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Long non-coding RNA FENDRR inhibits NSCLC cell growth and aggressiveness by sponging miR-761

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Abstract. – OBJECTIVE: The aim of this paper is to investigate the functions of long non-coding RNA (lncRNA) FOXF1 Adjacent Non-Coding Developmental Regulatory RNA (FENDRR) in the growth and aggressiveness of non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS: The expression of FENDRR in NSCLC tissues and cell lines was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and colony formation assays were conducted to explore the roles of FENDRR on the growth of NSCLC cell. The wound healing and transwell invasion assays were conducted to explore the impact of FENDRR on NSCLC cell migration and invasion. The apoptosis of NSCLC cell was detected using flow cytometer-based Annexin V/Propidium Iodide (PI) dual staining. The xenograft model was conducted to investigate the effect of FENDRR on the growth of NSCLC cell *in vivo*. The expression of Ki67 was measured by immunohistochemical (IHC) staining using Ki67 antibody. Bioinformatics analysis and Luciferase reporter assay were applied to identify that miR-761 was the target of FENDRR. Additional, colony formation and transwell experiments were utilized to confirm that FENDRR inhibited the growth and aggressiveness of NSCLC cell by regulating miR-761.

RESULTS: We found a marked down-regulation of FENDRR in NSCLC tissues compared to tumor-adjacent tissues. FENDRR down-expression was detected in four NSCLC cell lines (H1650, HCC827, H1975 and A549) compared to the human non-tumorigenic bronchial epithelial cell, BEAS-2B. Low expression of FENDRR was identified as a predictive factor for poor prognosis of patients with NSCLC. The over-regulation of FENDRR inhibited the proliferation, migration and invasion capacities of NSCLC cell and promoted the apoptosis of NSCLC cell *in vitro* whereas the down-regulation of FENDRR caused the opposite results. Moreover, the over-expression of FENDRR restrained the growth of NSCLC cell *in vivo*. We found that there were potential binding sites between FENDRR and miR-761 and the level of miR-761 was inversely associated with the expression of ENDRR in NSCLC tissues. Finally, the rescue experiments suggested that the anti-oncogenic role of FENDRR was at least partially mediated by miR-761 in NSCLC.

CONCLUSIONS: We found that FENDRR was down-expressed in NSCLC and the over-expression of FENDRR inhibited the malignant phenotypes of NSCLC cell by binding to miR-761 competitively.

Key Words:
FENDRR, MiR-761, NSCLC, Growth, Migration, Invasion.

Introduction

Non-small cell lung cancer (NSCLC), which accounts for nearly 85% of lung cancer cases, is one of the most common causes of cancer-associated death[1,2]. Hence, the exploration of effective options to increase the overall survival of patients is urgently needed. Currently, the therapeutic strategy of NSCLC includes chemotherapy and radiotherapy[3]. Although the great improvement in those therapies, the five-year overall survival (OS) rate still remains lower than 15%[4]. Recurrence, which is a common problem, is the main contributor to the poor clinical outcomes of patients[5]. Hence, it is urgent to explore underlying mechanisms of NSCLC progression. During the past years, intensive studies have identified a larger number of bio-markers for the characterization of NSCLC and clinical prognosis[6]. Recently, evidence suggested that long noncoding RNAs (lncRNAs) act as important modulators in the tumorigenesis and progression of NSCLC, and may become innovative treatment targets[7-9]. As a novel kind of controlling gene, lncRNAs exhibit

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limited or even no protein-coding ability[10,11]. Evidence demonstrates that lncRNAs have crucial functions in plenty of cellular processes, including cancer cell growth, epithelial-mesenchymal transition (EMT) and metastasis[12,13]. Studies reveal that lncRNAs are commonly dys-expressed in multiple cancer types. Few studies prove that lncRNAs mediate genes expression at either transcriptional level, post-transcriptional level or epigenetic level[14]. In addition, extensive lncRNAs act *via* competing with endogenous RNA (ceRNA) for miRNA targets, thereby preventing miRNAs from binding to their target genes[15]. FENDRR, which locates at chr3q13.31, consists of four exons and has a length of 3.099 nucleotides[16]. Xu et al[17] showed that the low level of FENDRR is associated with poor prognosis of patients with gastric cancer and the over-expression of FENDRR decreases the migration and invasion capacities of gastric carcinoma cell by down-regulating matrix metalloproteinase 2/9 (MMP2/MMP9) and fibronectin1 (FN1). In addition, FENDRR suppresses breast cancer cell growth, promotes cell apoptosis and is closely related to the advantageous prognosis in patients with breast carcinoma[18]. In prostate cancer, the expression of FENDRR is remarkably decreased in cancer tissues when compared to normal controls and the down-expression of FENDRR is associated with the adverse prognosis in patients[19]. The up-regulation of FENDRR inhibits the growth and decreases the aggressiveness ability of prostate cancer cell[19]. Nevertheless, the effects of FENDRR in NSCLC remain not yet investigated. In the current work, we revealed that FENDRR was significantly down-expressed in NSCLC. The over-expression of FENDRR suppressed the malignant phenotypes of NSCLC cell, including growth, migration and invasion whereas knocked-down FENDRR caused the completely opposite results. Furthermore, we demonstrated that the over-expression of FENDRR significantly increased the level of miR-761 in NSCLC cell. Bioinformatics analysis and Luciferase reporter assays showed that miR-761 bound to FENDRR in a sequence-specific manner. Altogether, these results suggested that lncRNA FENDRR inhibited the growth, migration and invasion of NSCLC cell by sponging miR-761.

Patients and Methods

Cell Lines and Clinical Tissues

Four NSCLC cell lines (H1650, HCC827, A549 and H1975) and human non-tumorigenic bronchial epithelial cell line BEAS-2B were purchased from the Guangzhou Jennio Biotech Co., Ltd., (Guangzhou, Guangdong, China). 293T cells were purchased from the Guangzhou Jennio Biotech Co., Ltd., (Guangzhou, Guangdong, China). Cancer cells were cultured in Roswell Park Memorial Institute-1640 medium (RPMI-1640; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 μg/ml streptomycin and 100 U/mL penicillin. 293T were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum, 100 μg/ml streptomycin and 100 U/mL penicillin. BEAS-2B cells were cultured in serum-free LHC-8 medium (Invitrogen, Carlsbad, CA, USA). All cells were maintained in an incubator with 95% air and 5% CO_2 at 37°C. The siRNA-FENDRR, pcDNA-FENDRR, miR-761 mimic and miR-761 inhibitor and their negative control were purchased from Ribobio Biotechnology Co. Ltd. (Guangzhou, Guangdong, China). Cell transfections were conducted using Lipofectamine 2000 kit (Thermo Fisher Scientific, Waltham, MA, USA). Fifty-six pairs of NSCLC tissues and corresponding non-cancerous tissues were obtained from patients who received surgery for NSCLC at the Affiliated Hospital of Weifang Medical University. None of the patients received chemotherapy or radiotherapy before the operation. NSCLC and non-cancerous tissues were confirmed by two pathologists. This research was approved by the Institutional Research Committee of the Affiliated Hospital of Weifang Medical University. The written informed consent for participating in the study was obtained from all patients.

3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide (MTT) Assay

The cell proliferation was detected using the cell proliferation reagent kit (Roche, Basel, Switzerland) according to the manufacturer's instruction.

Colony Formation

Cells (3×10^3 cells/well) were seeded into 35 mm plates. After four weeks, cells colony were fixed using 4% paraformaldehyde and stained by 0.1% crystal violet. The number of cells colony that diameter larger than 1.5 mm was counted.

Wound Healing Assay

The wound was generated after cells reached 90% confluent in 6-well plate by scratching using a 100 μl pipette tip. Then, cells were continually

cultured in medium containing only 2% FBS for 48 h. The wounded areas were photographed at 0 h and 48 h, respectively. The percentage of gap closure was calculated as the following formula: [1 - (empty area at 48 h/empty area at 0 h)]×100.

Invasion Analysis

1×10^5 cells were suspended in serum-free culture and were added into the upper chamber that was pre-coated with Matrigel (Sigma-Aldrich, St. Louis, MO, USA). The culture medium containing 20% fetal bovine serum was placed into the lower chamber of transwell. After 24 h, the invaded cells were stained by 0.1% crystal violet and were counted in five random fields.

Apoptosis Analysis

$1x10^6$ cells were collected and were re-suspended in 1 ml binding buffer (BioLegend, San Diego, CA, USA) and incubated with Annexin V (10 µl) and propidium iodide (PI) solution (5 µl) for 15 min at room temperature (RT). Then, cells were suspended in 500 µl binding buffer and the apoptosis was analyzed on the FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

RNA was extracted using TRIzol kit (Thermo Fisher Scientific, Waltham, MA, USA). The level of FENDRR was detected using SYBR Premix EX Taq™ II kit (TaKaRa, Otsu, Shiga, Japan) on ABI Prism®7500 (Applied Biosystems, Foster City, CA, USA). MiRNA was collected using PureLink™ miRNA isolation kit (Thermo Fisher Scientific, Waltham, MA, USA), and its level was measured using TaqMan microRNA assay kit. The relative level of miRNA and lncRNA were calculated using the $2^{-\Delta\Delta CT}$ method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were the internal controls. The primer sequences were the following: FENDRR-F: TAAAATTGCAGATCCTCCG; FENDRR-R: AACGTTCGCATTGGTTTAGC. GAPDH-F: GCTCTCTGCTCCTCCTGTTC; GAPDH-R: ACGACCAAATCCGTTGACTC. MiR-761-F: ACAGCAGGCACAGA and miR-761-R: GAGCAGGCTGGAGAA. U6-F: CTCGCTTCGGCAGCACA; U6-R: AACGCTTCACGAATTTGCGT.

Bioinformatics Analysis

The binding site between miR-761 and FENDRR was analyzed using the computer-aided algorithms obtained from starBase V2.0 (http://starbase.sysu.edu.cn/index.php).

Luciferase Reporter Assay

The pGL3-FENDRR wild-type (FENDRR-wt) plasmid was constructed by the insertion of FENDRR complementary DNA (cDNA) fragment which containing miR-761 binding sites into pGL3 Luciferase reporter vector (Promega, Madison, WI, USA). The mutant-type of pGL3-FENDRR (FENDRR-mut) was constructed by mutation of miR-761 binding site. 293T cells were co-transfected with FENDRR-wt or FENDRR-mut and miR-761 using Lipofectamine 2000. After 48 h, the Luciferase activity was detected in Luciferase reporter assay system (Promega, Madison, WI, USA).

Xenograft Model

100 µl 5×10^6 cells suspended in phosphate-buffered saline (PBS) were subcutaneously injected into nude mice. After 6 weeks, all mice were sacrificed and tumor tissues were harvested. The Animal Experimental was approved by the Ethics Committee of the Affiliated Hospital of Weifang Medical University in accordance with Institutional Guidelines and the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996).

Statistical Analysis

All data were presented as mean ± standard deviation (SD) and calculated using SPSS 22 (IBM, Armonk, NY, USA). Two-tailed Student's *t*-test or one-way ANOVA followed by post-hoc Dunnett's test was selected for statistical analysis. $p<0.05$ was considered statistically different.

Results

FENDRR is Down-Expressed in NSCLC and Associates with Prognosis

To identify the specific lncRNA that regulates NSCLC progression, microarray analysis (TCGA) was used to identify differential expressed lncRNA in NSCLC tissues (tumor) and its adjacent tissues (peritumor) (Figure 1A). LncRNA FENDRR, whose function was unknown, was markedly down-expressed in NSCLC (tumor) compared with the paired peritumor (Figure 1B). The prognosis analysis of lncRNA FENDRR suggested that the over-expression of lncRNA FENDRR was related to favorable overall-survival (Figure

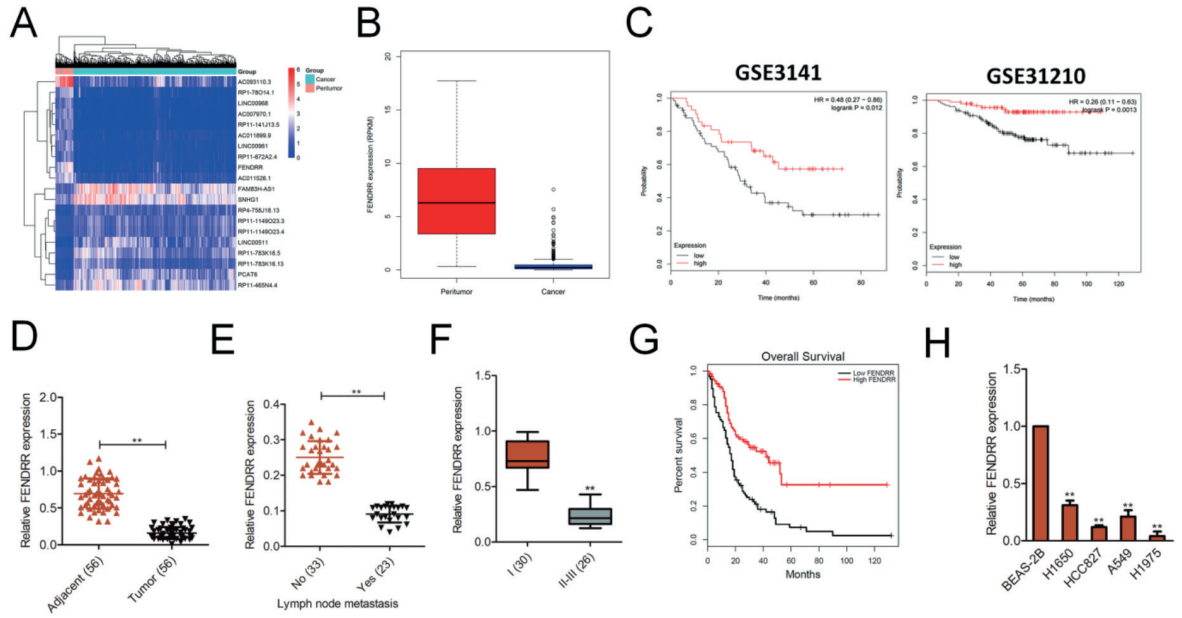

Figure 1. The level of FENDRR is low in NSCLC tissues and cells. ***A,*** Heatmap: The dys-expression pattern of lncRNAs in NSCLC tissues in comparison to non-cancer tissues. ***B,*** The level of FENDRR was down-regulated in tumor tissues compared to peritumor tissues. ***C,*** The Kaplan-Meier analysis using GEO datasets showed that high level of FENDRR was associated with better overall survival. ***D,*** The level of FENDRR was measured by qRT-PCR method in 56 paired of NSCLC tissues and adjacent non-cancerous tissues. $^{**}p<0.01$ compared to adjacent. ***E,*** The levels of FENDRR in metastatic and non-metastatic NSCLC tissues was detected by qRT-PCR. $^{**}p<0.01$ compared to no metastasis. ***F,*** Representative association between FENDRR expression and I or II/III tumor stage was shown. $^{**}p<0.01$ compared to I. ***G,*** Overall survival analysis of NSCLC patients with high or low level of FENDRR. ***H,*** The levels of FENDRR in four NSCLC cell lines and BEAS-2B cell were detected by qRT-PCR. $^{**}p<0.01$ compared to BEAS-2B cell.

1C). To explore the expression of FENDRR in NSCLC, we determined the level of FENDRR in 56 pairs of NSCLC tissues and matched non-cancer tissues using qRT-PCR. As shown in Figure 1D, the level of FENDRR was remarkably lower in tumor tissues than that in corresponding normal tissues ($p<0.01$). In addition, the lower level of FENDRR was associated with the distant metastasis (Figure 1E) and advanced stages of NSCLC (Figure 1F). We investigated the relationship between FENDRR and the clinical outcomes of patients with NSCLC. As shown in Figure 1G, patients who had a lower level of FENDRR exhibited an inferior overall survival. Finally, the levels of FENDRR in NSCLC cell lines, including H1650, HCC827, A549, H1975, normal cell and BEAS-2B were detected by qRT-PCR. As shown in Figure 1H, the levels of FENDRR in all NSCLC cell lines were significantly lower than that in a non-tumorigenic bronchial epithelial cell line, the BEAS-2B cell. Altogether, these findings suggested that FENDRR was down-expressed in NSCLC and was negatively associated with the prognosis of patients with NSCLC.

The Over-Expression of FENDRR Inhibits NSCLC Cells Proliferation and Promotes Apoptosis

To explore the effect of FENDRR in NSCLC, we transfected H1975 cell with pcDNA-FENDRR to increase the level of FENDRR, and A549 cell was transfected with siRNA-FENDRR to decrease the expression of FENDRR (Figure 2A-2B). Then, the MTT and colony formation assays were applied to assess the proliferation of H1975 and A549 cell that was transfected with FENDRR or siRNA-FENDRR, respectively. As shown in Figure 2C-2D, the over-expression of FENDRR inhibited the proliferation of H1975 cell, while the down-regulation of FENDRR promoted A549 cell proliferation. We also found that down-expression of FENDRR increased the colony formation of A549 cell, and the up-regulation of FENDRR inhibited the colony formation of H1975 cell (Figure 2E). Finally, the Annexin-FITC/PI staining was selected to determine the effect of FENDRR on the apoptosis of the NSCLC cell. As shown in Figure 2F, knockdown of FENDRR decreased the apoptosis of A549 cell and FENDRR over-expres-

sion increased the apoptosis of the H1975 cell. Altogether, these observations suggested that the knockdown of FENDRR inhibited the growth of the NSCLC cell and increased the apoptosis of NSCLC cell *in vitro*.

FENDRR Inhibits NSCLC Cell Migration and Invasion

To assess the roles of FENDRR on the aggressiveness of the NSCLC cell, we analyzed the migration capacity of the H1975 and A549 cell that transfected pcDNA-FENDRR or si-FENDRR using wound healing assay. As shown in Figure 3A-B, the over-expression of FENDRR suppressed H1975 cell migration, while the down-regulation of FENDRR promoted the A549 cell migration. The transwell invasion assay was utilized to determine the impact of FENDRR on NSCLC cell invasion. As shown in Figure 3C-3D, FENDRR over-expressing inhibited the invasion of H1975 cell and the knockdown of FENDRR increased the invasion of A549 cell. Altogether, these results indicated that FENDRR inhibited NSCLC cell migration and invasion. To investigate the roles of FENDRR on NSCLC cell growth, the xenografted model was established using BALB/c nude mice that were subcutaneously inoculated with pcDNA-FENDRR transfected H1975 cell. After six weeks, FENDRR over-expressing remarkably suppressed the growth of H1975 cells *in vivo* compared to the control vector transfected cells (Figure 3E-3F). Furthermore, we observed that the over-expression of FENDRR markedly decreased tumor weight (Figure 3G). Finally, tumor tissues were subjected to immunohistochemical (IHC) staining using Ki-67 antibody. As expected, the level of Ki-67 was lower in the tumor that was formed by FENDRR over-expressing H1975 cells compared to that in the tumor tissue which was formed by the control vector transfected H1975 cells (Figure 3H). Collectively, lncRNA FENDRR inhibited the growth and aggressiveness of H1975 cell.

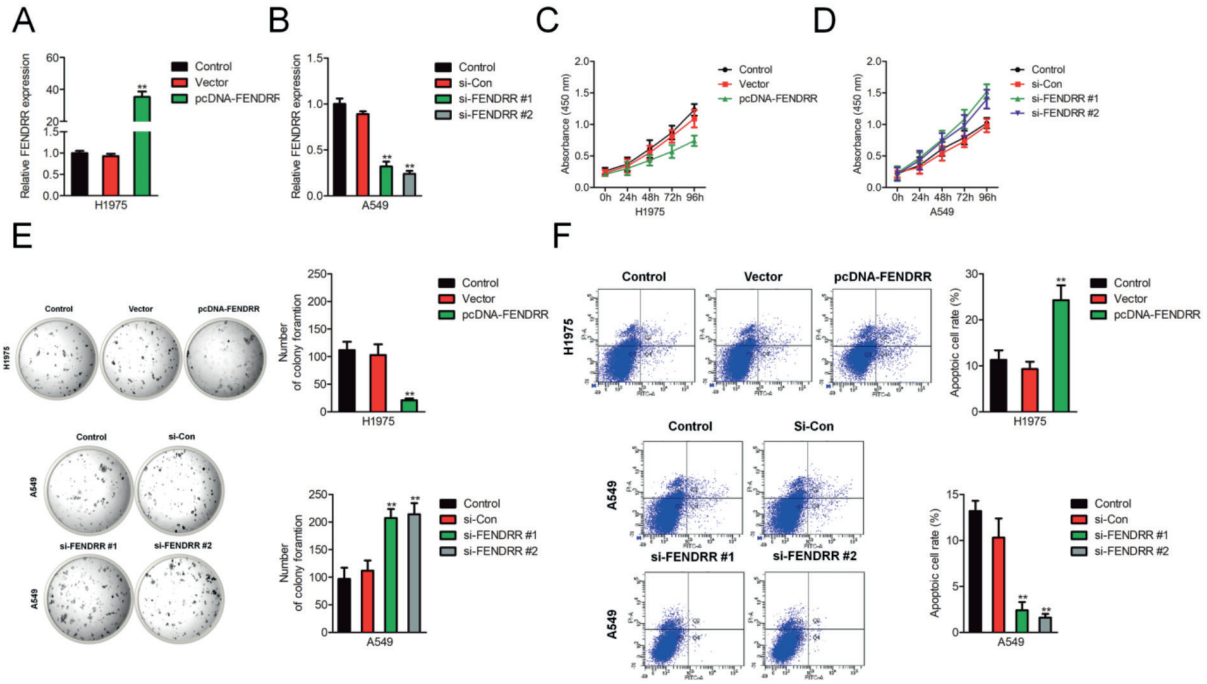

Figure 2. FENDRR suppresses growth and induces apoptosis of NSCLC cell. ***A***, H1975 cell was transfected with either control vector or pcDNA-FENDRR, and the level of FENDRR was detected by qRT-PCR. ***B***, A549 cell was transfected with si-Con, si-FENDRR #1 or si-FENDRR #2. The level of FENDRR was detected by qRT-PCR. ***C***, H1975 cell was transfected with either control vector or pcDNA-FENDRR, and the cell viability was detected by MTT assay. ***D***, The cell proliferation of A549 cell that was transfected with si-Con si-FENDRR #1 or si-FENDRR #2 was determined by MTT. ***E***, A549 cell was transfected with si-FENDRR. H1975 cell was transfected with pcDNA-FENDRR. The colony formation assay was conducted. ***F***, A549 was transfected with si-FENDRR. H1975 cell was transfected with pcDNA-FENDRR. The apoptosis of cell was analyzed. $^{**}p<0.01$ compared to control.

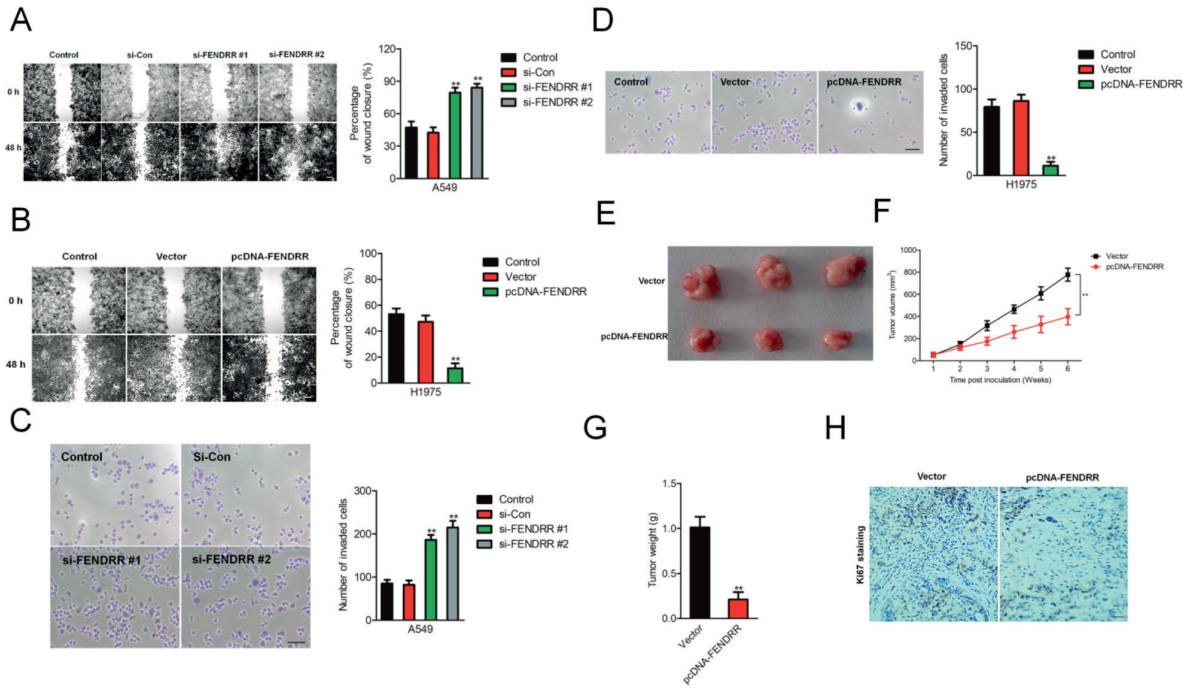

Figure 3. FENDRR suppresses the migration and invasion of NSCLC cell. ***A,*** A549 cell was transfected with either si-Con or si-FENDRR and the migration was detected using wound-healing assay. ***B,*** H1975 cell was transfected with control vector or pcDNA-FENDRR and the migration was detected using wound-healing assay. ***C,*** The transwell invasion assay of FENDRR down-expressing A549 cell. ***D,*** H1975 cell was transfected with control vector or pcDNA-FENDRR and the invasion was detected. ***E,*** Representative image of tumor that was formed by control vector or pcDNA-FENDRR transfected H1975 cell. ***F,*** The tumor growth was measured each week after inoculation H1975 cells. ***G,*** The weight of tumor tissues. ***H,*** Representative immunohistochemical (ICH) staining of Ki-67 in tumor tissues that was formed by H1975 cells. $^{**}p<0.01$ compared to control or vector.

The Over-Expression of MiR-761 Neutralizes the Inhibitory Effect of FENDRR on NSCLC Cells

Because of its vital roles in RNA homeostasis, one of the important functions of lncRNA is to compete with endogenous RNA (ceRNA)[20]. To better uncover the underlying mechanism by which FENDRR acted in NSCLC progression, we selected the online bioinformatics tool StarBase V 2.0 to predict the potential downstream target of FENDRR. As shown in Figure 4A, miR-761 was predicted to be the target of FENDRR with several binding sites. We then explored the level of miR-761 in both the A549 and H1975 cell that was transfected with FENDRR. The results suggested that the level of miR-761 was inhibited in FENDRR over-expressing NSCLC cell (Figure 4B). Next, we transfected two NSCLC cell lines with miR-761 and found that miR-761 remarkably inhibited the expression of FENDRR (Figure 4C and 4D). To prove that FENDRR directly binds to miR-761, the Luciferase reporter assay was conducted. The fragment of FENDRR containing a putative miR-761 binding site (wt-FENDRR) or mutant fragment (mut-FENDRR) was synthesized and inserted into the plasmid. The plasmid combination with miR-761 or miR-NC was co-transfected into the 293T cell. The Luciferase activity in 293T cell that was transfected with wt-FENDRR was decreased by miR-761. However, the inhibition effects of miR-761 were abolished in 292T cell that was transfected with mt-FENDRR (Figure 4E). Finally, the level of miR-761 was found negatively associated with the level of FENDRR in NSCLC tissues (Figure 4F). Therefore, these results demonstrated that lncRNA FENDRR directly bound to miR-761. Although we have identified that miR-761was a target of FENDRR, the precise roles of miR-761 in FENDRR-mediated on NSCLC cell growth and aggressiveness was unknown. To solve this problem, H1975 cell was co-transfected with miR-761 combination FENDRR (data not shown), and then colony formation as well as invasion assays were conducted. As shown in Figure 4G-4H, the over-expression of miR-761 remarkably counteracted the inhibitory

effects of FENDRR on the colony formation and invasion of H1975 cell. Moreover, the A549 cell was co-transfected with miR-761 inhibitor (miR-761^{inhi}) and si-FENDRR (data not shown), and both colony formation and transwell assays were conducted. As expected, miR-761^{inhi} significantly decreased the growth and invasion of H1975 cell in the presence of si-FENDRR (Figure 4I-4J). Our study demonstrated that FENDRR regulated the growth and aggressiveness of NSCLC cell by sponging miR-761.

Discussion

Recently, substantive genes expression investigations of cancer have identified that a great quantity of lncRNAs are dysregulated in diverse cancer types[21]. For example, lncRNA H19 inhibits the growth, migration and invasion of thyroid cancer cell through the down-regulation of insulin receptor substrate 1 (IRS-1)[22]. LncRNA activated by transforming growth factor (TGF)-β (ATB) is up-regulated in osteosarcoma and is positively associated with Enneking stage, metastasis and recurrence[23]. Meanwhile, the over-expression of lncRNA ATB promotes osteosarcoma cell growth and aggressiveness by inhibiting miR-200s[23]. In addition, lncRNA X Inactive Specific Transcript (XIST) participates into the metastasis and regulates the EMT process of colorectal carcinoma cell by competing for miR-200b-3p[24]. In the current research, we revealed that lncRNA FENDRR was remarkably down-expressed in NSCLC and was correlated with the growth and malignant phenotypes of NSCLC cell, which suggested that FENDRR played vital roles in NSCLC progression. Previous studies have revealed that the level of FENDRR is lower in breast cancer tissues than that in the matched non-cancerous tissues, and the down-expression of FENDRR is correlated with the poor overall survival rate of

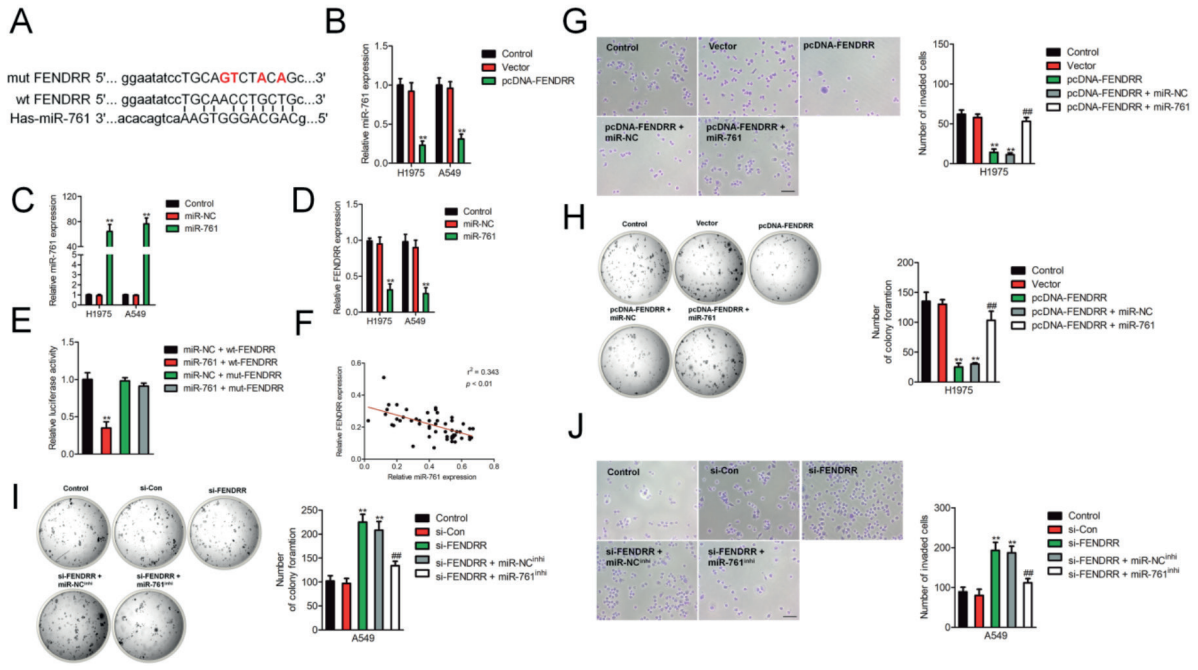

Figure 4. FENDRR is the downstream target of miR-761. ***A,*** The binding sites were identified between FENDRR and miR-761. ***B,*** The over-expression of FENDRR inhibited the level of miR-761 in H1975 and A549 cell. ***C,*** Cell was transfected with miR-NC or miR-761, and the level of miR-761 was detected by qRT-PCR. ***D,*** MiR-761 transfection remarkably suppressed the level of FENDRR in H1975 and A549 cell. ***E,*** Luciferase reporter analysis suggested that FENDRR directly bound to miR-761. ***F,*** The association between FENDRR and miR-761 in NSCLC tissues was evaluated. ***G,*** The transwell invasion assay was conducted in H1975 cell that was transfected with pcDNA-FENDRR alone, or cotransfected with pcDNA-FENDRR and miR-761. ***H,*** The colony formation assay was conducted in H1975 cell that was transfected with pcDNA-FENDRR alone or co-transfected with pcDNA-FENDRR and miR-761. ***I,*** The colony formation assay was executed in A549 cell that was transfected with si-FENDRR alone or cotransfected with miR-761^{inhi} and si-FENDRR. ***J,*** The transwell invasion assay was conducted in A549 cell that was transfected with si-FENDRR alone, or cotransfected with miR-761^{inhi} and si-FENDRR. $^{**}p<0.01$ compared to control, $^{\#\#}p<0.01$ compared to pcDNA-FENDRR or si-FENDRR.

patients with breast cancer[18]. *In vitro*, the up-regulation of FENDRR inhibits breast carcinoma cell growth and increases cellular apoptosis while FENDRR knockdown increases breast carcinoma cell growth and restrains apoptosis[18]. In addition, FENDRR is down-regulated in gastric carcinoma cells and clinical tissues, when compared to the gastric epithelial cells and the matched non-cancerous tissues[25]. Importantly, the low level of FENDRR is associated with the higher cancer stage and lymphatic metastasis. The over-expression of FENDER inhibits the migration and invasion of gastric carcinoma cell *in vitro*, by suppressing MMP2/MMP9 and Fibronectin 1 (FN1)[17]. Similarly, in this work, down-expressed FENDRR was observed in both NSCLC tissues and cell lines, when compared to the corresponding normal tissues and normal epithelial cell line. To explore the precise roles of FENDRR in NSCLC cell, H1975 and A549 cell were selected and transfected with siRNA targeting FENDRR or pcDNA-FENDRR, respectively. The over-expression of FENDRR inhibited the growth of H1975 cell as demonstrated in MTT and colony formation analysis whereas the down-expression of FENDRR facilitated cell viability and colony formation. We identified that FENDRR inhibited the growth of NSCLC cells *in vitro* and we next investigated the role of FENDRR on the tumor growth of NSCLC cell *in vivo*. As expected, the over-expression of FENDRR remarkably decreased NSCLC cell growth *in vivo*. Cancer cells metastasis is the major cause of death in patients with cancer. The initial steps of metastatic cascade include local invasion and migration, EMT and intravasation[26]. LncRNAs are emerging as key regulators governing biological processes of metastasis, including migration and invasion. In this work, wound healing as well as transwell assays were applied to explore whether FENDRR regulated the migration and invasion ability of NSCLC cell. All the results demonstrated that both migration and invasion abilities of NSCLC cell were significantly inhibited by the FENDRR over-expression. Emerging evidence proves that lncRNAs are the endogenous miRNA sponges and competitively bind to miRNAs to mediate genes expression[27,28]. To explore whether FENDRR acted as a miRNA sponge, the bioinformatics method was utilized and the result suggested that there were binding sites between miR-761 and FENDRR, and the following investigations confirmed that miR-761 was negatively regulated by FENDRR. The level of miR-761 was up-regulated in FENDRR siRNA transfected cell, whereas miR-761 was decreased in cell that was FENDRR over-expressed. The Luciferase activity assays verified that the Luciferase activity was remarkably weakened in cell that was cotransfected with miR-761 and lncRNA FENDRR. Finally, miR-761 transfection reversed the suppressive roles of FENDRR on the growth and malignant phenotypes of NSCLC cell. MiR-761 inhibitor transfected significantly decreased the growth and invasion of NSCLC cell that was increased by si-FENDRR whereas the over-expression of miR-761 reversed the inhibitory effect of FENDRR on NSCLC cell.

Conclusions

We demostrated that FENDRR was down-expressed in NSCLC tissues and cells. The over-expression of FENDRR inhibited the growth and aggressiveness of NSCLC cell. In addition, miR-761 was a target of FENDRR and alternation of miR-761 reversed the functions of FENDRR on the growth and aggressiveness of NSCLC cell. Our study revealed the possible relationship between FENDRR and miR-761 in NSCLC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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