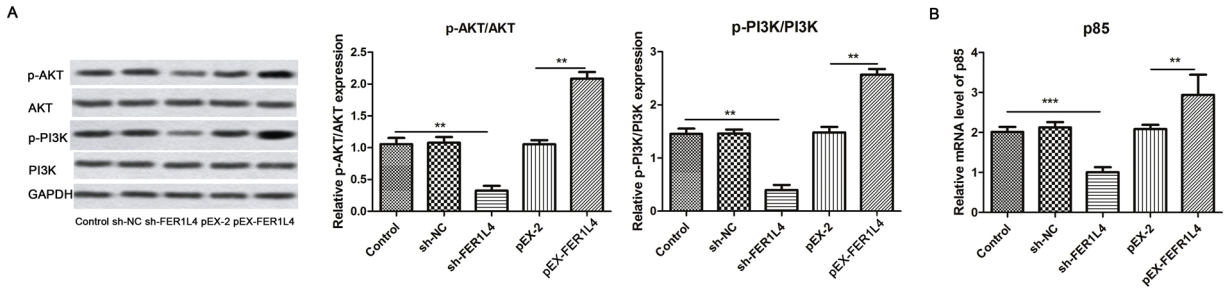

Fig. 4. FER1L4 knockdown inactivated the PI3K/AKT pathway. The expression levels of p-AKT, AKT, p-PI3K and PI3K were analyzed using western-blot. ($^{**}P < 0.01$).

that overexpression of FER1L4 promoted the apoptosis and suppresses EMT and stemness markers via activating PI3K/AKT signaling pathway in osteosarcoma cells.

At first, we demonstrated that lncRNA FER1L4 was aberrantly expressed in osteosarcoma cell lines (US-O2, MG63 and SaOS cells), especially in MG63 cells which was chosen for the subsequent experiments, with help of CCK-8 assay. An array of studies have indicated that the abnormal expression of lncRNAs played an vital role in the occurrence and progression of cancers [16]. For instance, the restrained expression of TUG1 significantly suppressed the growth and migratory ability of PC cells in vitro and in vivo via targeting miR-29c [17]. LncRNA DLX6 AS1 is closely related to the malignant progression, proliferation and invasion of esophageal squamous cell carcinoma [18]. Some researchers have demonstrated that lncRNA PRNCR12 may promote the proliferation, migration, invasion and cell cycle progression of breast cancer cell [19]. Moreover, studies have indicated that FER1L4 served as a tumor suppressors in various cancer types, including endometrial carcinoma, ovarian cancer, hepatocellular carcinoma and esophageal squamous cell carcinoma [20–23]. In addition to this, Chen et al. have demonstrated that down-regulated of lncRNA FER1L4 might be a prognostic marker in osteosarcoma [24]. However, how FER1L4 worked in osteosarcoma has not been investigated. In our study, results illustrated that up-regulated FER1L4 could tremendously restrain the MG63 cell proliferation and stimulate apoptosis. Further experiment proved that down-regulated FER1L4 could promote the migration and invasion of MG63 cells. These findings preliminarily revealed the correlation between FER1L4 and osteosarcoma.

Additionally, the current study observed that up-regulated FER1L4 could significantly repress the EMT of MG63 cells via increasing the expression of E-cadherin and decreasing fibronectin and vimentin level. It is well known that EMT go hand in hand with the metastasis ability of cancer cell. EMT involves multiple components, including fibronectin, vimentin and E-cadherin [3]. Vimentin expression is a late event in EMT, before which epithelial function is lost, leading to the up-regulation of mesenchymal genes [4]. E-cadherin, an important EMT transcription factor and epithelial factor, is related to the invasiveness of cancer cells, which can promote cell-cell contact and inhibit malignant invasion and metastasis of epithelial cells [6]. Fibronectin played a vital role in cancer cell mobility and invasion [10]. These results demonstrated that FER1L4 could restrain the malignant invasion and metastasis of osteosarcoma cells partly through EMT suppression.

Growing evidences have indicated that tumor cells contain a subpopulation of cells called cancer stem cells, which are responsible for initiating tumor growth and triggering recurrence after chemotherapy [25]. To further evaluate the role of FER1L4 in the mechanisms of modulating the proliferation, invasion and metastasis of osteosarcoma cells, cell stemness was measured. Transcription factor SOX9, Nanog and Oct4 have been reported to accelerated cell growth and migration in osteosarcoma [26–28]. CD44, a receptor for hyaluronic acid (HA) and HA-binding, has been demonstrated to be involved in osteosarcoma cell activities, including tumor progression, metastasis and drug resistance [29]. Besides, $ALDH1^+$ cells could activate cell autophagy and decrease cell death [30]. Thus, we used qRT-PCR to analyze these stemness markers. Not surprisingly, increased FER1L4 obviously down-regulated the SOX9, CD44, ALDH1, Nanog and Oct4 level.

Accumulated researches have indicated that lncRNAs could modulate core components of signaling pathway and play a blocking or promoting role in the pathogenesis of cancer by regulating its activity or stability [31]. Among the signaling pathways, PI3K/AKT pathway has been demonstrated to be involved in regulating lots of biological processes such as stem cell maintenance [32], cell proliferation [33], EMT [34], cell apoptosis [35] in osteosarcoma. PI3K was heterodimer composed of a regulatory subunit, p85, and a catalytic subunit, p110 [25]. Wang et al. have proved that overexpression of FER1L4 inhibited the migration and proliferation of hepatocellular cancer through regulating PI3K/AKT pathway [22]. Thus, we hypothesized that FER1L4 might contribute to progression of osteosarcoma through PI3K/AKT signaling pathway. As expected, up-regulated FER1L4 increased the p-AKT, p-PI3K and p85 activation, and consistent with this assumption, PI3K/AKT signaling pathway was positive correlated to the FER1L4 expression.

All in all, our study indicated that FER1L4 acted as a critical anticancer role in osteosarcoma. FER1L4 was lowly expressed in osteosarcoma cell lines. Over-expression of FER1L4 may inhibit cell proliferation, migration, invasion, EMT, stemness and promote cell apoptosis through activating PI3K/AKT pathway. The present research provided novel evidence that the therapeutic targeting of FER1L4 or PI3K/AKT may be a promising strategy for the treatment of osteosarcoma in clinic. We have pointed out that this study has examined only one cell line and no clinical specimens was involved. However, the clinical trial will be carried out to make up for the deficiency in the future.

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Conflicts of interest

None.

References

[1] L. Mirabello, R.J. Troisi, S.A. Savage, Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program, Cancer 115 (2009) 1531–1543.
[2] L. Mirabello, R.J. Troisi, S.A. Savage, International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons, Int. J. Cancer 125 (2009) 229–234.
[3] N. Omer, M.C. Le Deley, S. Piperno-Neumann, P. Marec-Berard, A. Italiano, N. Corradini, C. Bellera, L. Brugieres, N. Gaspar, Phase-II trials in osteosarcoma recurrences: a systematic review of past experience, Eur. J. Cancer 75 (2017) 98–108.
[4] G.N. Yan, Y.F. Lv, Q.N. Guo, Advances in osteosarcoma stem cell research and opportunities for novel therapeutic targets, Cancer Lett. 370 (2016) 268–274.
[5] J.R. Mortus, Y. Zhang, D.P. Hughes, Developmental pathways hijacked by

osteosarcoma, Adv. Exp. Med. Biol. 804 (2014) 93–118.
[6] C. Wang, J. Jing, L. Cheng, Emerging roles of non-coding RNAs in the pathogenesis, diagnosis and prognosis of osteosarcoma, Invest. New Drugs 36 (2018) 1116–1132.
[7] T. Lu, Y. Wang, D. Chen, J. Liu, W. Jiao, Potential clinical application of lncRNAs in non-small cell lung cancer, Onco. Targets Ther. 11 (2018) 8045–8052.
[8] Y. Gao, H. Meng, S. Liu, J. Hu, Y. Zhang, T. Jiao, Y. Liu, J. Ou, D. Wang, L. Yao, S. Liu, N. Hui, LncRNA-HOST2 regulates cell biological behaviors in epithelial ovarian cancer through a mechanism involving microRNA let-7b, Hum. Mol. Genet. 24 (2015) 841–852.
[9] R. Tang, J.C. Wu, L.M. Zheng, Z.R. Li, K.L. Zhou, Z.S. Zhang, D.F. Xu, C. Chen, Long noncoding RNA RUSC1-AS-N indicates poor prognosis and increases cell viability in hepatocellular carcinoma, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 388–396.
[10] D. Fei, X. Zhang, J. Liu, L. Tan, J. Xing, D. Zhao, Y. Zhang, Long noncoding RNA FER1L4 suppresses tumorigenesis by regulating the expression of PTEN targeting miR-18a-5p in Osteosarcoma, Cell. Physiol. Biochem. 51 (2018) 1364–1375.
[11] T. Xia, S. Chen, Z. Jiang, Y. Shao, X. Jiang, P. Li, B. Xiao, J. Guo, Long noncoding RNA FER1L4 suppresses cancer cell growth by acting as a competing endogenous RNA and regulating PTEN expression, Sci. Rep. 5 (2015) 13445.
[12] S. Rastogi, A. Aggarwal, A. Tiwari, V. Sharma, Chemotherapy in nonmetastatic osteosarcoma: recent advances and implications for developing countries, J. Glob. Oncol. (2018) 1–5.
[13] C. Adamopoulos, A.N. Gargalionis, C. Piperi, A.G. Papavassiliou, Recent advances in Mechanobiology of Osteosarcoma, J. Cell. Biochem. 118 (2017) 232–236.
[14] E. Malek, S. Jagannathan, J.J. Driscoll, Correlation of long non-coding RNA expression with metastasis, drug resistance and clinical outcome in cancer, Oncotarget 5 (2014) 8027–8038.
[15] R. Deng, J. Zhang, J. Chen, lncRNA SNHG1 negatively regulates miRNA1013p to enhance the expression of ROCK1 and promote cell proliferation, migration and invasion in osteosarcoma, Int. J. Mol. Med. (2018).
[16] J.C. de Oliveira, L.C. Oliveira, C. Mathias, G.A. Pedroso, D.S. Lemos, A. Salviano-Silva, T.S. Jucoski, S.C. Lobo-Alves, E.P. Zambalde, G.A. Cipolla, D.F. Gradia, LncRNAs in Cancer: another layer of complexity, J. Gene Med. (2018) e3065.
[17] Y. Lu, L. Tang, Z. Zhang, S. Li, S. Liang, L. Ji, B. Yang, Y. Liu, W. Wei, Long non-coding RNA TUG1/miR-29c Axis Affects cell proliferation, invasion, and migration in human pancreatic cancer, Dis. Markers 2018 (2018) 6857042.
[18] M. Wang, Y. Li, Y. Yang, X. Liu, M. Zang, Y. Li, K. Yang, W. Yang, S. Zhang, Long noncoding RNA DLX6AS1 is associated with malignant progression and promotes proliferation and invasion in esophageal squamous cell carcinoma, Mol. Med. Rep. (2018).
[19] D. Pang, Q. Hu, X. Lan, Y. Lin, H. Duan, S. Cao, Y. Lin, L. Li, F. Peng, F. Pan, The novel long noncoding RNA PRNCR12 is involved in breast cancer cell proliferation, migration, invasion and cell cycle progression, Mol. Med. Rep. (2018).
[20] Y. Kong, Z. Ren, Overexpression of LncRNA FER1L4 in endometrial carcinoma is associated with favorable survival outcome, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 8113–8118.
[21] S. Liu, B. Zou, T. Tian, X. Luo, B. Mao, X. Zhang, H. Lei, Overexpression of the lncRNA FER1L4 inhibits paclitaxel tolerance of ovarian cancer cells via the regulation of the MAPK signaling pathway, J. Cell. Biochem. (2018).
[22] X. Wang, K. Dong, Q. Jin, Y. Ma, S. Yin, S. Wang, Upregulation of lncRNA FER1L4 suppresses the proliferation and migration of the hepatocellular carcinoma via regulating PI3K/AKT signal pathway, J. Cell. Biochem. (2018).
[23] W. Ma, C.Q. Zhang, H.L. Li, J. Gu, G.Y. Miao, H.Y. Cai, J.K. Wang, L.J. Zhang, Y.M. Song, Y.H. Tian, Y.H. Song, LncRNA FER1L4 suppressed cancer cell growth and invasion in esophageal squamous cell carcinoma, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 2638–2645.
[24] Z.X. Chen, C.P. Chen, N. Zhang, T.X. Wang, Low-expression of lncRNA FER1L4 might be a prognostic marker in osteosarcoma, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 2310–2314.
[25] P.T. Hawkins, L.R. Stephens, PI3K signalling in inflammation, Biochim. Biophys. Acta 1851 (2015) 882–897.
[26] H. Pei, L. Chen, Q.M. Liao, K.J. Wang, S.G. Chen, Z.J. Liu, Z.C. Zhang, SUMO-specific protease 2 (SENP2) functions as a tumor suppressor in osteosarcoma via SOX9 degradation, Exp. Ther. Med. 16 (2018) 5359–5365.
[27] G.N. Yan, X.F. Tang, X.C. Zhang, T. He, Y.S. Huang, X. Zhang, G. Meng, D.Y. Guo, Y.F. Lv, Q.N. Guo, TSSC3 represses self-renewal of osteosarcoma stem cells and Nanog expression by inhibiting the Src/Akt pathway, Oncotarget 8 (2017) 85628–85641.
[28] D.I. Paiva-Oliveira, S.R. Martins-Neves, A.J. Abrunhosa, C. Fontes-Ribeiro, C.M.F. Gomes, Therapeutic potential of the metabolic modulator Metformin on osteosarcoma cancer stem-like cells, Cancer Chemother. Pharmacol. 81 (2018) 49–63.
[29] Z. Xiao, J. Wan, A.A. Nur, P. Dou, H. Mankin, T. Liu, Z. Ouyang, Targeting CD44 by CRISPR-Cas9 in multi-drug resistant osteosarcoma cells, Cell. Physiol. Biochem. 51 (2018) 1879–1893.
[30] J. Zhou, S. Wu, Y. Chen, J. Zhao, K. Zhang, J. Wang, S. Chen, microRNA-143 is associated with the survival of ALDH1 + CD133 + osteosarcoma cells and the chemoresistance of osteosarcoma, Exp. Biol. Med. (Maywood) 240 (2015) 867–875.
[31] M. Kretz, Z. Siprashvili, C. Chu, D.E. Webster, A. Zehnder, K. Qu, C.S. Lee, R.J. Flockhart, A.F. Groff, J. Chow, D. Johnston, G.E. Kim, R.C. Spitale, R.A. Flynn, G.X. Zheng, S. Aiyer, A. Raj, J.L. Rinn, H.Y. Chang, P.A. Khavari, Control of somatic tissue differentiation by the long non-coding RNA TINCR, Nature 493 (2013) 231–235.
[32] T. Kashyap, K.K. Pramanik, N. Nath, P. Mishra, A.K. Singh, S. Nagini, A. Rana, R. Mishra, Crosstalk between Raf-MEK-ERK and PI3K-Akt-GSK3beta signaling networks promotes chemoresistance, invasion/migration and stemness via expression of CD44 variants (v4 and v6) in oral cancer, Oral Oncol. 86 (2018) 234–243.
[33] Y.A. Chen, D.T.W. Tzeng, Y.P. Huang, C.J. Lin, U.G. Lo, C.L. Wu, H. Lin, J.T. Hsieh, C.H. Tang, C.H. Lai, Antrocin sensitizes prostate cancer cells to radiotherapy through inhibiting PI3K/AKT and MAPK signaling pathways, Cancers (Basel) (2018) 11.
[34] H. Chen, D. Zhu, Z. Zheng, Y. Cai, Z. Chen, W. Xie, CEP55 promotes epithelial-mesenchymal transition in renal cell carcinoma through PI3K/AKT/mTOR pathway, Clin. Transl. Oncol. (2019).
[35] L. Liu, Y. Huang, X. Feng, J. Chen, Y. Duan, Overexpressed Hsp70 alleviated formaldehyde-induced apoptosis partly via PI3K/Akt signaling pathway in human bronchial epithelial cells, Environ. Toxicol. (2019).