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Long non-coding RNA Fer-1-like family member 4 suppresses hepatocellular carcinoma cell proliferation by regulating PTEN *in vitro* and *in vivo*

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Abstract. The aim of the present study was to investigate the potential role of long non-coding RNA Fer-1-like family member 4 (*FER1L4*) in the proliferation of hepatocellular carcinoma (HCC) through the regulation of phosphatase and tensin homolog (PTEN) expression. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used to detect the expression levels of *FER1L4* and *PTEN* mRNA in HCC tissues, and western blotting was performed to measure the protein expression level of PTEN; MTT and colony formation assays were performed to detect the cell proliferative ability. Furthermore, nude mice were injected with transfected HCC cells and the tumor volume and weight were measured. The results indicated that *FER1L4* was expressed at a low level in human HCC tissues compared with adjacent normal tissues. Functional studies indicated that *FER1L4* may inhibit the proliferative ability of HCC cells. In addition, *PTEN* was highly expressed in HCC tissues compared with normal adjacent tissues and was positively associated with *FER1L4*. In addition, it was demonstrated that *FER1L4* inhibited the proliferative ability of HCC cells *in vitro*, and silencing *FER1L4* expression by small interfering RNAs promoted the growth of HCC tumors *in vivo*. Therefore, *FER1L4* may be a potent therapeutic target for HCC.

Introduction

Hepatocellular carcinoma (HCC) is a common type of cancer, and its incidence is increasing rapidly, causing ~745,000 mortalities in 2012, worldwide (1-4). As a result of the low detection rate, a number of HCC patients are at advanced stages of the disease at the time of diagnosis with limited sensitivity biomarkers (5). Long non-coding RNAs (lncRNAs) are a novel class of non-coding RNAs (ncRNAs) that contribute to the development and progression of cancer and that may serve as novel biomarkers (6). However, the molecular and functional mechanisms of lncRNAs in HCC remain unknown. Therefore, studies on the role of lncRNA in HCC are urgent. LncRNAs are >200 nucleotides in length, and previous studies have demonstrated that lncRNAs exert important effects on diverse cellular functions through gene expression regulation, including chromatin modification, transcription regulation and genomic imprinting (7,8). A number of previous studies have demonstrated that lncRNAs participate in tumor development, including cell cycle (9), differentiation (10), apoptosis (11,12), migration and invasion (13). Therefore, lncRNAs may be important therapeutic targets for diseases.

Fer-1-like family member 4 (*FER1L4*) is a tumor-associated lncRNA involved in the development of tumors, and it has been demonstrated that *FER1L4* expression is downregulated in patients with gastric cancer (14,15). It was reported that *FER1L4* inhibits proliferation, migration and invasion of gastrointestinal cancer (16,17). A recent study also revealed that *FER1L4* inhibits proliferation and the cell cycle through phosphatase and tensin homolog (*PTEN*) in endometrial carcinoma (18). Therefore, the interactions between lncRNAs and protein-coding genes are popular topics in cancer biology that provide an important theoretical basis for the diagnosis and treatment of cancers. In the present study, the biological mechanism of *FER1L4* for proliferation in regulating *PTEN* were demonstrated in HCC.

The tumor suppressor gene *PTEN* is located at chromosome 10q23.31 and is frequently inactivated in cancer (19-22). A recent study also demonstrated that PTEN is closely linked to HCC tumorigenesis (23). As previously noted, downregulated FER1L4 in endometrial carcinoma (EC) tissues was positively correlated with decreased PTEN expression by using RT-qPCR assay; FER1L4 inhibited cell proliferation, promoted cell cycle arrest at G_0/G_1 phase and cell apoptosis by upregulating PTEN expression with MTT, colony-formation and flow cytometry detection (18). Based on the previous study that *FER1L4* serves a potential role in regulating *PTEN* expression (18), it was hypothesized that *FER1L4* may also function as a *PTEN* regulator in HCC.

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Key words: long non-coding RNA Fer-1-like family member 4, phosphatase and tensin homolog, hepatocellular carcinoma, proliferation

Materials and methods

Clinical specimens. HCC cancer tissues and adjacent normal tissues (a distance of at least 5 cm from the tumor) were collected from 35 patients with HCC at the Center Hospital of Cangzhou (Cangzhou, China) between March 2014 and October 2015. These HCC cases without other clinicopathological characteristics included 19 male patients and 16 female patients with a mean age of 67.2 years (age, 36-84 years). All patients had not received preoperative treatment, such as radiotherapy or chemotherapy. The inclusion/exclusion criteria of HCC patients were as follows: i) All enrolled patients were diagnosed with HCC, ii) VEGF evaluation, iii) correlation of VEGF with OS or DFS, iv) all patients were carefully evaluated to identify duplicate patient populations, and v) criteria used to judge duplicate populations, such as treatment information, study period and admission hospital. The present study was approved by the ethics committee of the Center Hospital of Cangzhou, and written informed consent was obtained from all patients prior to enrolment in the study. Histological diagnosis of HCC was evaluated according to the World Health Organization criteria. All collected tissues were immediately stored at -80˚C until use.

Cell culture. The normal hepatocyte LO2 cell line was purchased from American Type Culture Collection (Manassas, VA, USA). The HCC cell lines Hep3B and Huh7, and 293T cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). LO2, Hep3B, Huh7 and 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM; High glucose; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma Aldrich; Merck KGaA, Darmstadt, Germany) and 100 U/ml penicillin/streptomycin (Invitrogen, Thermo Fisher Scientific, Inc.). All cells were cultured in a 37˚C, 5% CO_2 cell culture incubator.

Lentiviral vector construction, production and transfection. To obtain an *FER1L4* expression vector, a full-length *FER1L4* DNA fragment was amplified by polymerase chain reaction (PCR) using the Takara *Ex Taq* Hot Start Version kit (Takara Bio, Inc., Otsu, Japan). The PCR reaction is presented in Table I and the reaction steps were as follows: 94˚C for 5 min; 94˚C for 45 sec, 60˚C for 45 sec, 72˚C for 1 min, for a total of 30 cycles and 72˚C for 10 min.

The primer sequences of *FER1L4* were: Forward, 5'-GATTCAGGTGGGCGGGCTGGTG-3' and reverse, 5'-TCAGTGGCTGTGATAGGTTTA-3'. The PCR products were inserted into the mammalian expression vector pCDNA3.1 (Invitrogen; Thermo Fisher Scientific, Inc.). A lentiviral vector expressing Enhanced Green Fluorescent Protein (enhanced green fluorescent protein; EGFP, Gene Bank Accession, no. U57607) was used as an empty vector (pCDNA3.1-expressing EGFP) was selected as a control. 293T cells ($1x10^6$ cells/well) were seeded in 10 cm plates and co-transfected with lentiviral packaging vectors [pMD2.G (Addgene; cat. no. 12259), pMDL-G/P-RRE (Addgene; cat. no. 12251) and pRSV-REV (Addgene; cat. no. 12253)] and either the constructed pCDNA3.1-FER1L4 overexpression vector or the Control vector for 48 h. Lentiviral particles were harvested, purified and transfected into cells ($1x10^4$ cells/well) with 8 μg/ml polybrene (Sigma-Aldrich; Merck KGaA) in 24-well plates. Finally, the cells with stable expression were screened using 800 μg/ml G418 (Sigma-Aldrich; Merck KGaA).

Small interfering (si)RNA transfection. siRNA sequences were designed to target the human *FER1L4* gene and siRNAs were purchased from GenePharma (Shanghai GenePharma Co., Ltd., Shanghai, China). The sequences of siRNA-*FER1L4* were 5'-CAGGACAGCUUCGAGUUAATT-3' (sense) and 5'-UUAACUCGAAGCUGUCCUGTT-3' (antisense). The sequences of the negative control (NC) siRNA were 5'-UUCUCCGAACGUGUCACGU-3' (sense), 5'-ACGUGACACGUUCGGAGAA-3' (antisense). Cells ($2x10^5$ cells/well) were seeded in 6-well plates and transfected with FER1L4 siRNAs (50 nM) or NC siRNAs (50 nM) for 48 h by using Lipofectamine® 3000 (Invitrogen; Thermo Fisher scientific, Inc.) according to the manufacturer's protocol.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from 35 HCC tissues or treated cells ($1x10^6$ cells) by using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was reverse transcribed to cDNA using a cDNA synthesis kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. RT-qPCR was performed using the standard SYBR Green PCR Master Mix kit (Takara Bio, Inc.) on an ABI 7500 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. The reaction steps were as follows: Initial denaturation at 95˚C for 30 sec; followed by 40 cycles of 95˚C for 5 sec and 60˚C for 34 sec. Relative expression was determined based on the $2^{-\Delta\Delta Cq}$ method (24). The following primer sequences were used: *FER1L4*, forward 5'-CCGTGTTGGGTGCTGTTC-3', reverse 5'-GGCAAGTCCACTGTCAGATG-3'; *PTEN*, forward 5'-GTTTACCGGCAGCATCAAAT-3', reverse 5'-CCCCCACTTTAGTGCACAGT-3'; *GAPDH*, forward 5'-CCTCGTCTCATAGACAAGATGGT-3', reverse 5'-GGGTAGAGTCATACTGGAACATG-3'; *GAPDH* was used as an internal control.

Western blot assay. Protein expression levels of PTEN were measured by western blot assay. Briefly, the treated cells ($1x10^6$ cells) were lysed in Radioimmunoprecipitation Assay buffer (Beyotime Institute of Biotechnology, Shanghai, China) containing a protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). Protein concentrations were measured using a Bicinchoninic Acid Protein Assay kit (Thermo Fisher Scientific, Inc.). Equal concentrations of protein (30 μg) were separated by 10% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA). All the membranes were blocked with 5% skim milk (Shanghai solarbio Bioscience & Technology Co., LTD, Shanghai, China.) for 1.5 h at room temperature and incubated overnight at 4˚C with primary antibodies against PTEN (1:500; Abcam, Cambridge, UK, cat. no. ab31392) and beta-actin (1:3,000; Sigma-Aldrich; Merck KGaA; cat. no. A2066)). Subsequently, membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse (1:5,000; cat. no. CW102) and goat anti-rabbit (1:5,000; cat. no. CW103)

Table I. Reaction components used in the RT-qPCR.

Reagent	Consumption
Premix Ex Taq Hot Start Version	25.0 μl
PCR Forward Primer	1.0 μM
PCR Reverse Primer	1.0 μM
Template	0.2 μl
ddH_2O	up to 50 μl

secondary antibody for 1 h at room temperature. The results were analyzed using an Enhanced Chemiluminescence Detection kit (Amersham Biosciences Inc., Piscataway, NJ, USA) an enhanced chemiluminescence system (Amersham Biosciences Inc.). The results were analyzed by using Image-Pro Plus 6.0 software (National Institutes of Health, Bethesda, MD, USA).

MTT assay. The MTT assay was used to measure the cell viability by using MTT reagent (Cat. number M2128) according to the manufacturer's protocol. Briefly, the treated cells (4,000 cells/well) were seeded in 96-well plates and incubated at 37˚C for 0, 12, 24 and 72 h. Subsequently, 20 μl MTT (5 mg/ml) solution was added to each well at the prescribed time and the cells were incubated for an additional 4 h at 37˚C. Dimethyl sulfoxide was used to dissolve the formazan crystals and the absorbance was detected at 570 nm under the micro-plate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Colony-formation assay. A colony formation assay was also performed to measure the cell viability. Transfected cells ($5x10^3$ cells/plate) were seeded onto 6-cm culture dishes and were maintained in the DMEM (High glucose; Invitrogen; Thermo Fisher Scientific, Inc.) including 10% FBS for 2 weeks; the medium was replaced every 3 days. Colonies were stained with Coomassie blue (cat: B-2025, Sigma-Aldrich; Merck KGaA) at 37˚C for 1 h, and colony formation was calculated under the stereomicroscope by counting the number of stained colonies.

Tumor formation in nude mice. Prior to the beginning of the experiments, specific criteria for humane endpoints were established as previously described, including labored breathing, reduced food or drinking water intake, inability to stay upright, unresponsive or unconscious to external stimuli and shaggy coat (25,26); animal reactions were confirmed by an animal specialist. If mice presented one or more of the above reactions, the animals were assumed to have reached the humane endpoint. When mice met humane endpoints, they were anesthetized with sodium pentobarbital (50 mg/kg) by intraperitoneal injection and sacrificed by cervical dislocation immediately.

A total of 14 Female athymic BALB/c mice (age, 4-6 weeks; weight, 20-22 g) were obtained from Yangzhou University Medical Centre (Yangzhou, China). Specific pathogen free (SPF) were provided by the Cangzhou Central Hospital Animal Center, and they were housed in a controlled environment (food and water available ad libitum, 21±1˚C, humidity 60%, lights on from 7:00 a.m.-7:00 p.m.). The study was approved by the ethics committee of the Cancer Institute. All the animals were treated humanely in accordance with the guidelines laid down by the Institutional Animal Ethics Committee. siRNA-*FER1L4*- or NC-siRNA-transfected cells ($1x10^7$ cells in 100 μl) were injected into nude mice. Tumor volume and weights were measured and calculated at 0, 5, 10, 15, 20, 25, and 30 days; tumor volume was calculated as the volume of a sphere: (4/3) x (π) x (r^3), where radius (r) =½ diameter.

Statistical analysis. Significant differences between two groups were analyzed by Student's t-test. FER1L4 expression was analyzed by using one-way analysis of variance followed by a Dunnett post-hoc test in HCC cell lines. The correlation between *FER1L4* and *PTEN* mRNA expression was analyzed by Pearson's correlation coefficient. All data are presented as the mean ± standard deviation, and analyzed by using IBM SPSS Statistics version 21 (IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference. All experiments were performed in triplicate.

Results

FER1L4 is expressed at low levels in human HCC. *FER1L4* mRNA expression levels were examined in 35 pairs of HCC and paired adjacent normal tissues by RT-qPCR. The results indicated that *FER1L4* expression was decreased in HCC tissues (n=34/35) compared with the adjacent non-cancerous tissues (Fig. 1A). Similarly, *FER1L4* mRNA expression levels were demonstrated to be significantly lower in Hep3B and Huh7 HCC cell lines compared with expression in LO2 normal hepatocyte cells (P<0.001; Fig. 1B). These results suggested that *FER1L4* may function as a tumor suppressor in HCC.

FER1L4 inhibits the proliferative ability of HCC cells. To further identify the effects of *FER1L4* on the proliferative ability of HCC cells, Hep3B cells were transfected with lentiviral *FER1L4* overexpression vectors or siRNA-*FER1L4*. RT-qPCR was used to validate the efficiency of lentiviral vector and siRNA transfections. The results demonstrated that Hep3B cells transfected with *FER1L4* overexpression vectors expressed significantly higher levels of *FER1L4* mRNA, and cells transfected with siRNA-*FER1L4* expressed a significantly lower level of *FER1L4*, compared with the respective empty vector-transfected or siRNA-NC-transfected control cells (P<0.001; Fig. 2A). Results from MTT assays indicated that *FER1L4* overexpression significantly inhibited the proliferative ability of Hep3B cells compared with the empty vector-transfected control cells (P<0.001; Fig. 2B). However, the silencing of *FER1L4* expression by siRNAs significantly promoted the proliferative ability of Hep3B cells compared with the siRNA-NC-transfected cells (P<0.05; Fig. 2C). In addition, a colony-forming assay was performed to examine the effects of *FER1L4* on the long-term proliferative ability of Hep3B cells. It was demonstrated that *FER1L4* over expression significantly suppressed the long-term proliferative abilities of Hep3B cells compared with control cells (P<0.001; Fig. 2D), whereas reduced *FER1L4* expression significantly increased

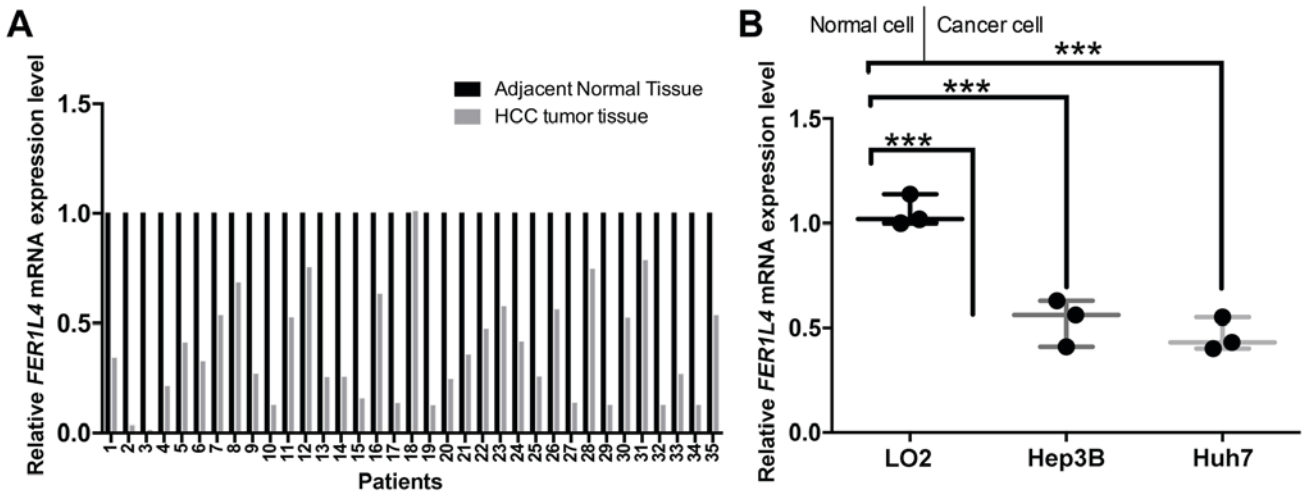

Figure 1. *FER1L4* is expressed at a low level in human HCC tissues and cell lines. (A) RT-qPCR was performed to analyze the mRNA expression levels of *FER1L4* in 35 pairs of HCC and paired adjacent normal tissues. (B) RT-qPCR was used to compare the mRNA expression levels of *FER1L4* in HCC cell lines (Hep3B and Huh7) with expression in the normal hepatocyte LO2 cell line. ***P<0.001 vs. LO2 cells. *FER1L4*, Fer-1-like family member 4; HCC, hepatocellular carcinoma; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

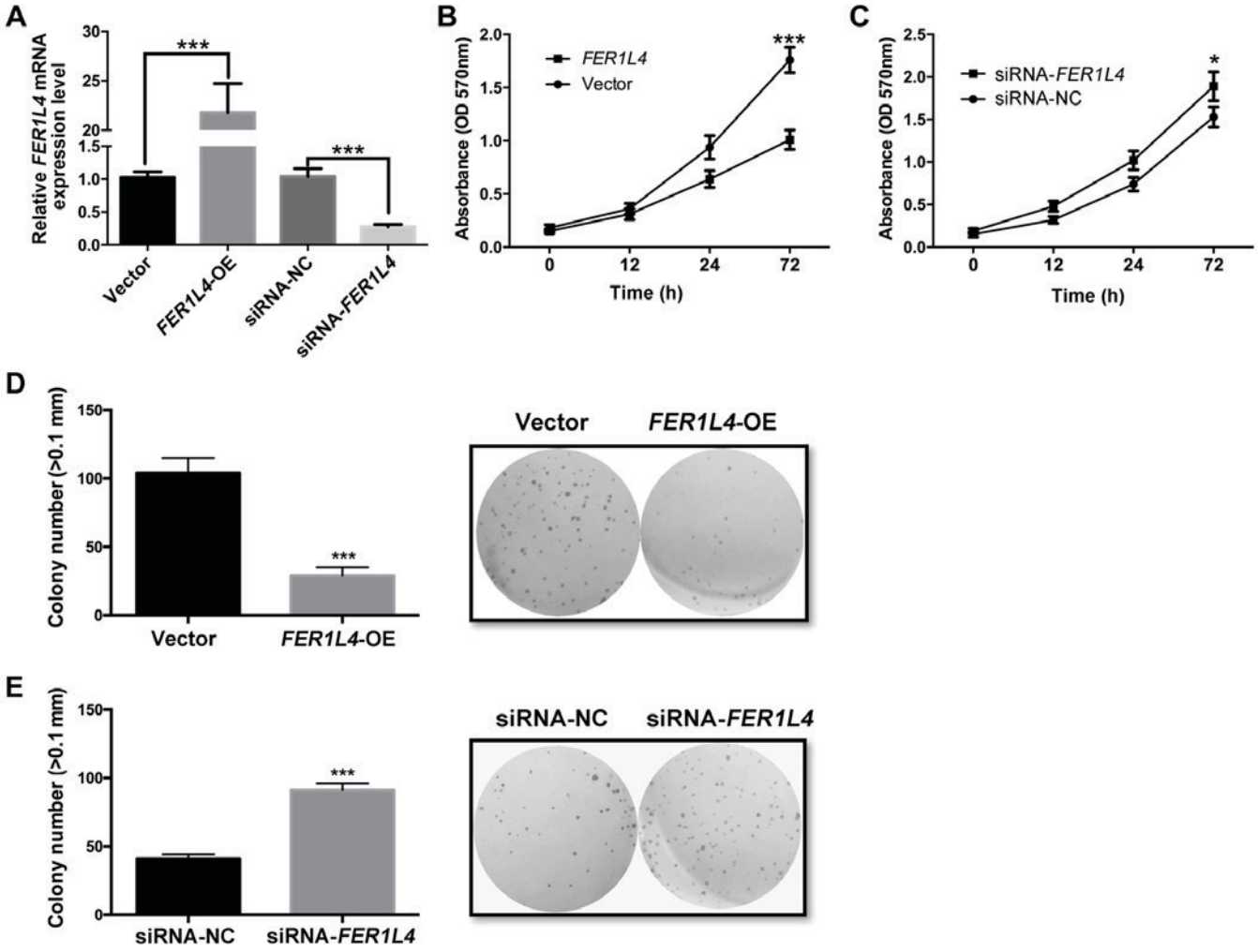

Figure 2. *FER1L4* inhibits the proliferative ability of HCC cells. Hep3B HCC cells were transfected with *FER1L4*-OE or with siRNA-*FER1L4*. (A) Reverse transcription-quantitative polymerase chain reaction was used to measure the mRNA expression level of *FER1L4* in the treated Hep3B cells; ***P<0.001. (B) The proliferative ability was detected by MTT assay in Hep3B cells transfected with *FER1L4*-OE or with empty vector control; ***P<0.001. (C) The MTT assay was performed to measure the proliferation ability of Hep3B cells transfected with siRNA-*FER1L4* or siRNA-NC at 0, 12, 24, 72 h, respectively; *P<0.05. (D and E) Clonal colony-forming experiments were performed to measure the proliferative ability of Hep3B cells that were transfected with (D) *FER1L4*-OE or the empty vector control or (E) siRNA-*FER1L4* or siRNA-NC magnification, x100; ***P<0.001. *FER1L4*, Fer-1-like family member 4; NC, negative control; OD, optical density; OE, overexpression; siRNA, small interfering RNA.

Table II. Volume and the longest diameter of subcutaneous tumors in nude mice injected with Hep3B cells transfected with siRNA-*FER1L4* or siRNA-NC.

	siRNA-NC			siRNA-*FER1L4*		
Day	Tumors (n)	Volume (mm^3)	Longest diameter (mm)	Tumors (n)	Volume (mm^3)	Longest diameter (mm)
0	2	32.12	1.97	1	42.12	4.32
5	1	64.28	4.97	1	108.30	5.91
10	1	128.32	6.26	2	211.10	3.70
15	2	246.32	3.89	1	444.60	9.47
20	1	503.20	9.87	1	875.32	11.87
25	1	785.40	11.45	2	1,423.40	6.98
30	3	1,132.40	4.31	2	2,552.40	8.48

FER1L4, Fer-1-like family member 4; NC, negative control; siRNA, small interfering RNA.

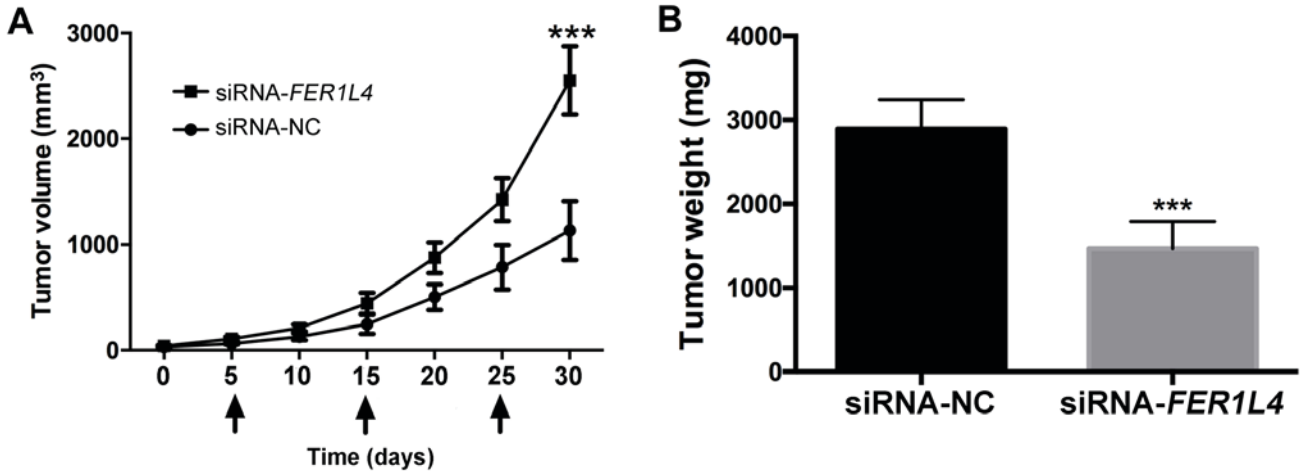

Figure 3. Reduced *FER1L4* expression promotes the growth of HCC tumors in nude mice *in vivo*. (A) Tumor volume was detected at 0, 5, 10, 15, 20, 25 and 30 days. Arrow indicates that nude mice were injected with Hep3B cells that were transfected with siRNA-*FER1L4* or siRNA-NC at 5, 15 and 25 days. ***P<0.001. (B) When animals met humane endpoints, they were sacrificed and tumor weights were measured; ***P<0.001. *FER1L4*, Fer-1-like family member 4; HCC, hepatocellular carcinoma; NC, negative control; siRNA, small interfering RNA.

the long-term proliferative abilities of Hep3B cells compared with control cells (P<0.001; Fig. 2E).

Reduced FER1L4 expression promotes the growth of HCC tumors in vivo. Based on the higher efficiency of *FER1L4* siRNAs compared with the *FER1L4* overexpression vector (data not shown), *FER1L4* was chosen to be silenced by siRNAs for the *in vivo* study. Transfected Hep3B cells were implanted subcutaneously into nude mice. The tumor volume was calculated at 0, 5, 10, 15, 20, 25 and 30 days; tumor volumes and longest diameters are presented in Table II (the values correspond to the tumors at the end of the experiment). The results indicated that the silencing of *FER1L4* expression led to a significant increase in tumor volume compared with siRNA-NC (P<0.001; Fig. 3A). Tumor weight was also calculated at 30 days. The results demonstrated that the tumor weights were 2,893±345 and 1,468±321 mg in nude mice implanted with Hep3B cells transfected with siRNA-*FER1L4* and siRNA-NC at 30 days, respectively; these data revealed that the silencing of *FER1L4* significantly decreased the tumor weight (P<0.001; Fig. 3B).

FER1L4 positively regulates PTEN expression in HCC. To investigate the interaction between *FER1L4* and PTEN, RT-qPCR and western blot assays were performed. *FER1L4* overexpression significantly upregulated the mRNA expression level of PTEN and the silencing of *FER1L4* by siRNAs significantly downregulated the mRNA expression level of *PTEN* in transfected Hep3B cells, compared with the respective controls (both P<0.001; Fig. 4A). *FER1L4* overexpression significantly increased PTEN protein expression whereas the silencing of *FER1L4* by siRNAs significantly decreased PTEN protein expression in transfected Hep3B cells compared with the respective controls (both P<0.001; Fig. 4B). In addition, the mRNA expression level of *PTEN* was measured in HCC tissues with either high *FER1L4* (n=21) or low *FER1L4* (n=14) expression levels (Cut off=0.435) by using SigmaPlot 10.0 (SigmaPlot Software, La Jolla, CA, USA). The results demonstrated that high *PTEN* mRNA expression may be associated with high

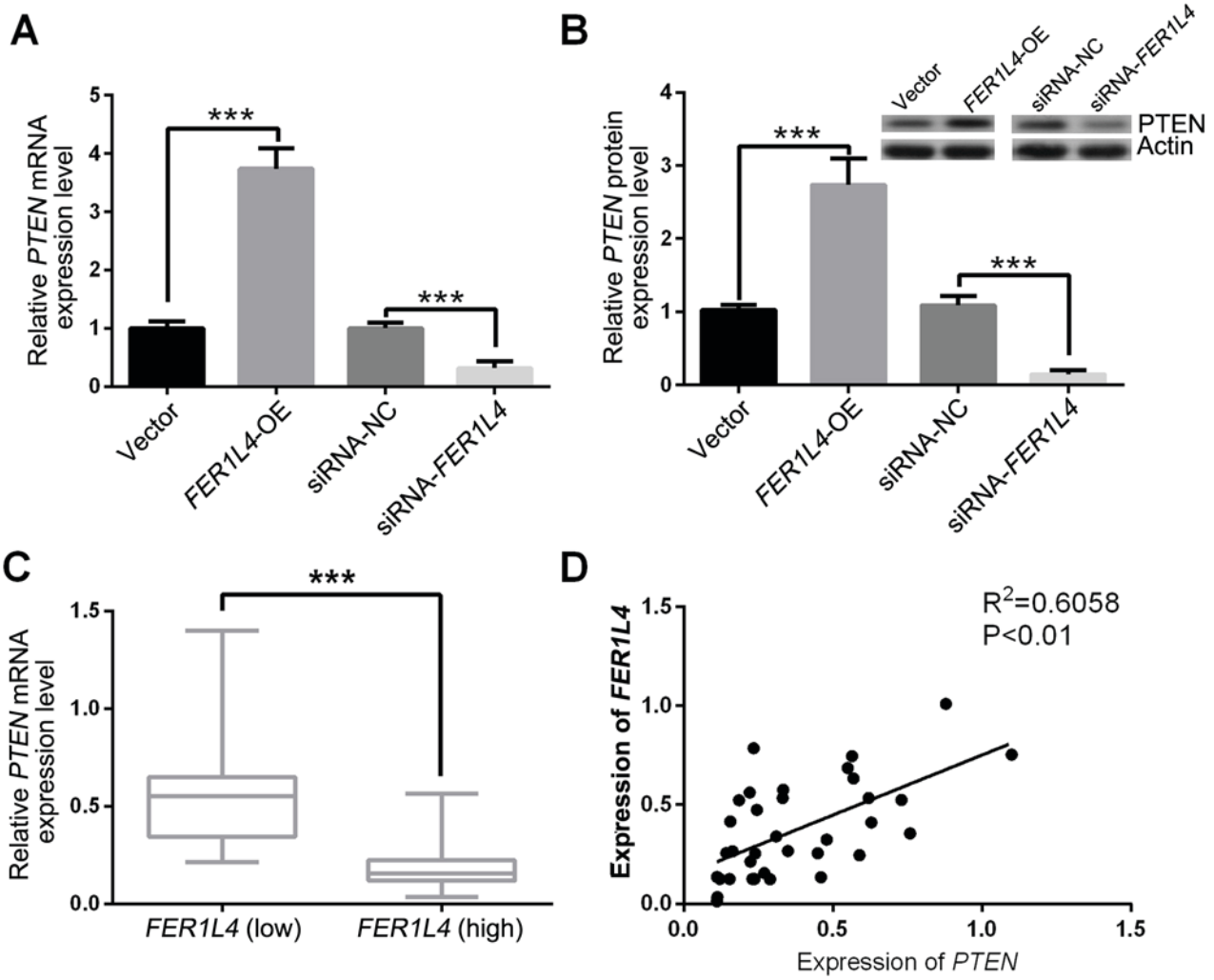

Figure 4. FER1L4 positively regulates PTEN expression in HCC. (A) The mRNA expression level of PTEN was measured by RT-qPCR in Hep3B cells that were transfected with lentiviral vectors expressing FER1L4 or control vector and FER1L4 siRNA or NC siRNA. ***P<0.001. (B) Western blotting detected the protein expression level of PTEN in Hep3B cells that were treated as in A; beta-actin was used as a protein-loading control. ***P<0.001. (C) RT-qPCR assay was used to detect PTEN expression in HCC tissues with high FER1L4 expression and low FER1L4 expression. The transcript level was normalized to GAPDH expression (n=35, ***P<0.001). (D) The correlation between FER1L4 and PTEN mRNA expression was analyzed by Pearson's correlation coefficient (R^2=0.6058; P<0.01). FER1L4, Fer-1-like family member 4; PTEN, phosphatase and tensin homolog; NC, negative control; siRNA, small interfering RNA; HCC, hepatocellular carcinoma.

FER1L4 expression in clinical HCC tissues (P<0.001; Fig. 4C) and the expressions were positively correlated (R^2=0.6058; P<0.01; Fig. 4D).

Discussion

Previous studies have demonstrated that a number of ncRNAs serve important roles in human diseases by activating or repressing genes (24,27,28). Additional studies have demonstrated that the dysregulated expression or the dysfunction of specific ncRNAs might drive tumorigenesis (29). There are three forms of ncRNAs that are especially important for the regulation of gene expression: MicroRNAs (miRNAs), lncRNAs and circular RNAs (30), of which miRNAs have gained much attention and have been considered to serve important roles in cancer through the transcriptional regulation of specific target mRNAs (31). lncRNAs are one of the emerging fields in ncRNA research that have been reported to participate in various biological processes, including transcription, mRNA splicing, RNA decay and translation (32-34). Previous studies indicated that lncRNAs may be associated with the development and progression of human cancer. For example, one study demonstrated that *FER1L4* serves as a potential biomarker of gastric cancer with lymph node invasion (35). *FER1L4* was reported to inhibit proliferation in endometrial carcinoma (18) and *FER1L4* was expressed at a low level in gastric cancer patients (15). Another study demonstrated that *FER1L4* inhibits cancer cell growth by regulating *PTEN* expression (17). In the preset study, *FER1L4* was revealed to be expressed at a low level in human HCC tissues. *FER1L4* was demonstrated to inhibit the proliferative ability of HCC cells *in vitro*, and silencing of *FER1L4* promoted the proliferative ability of HCC *in vivo*. Therefore, the results of the present study strongly supported the potential tumor suppressor role of *FER1L4* in cancer. Additionally, it may serve as a target for HCC therapy.

A number of previous studies have demonstrated that PTEN serves crucial roles in the progression of tumors, including cell

proliferation, differentiation, migration and apoptosis (36-38). Additional studies suggested that *PTEN* is associated with miRNA (miR)-21 and affects the prognosis of colorectal cancer (39), whereas PTEN may inhibit the epithelial-mesenchymal transition and invasive ability of tongue cancer cells (40). In addition, low expression of *PTEN* is also considered to be a potential biomarker for resistance to human epidermal growth factor receptor 2-targeted therapy in advanced gastric cancer (41). Recent work has demonstrated that there is connection between lncRNA and PTEN by the lncRNA-GAS5/PTEN/miR-103 axis in endometrial cancer cells (42). In addition, a previous study reported that the *FER1L4*/PTEN axis has significant effect in regulating the function of endometrial carcinoma (18). In the present study, it was demonstrated that *PTEN* was expressed at high levels in HCC tissues with high *FER1L4* expression. Additionally, both overexpression and knockdown of *FER1L4* may be able to regulate *PTEN* expression in HCC. Therefore, the results of the present study indicated that *FER1L4* may also act as a *PTEN* regulator in HCC, inhibiting the proliferative ability of HCC cells.

In conclusion, the present study results may be summarized as follows: i) *FER1L4* was expressed at a low level in human HCC tissues; ii) *FER1L4* inhibited the proliferative ability of HCC cells; iii) *PTEN* was positively associated with *FER1L4* expression in HCC; and iv) *FER1L4* silencing by siRNAs promoted the growth of HCC tumors *in vivo*. Therefore, *FER1L4* may be a potential therapeutic target for HCC.

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Not applicable.

Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

XS and GQZ contributed to study design, the majority of the experiments and data collection. CYL and CDL contributed to data analysis and drafted the manuscript. GQZ and CYL were responsible for figure preparation, improving the manuscript and helping to conceive the study. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the ethics committee of the Center Hospital of Cangzhou, (Cangzhou, China). Written informed consent was obtained from all patients prior to enrolment in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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