Downregulation of circATXN7 represses non-small cell lung cancer growth by releasing miR-7-5p

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Abstract

**Background:** Circular RNAs (circRNAs) participate in the occurrence and progression of many cancers. CircRNA ataxin 7 (circATXN7) (circBase ID: hsa_circ_0066436) plays a promoting influence on gastric cancer progression. However, the biological role of circATXN7 in non-small cell lung cancer (NSCLC) is indistinct.

**Methods:** Levels of circATXN7, microRNA (miR)-7-5p, and profilin 2 (PFN2) mRNA were detected using quantitative real-time polymerase chain reaction (RT-qPCR). Proliferation, apoptosis, metastasis, and invasion were analyzed using cell counting kit-8 (CCK-8), colony formation, 5-ethynyl-2'-deoxyuridine (EdU), flow cytometry, and transwell assays. Protein levels were analyzed using western blotting (WB) and immunohistochemistry (IHC). The relationship between circATXN7 or PFN2 and miR-7-5p was analyzed by dual-luciferase reporter and RNA immunoprecipitation (RIP) assays. The biological function of circATXN7 was verified by xenograft assay.

**Results:** CircATXN7 and PFN2 were highly expressed in NSCLC, whereas miR-7-5p expression had the opposite trend. CircATXN7 overexpression constrained apoptosis and promoted proliferation, metastasis, invasion, and epithelial-mesenchymal transition of NSCLC cells, but circATXN7 silencing played the opposing influence and repressed xenograft tumor growth in vivo. CircATXN7 served as a miR-7-5p sponge, and circATXN7 regulated malignant behaviors of NSCLC cells through sponging miR-7-5p. PFN2 acted as a miR-7-5p target. PFN2 silencing overturned the promoting effect of miR-7-5p inhibitor on NSCLC cell malignancy, while PFN2 overexpression reversed the inhibitory impact of miR-7-5p mimic on NSCLC cell malignancy.

**Conclusion:** CircATXN7 accelerated the malignancy of NSCLC cells through adsorbing miR-7-5p and upregulating PFN2, offering evidence to support circATXN7 as a target for NSCLC treatment.

**KEYWORDS**
circATXN7, miR-7-5p, NSCLC, PFN2

INTRODUCTION

Lung cancer (LC) is a malignant tumor originating from the bronchial mucosa or glands of the lungs. The occurrence of LC is related to family history, smoking, chronic inflammation of the lungs, tuberculosis, environmental pollution, and occupational exposure. Non-small cell lung cancer (NSCLC) accounts for up to 85% of all LC. Many NSCLC patients are already in the advanced stage when they are diagnosed. Currently, the mechanism of NSCLC progression has not been fully elucidated. Therefore, exploring the mechanisms related to NSCLC progression is conducive to the development of new treatment strategies.

Circular RNAs (circRNAs) are a novel class of RNA transcripts that have closed-loop structures generated by back-splicing. Also, the special structure of circRNAs...
Most circRNAs show cell type- or tissue-specific MiRs, small non-coding RNA molecules, exert a pivotal role in post-transcriptional gene regulation by means of mRNA degradation or translational repression. Cellular studies have confirmed that miRs dysregulation is the cause of many cancers. MiR-7-5p has been found to exert an anticancer effect or promoting effect in different types of cancer. In NSCLC, miR-7-5p exerts an oncogenic role in cervical cancer. Also, miR-7-5p exerts an oncogenic role in cervical cancer. The overexpression plasmids of circATXN7 (circATXN7) were generated with empty pCD5-ciR vectors (Vector) (Geneseeed), while the overexpression plasmids of PFN2 (PFN2) were produced with empty pCDNA vectors (Invitrogen). The oligonucleotide sequences were synthesized by AoKe Biotech, including short hairpin (sh) RNA against circATXN7 (sh-circATXN7), negative control for sh-circATXN7 (sh-NC), small interference (si) RNA targeting PFN2 (si-PFN2), negative control for si-PFN2 (si-con), miR-7-5p inhibitor (in-miR-7-5p), negative control for miR inhibitor (in-miR-NC), miR-7-5p mimic (miR-7-5p), and negative control for miR mimic (miR-NC). Cell transfection was conducted using lipofectamine RNAiMAX (Invitrogen).

Quantitative real-time polymerase chain reaction

The isolation of cytoplasmic RNA and nuclear RNA was carried out using the PARIS kit (Invitrogen). Total RNA was isolated with the TRIzol kit (Invitrogen) and quantified using the NanoDrop 2000c spectrophotometer (Invitrogen). For RNA digestion, total RNA derived from NSCLC cells was treated with RNase R (3 U/µg, BioVision), and dithylypyrocarbonate-treated water (Invitrogen) was utilized as a negative control. Complementary DNA was generated using the SuperScript III (Invitrogen) or miScript II RT Kit (Qiagen), followed by performing qPCR with the SYBR qPCR Master Mix (Roche). All primer sequences are presented in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were used as internal references. Relative expression of each gene was figured with the threshold cycle method (2^ΔΔCt).

METHODS

Clinical specimens

Collection of clinical samples from 34 NSCLC patients was done at Shanxi Province Cancer Hospital, Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences, Cancer Hospital Affiliated to Shanxi Medical University, including NSCLC tissues and adjacent nontumor tissues. Registered patients signed informed consents. The utilization of human clinical specimens followed the procedures authorized by the Ethics Committee of Shanxi Province Cancer Hospital, Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences, Cancer Hospital Affiliated to Shanxi Medical University. Patient information is shown in Table S1.

Cell culture

Human tracheobronchial epithelial cells 16HBE (Procell) and NSCLC cell lines (NCI-H460, NCI-H1299, LK-2, and A549) (Coboer) were cultured in Roswell Park Memorial Institute-1640 medium (Invitrogen) or Ham’s F-12K medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Sigma) at 37°C with 5% CO2.

Oligonucleotides and plasmids

The overexpression plasmids of circATXN7 (circATXN7) were generated with empty pCD5-ciR vectors (Vector) (Geneseeed), while the overexpression plasmids of PFN2 (PFN2) were produced with empty pCDNA vectors (Invitrogen). The oligonucleotide sequences were synthesized by AoKe Biotech, including short hairpin (sh) RNA against circATXN7 (sh-circATXN7), negative control for sh-circATXN7 (sh-NC), small interference (si) RNA targeting PFN2 (si-PFN2), negative control for si-PFN2 (si-con), miR-7-5p inhibitor (in-miR-7-5p), negative control for miR inhibitor (in-miR-NC), miR-7-5p mimic (miR-7-5p), and negative control for miR mimic (miR-NC). Cell transfection was conducted using lipofectamine RNAiMAX (Invitrogen).
Cell counting kit-8 assay

The transfected NSCLC cells were seeded in 96-well plates (1 × 10^3). Subsequently, the cell counting kit-8 (CCK-8) reagent (10 μl) (Beyotime) was added at the specified time. The optical density (OD) value was evaluated with the SpectraMax M2 microplate detection system (MD).

Colony formation assay

NSCLC cells (3 × 10^3) were cultured in 6-well plates for 12 days. Subsequently, the colonies were fixed with 4% paraformaldehyde (Beyotime), followed by staining with 0.5% crystal violet (Beyotime) and counting and capturing with a fluorescent microscope (Olympus).

5-ethynyl-2'-deoxyuridine assay

Cell proliferation was determined by 5-ethynyl-2'-deoxyuridine (EdU) assay with the Cell-Light EdU DNA Cell Proliferation Kit (RiboBio). In brief, the EdU solution (50 mM) was added and then 2 h later, the cells were fixed with 4% paraformaldehyde (Beyotime) and then stained with Apollo Dye Solution and 4',6-diamidino-2-phenylindole (DAPI).

A fluorescent microscope (Olympus) was utilized to visualize the cells.

Flow cytometry assay

The transfected NSCLC cells were collected and then detached with 0.025% trypsin (Sigma). Cell apoptosis was evaluated with the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Sigma) based on the manufacturer’s instructions. The apoptotic rate was determined using cytoreflex flow cytometry (Beckman Coulter).

Transwell assay

Transwell chambers without Matrigel (#3422, Costar) were used for analysis of cell metastasis. In summary, the cells (1 × 10^5) in 200 μl serum-free cell culture medium were placed on the apical chamber and 600 μl cell culture medium encompassing 10% fetal bovine serum (Sigma) was added to the basolateral chamber. Then, 24 h later, the migrated cells were stained with 0.1% crystal violet (Beyotime) and counted with a microscope (Olympus). Notably, the experimental procedures for cell metastasis and invasion were the same, the only difference was that the invasion experiment was performed with the transwell chamber with Matrigel (#354480, Costar).

Western blotting

Total protein was extracted using the radioimmuno-precipitation buffer (Beyotime). Western blotting (WB) was executed as previously described. Antibodies utilized for WB were as follows: E-cadherin (#PA5-32178, 1:3000, Invitrogen), N-cadherin (#PA5-29570, 1:1000, Invitrogen), vimentin (#10366-1-AP, 1:3000, Invitrogen), GAPDH (#PA1-16777, 1:4000, Invitrogen), NOVA2 (#PA5-69076, 1.25 μg/ml, Invitrogen), Lamin A/C (#MA3-1000, 1:500, Invitrogen), Calreticulin (#MA5-15382, 1:1000, Invitrogen), goat rabbit IgG (#31460, 1:10,000, Invitrogen), and goat anti-mouse IgG (#31430, 1:5000, Invitrogen). The blots were developed using the Pierce ECL WB Substrate (Invitrogen).

Dual-luciferase reporter assay

MiRs that might interact with circATXN7 were jointly predicted using circBank, starBase, and CircInteractome databases. The binding sites between miR-7-5p and PFN2 3' untranslated region (UTR) were predicted using the TargetScan database. To verify the relationship between circATXN7 or PFN2 and miR-7-5p, the wild-type (WT) sequences of circATXN7 and PFN2 3'UTR and their

**Table 1** Primer sequences used for qRT-PCR

| Genes   | Primer sequences (5’-3’)                          |
|---------|---------------------------------------------------|
| circATXN7 | Forward (F): 5’-CACAGCTATGGAAACCA CATCCCT-3’       |
|         | Reverse (R): 5’-GAGTCGGATGGCGAATCAA-3’             |
| ATXN7   | F: 5’-CAACCCTCATGGTGAGAAGCATC-3’                  |
|         | R: 5’-ATGGTCGCCACAGAGGTTGCG-3’                    |
| PFN2    | F: 5’-TCCGCTACTGCGACGGATCCAA-3’                   |
|         | R: 5’-GATCAGACTTCTTGAGCCAAGG-3’                   |
| GAPDH   | F: 5’-GTCCTGGATGCTTCAACCAGC-3’                    |
|         | R: 5’-ACCACCCGTGGTCTGAGC-3’                       |
| miR-7-5p| F: 5’-CGCGTGGGAGACTAGTGATT-3’                     |
|         | R: 5’-AGTGCAGGGTCCGAGGTT-3’                       |
| miR-145-5p| F: 5’-CCGGTGATTGTCGAGGAAGG-3’                     |
|         | R: 5’-AGTGCAAGGTTCCGAGGTT-3’                      |
| miR-149-5p| F: 5’-CGTCTGGCTCCGGTTGCTCTC-3’                    |
|         | R: 5’-AGTGCAAGGTTCCGAGGTT-3’                      |
| miR-421 | F: 5’-GGCGGATCTACAGACATTATTT-3’                   |
|         | R: 5’-AGTGCAAGGTTCCGAGGTT-3’                      |
| miR-1270| F: 5’-CGCGCTGAGGATATAGGAAGA-3’                    |
|         | R: 5’-AGTGCAAGGTTCCGAGGTT-3’                      |
| miR-1294| F: 5’-CGCGGCTGGAGATATGGAAGA-3’                    |
|         | R: 5’-AGTGCAAGGTTCCGAGGTT-3’                      |
| U6      | F: 5’-CTCGGCTCCGGAGCAACAAC-3’                     |
|         | R: 5’-AACGCTACGAAATTTGGCT-3’                      |
mutant (MUT) sequences were inserted into the pMIRREPORT reporter (Applied Biosystems), respectively. NSCLC cells were transfected with miR-7-5p mimic or miR-NC together with a luciferase reporter. The luciferase activity was assessed using the Pierce Renilla-Firefly Luciferase Dual Assay Kit (Invitrogen).

RNA immunoprecipitation assay

The interaction between circATXN7 or PFN2 and miR-7-5p was verified by RNA immunoprecipitation (RIP) analysis with the Magna RIP kit (Millipore). In brief, NSCLC cells were lysed and then centrifuged at 4°C (14,000 rpm, 10 min). The supernatant was incubated with magnetic beads conjugated to IgG antibody (Abcam) or Ago2 antibody (Abcam). The abundance of circATXN7 or PFN2 and miR-7-5p in immunoprecipitated RNA was assessed by RT-qPCR.

Xenograft assay

Twelve BALB/c nude mice (4–6 weeks old) were purchased from Vital River Laboratory. The execution of animal experiments followed the procedures approved by the Animal Ethics Committee of Shanxi Province Cancer Hospital, Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences, Cancer Hospital Affiliated to Shanxi Medical University. Briefly, A549 cells carrying lentivirus-mediated sh-circATXN7 or sh-NC were subcutaneously injected into the right flank of nude mice (n = 6 mice per group). Tumor volume was measured every 4 days from day 7 (volume = [length × width^2]/2), and then 27 days later, all mice were sacrificed to strip the xenograft tumors and weighed.

Immunohistochemistry

Paraffin-embedded mice tissue sections (5 μm thick) were incubated with anti-PFN2 antibody (#PA5-21959, 1:500, Invitrogen) at 4°C for 12 h. The Vectastain Universal Elite ABC Kit (Vector Laboratories) was used for immunohistochemistry (IHC) staining based on the manufacturer’s instructions.

Statistical analysis

At least three repeats were conducted for each experiment. SPSS Statistical software version 17.0 (SPSS) was utilized for statistical analyses. All data are presented as mean ± SD. Student’s t-test and analysis of variance were used to compare the differences between two groups or more groups. A value of p < 0.05 was considered statistically significant.

RESULTS

CircATXN7 was highly expressed in NSCLC

CircATXN7 (circBase ID: hsa_circ_0066436) contains 587 nucleotides and is produced from the ATXN7 gene (exons 9–11) in chr3: 63973734–63976535 (Figure 1a). To validate the expression trend of circATXN7 in NSCLC, we detected circATXN7 expression in NSCLC tissues and cells. As exhibited in Figure 1b,c, there was observable upregulation of circATXN7 in NSCLC tissues and cells (NCI-H460, NCI-H1299, LK-2, and A549) compared to their matched negative controls. A significant correlation between TNM grade, lymph node metastasis, or tumor size and high circATXN7 level was obtained by Chi-square analysis, as shown in Table S1. To verify the stability of circATXN7, we treated RNA extracted from NCI-H460 and A549 cells with RNase R. RT-qPCR showed that RNase R treatment did not affect the level of circATXN7, whereas linear ATXN7 was significantly degraded (Figure 1d,e). Nuclear mass separation with RT-qPCR revealed that circATXN7 was preferentially localized in the cytoplasm of NCI-H460 and A549 cells, and western blotting confirmed good nucleus/cytoplasm fractionation (Figure 1f). These results uncovered that circATXN7 was preferentially localized in the cytoplasm and might participate in the progression of NSCLC.

CircATXN7 facilitated proliferation and curbed apoptosis of NSCLC cells

Next, we carried out gain/loss-of-function experiments to verify the impacts of circATXN7 on NSCLC cell proliferation and apoptosis. RT-qPCR displayed that circATXN7 expression was increased in NCI-H460 cells after circATXN7 transfection and decreased in A549 cells after sh-circATXN7 transfection (Figure 2a,b). CCK-8 and colony formation assays exhibited that circATXN7 overexpression promoted NCI-H460 cell growth and colony formation, whereas circATXN7 knockdown repressed A549 cell growth and colony formation (Figure 2c–f). EdU assay showed that the number of positive cells was elevated in NCI-H460 cells with overexpression of circATXN7 and reduced in A549 cells with knockdown of circATXN7 (Figure 2g–j). Flow cytometry assay revealed that the transfection of circATXN7 repressed the apoptosis of NCI-H460 cells, but the transfection of sh-circATXN7 elevated the apoptosis of A549 cells (Figure 2k,l). Collectively, these findings manifested that circATXN7 accelerated cell proliferation and constrained cell apoptosis in NSCLC cells.

CircATXN7 promoted metastasis, invasion, and epithelial-mesenchymal transition of NSCLC cells

Subsequently, the influence of circATXN7 on NSCLC cell metastasis, invasion, and epithelial-mesenchymal transition
(EMT) was surveyed. Transwell assay revealed that the number of metastasizing and invading cells was elevated in circATXN7-transfected NCI-H460 cells and reduced in sh-circATXN7-transfected A549 cells (Figure 3a,d). Also, circATXN7 upregulation reduced E-cadherin protein level and elevated N-cadherin and vimentin protein levels in NCI-H460 cells, but circATXN7 knockdown elevated E-cadherin protein level and decreased N-cadherin and vimentin protein levels in A549 cells (Figure 3e,f). Together, these results indicated that circATXN7 accelerated NSCLC cell metastasis, invasion, and EMT.

CircATXN7 was identified as a miR-7-5p decoy

Since circATXN7 was mainly located in the cytoplasm, we speculated that circATXN7 might function as a decoy for miRs. Bioinformatic analysis (circBank, starBase, and CircInteractome) exhibited that 6 miRs (miR-7-5p, miR-145-5p, miR-149-5p, miR-421, miR-1270, and miR-1294) might interact with circATXN7 (Figure 4a). RT-qPCR showed that miR-7-5p, miR-145-5p, and miR-149-5p were downregulated in 6 random NSCLC tissues, while miR-421 and miR-1270 were upregulated (Figure 4b). Also, miR-7-5p and miR-145-5p expression were decreased in circATXN7-overexpressed NCI-H460 cells and elevated in circATXN7-suppressed A549 cells, especially the expression of miR-7-5p (Figure 4c,d). The putative binding sites of circATXN7 in miR-7-5p are shown in Figure 4e. Furthermore, the luciferase activity of the circATXN7 WT reporter was suppressed in miR-7-5p-overexpressed NCI-H460 and A549 cells (Figure 4f,g). The abundance of miR-7-5p and circATXN7 was significantly increased in the immunoprecipitates pulled down by the Ago2 antibody (Figure 4h,i). As expected, miR-7-5p expression was decreased in NSCLC tissues and was negatively correlated with circATXN7 (Figure 4j,k). In addition, miR-7-5p was overtly downregulated in NCI-H460 and A549 cells (Figure 4l). Collectively, these findings suggested that circATXN7 acted as a miR-7-5p sponge in NSCLC cells.

CircATXN7 regulated NSCLC cell malignancy by sponging miR-7-5p

Based on circATXN7 served as a miR-7-5p sponge, we wondered whether circATXN7 regulated NSCLC cell malignancy by sponging miR-7-5p. The overexpression efficiency of miR-7-5p in NCI-H460 cells and the knockdown efficiency of in-miR-7-5p in A549 cells were presented in Figure 5a,b. Moreover, the promotion of proliferation and the repression of apoptosis of NCI-H460 cells caused by circATXN7 upregulation were overturned by miR-7-5p overexpression. However, the silence of miR-7-5p reversed the repression of proliferation and the promotion of apoptosis of A549 cells mediated by circATXN7 knockdown (Figure 5c–j). Also, miR-7-5p elevation offset the promoting impact of circATXN7 overexpression on NCI-H460 cell metastasis and invasion, whereas miR-7-5p inhibitor counteracted the inhibitory influence of circATXN7...
silencing on A549 cell metastasis and invasion (Figure 5k–n). Furthermore, the protein trends of E-cadherin, N-cadherin, and vimentin in NCI-H460 cells transfected with circATXN7 were overturned by miR-7-5p overexpression, while the protein trends of E-cadherin, N-cadherin, and vimentin in A549 cells mediated by circATXN7 inhibition were restored after miR-7-5p knockdown (Figure 5o,p). These results indicated that circATXN7 promoted NSCLC cell malignancy by sponging miR-7-5p.

PFN2 served as a miR-7-5p target

To further explore the regulation mechanism of circATXN7 in NSCLC, we sought the targets of miR-7-5p. Through bioinformatic analysis (TargetScan database), we discovered that PFN2 might be a miR-7-5p target (Figure 6a). Moreover, the level of PFN2 protein was decreased in miR-7-5p-overexpressed NCI-H460 cells and elevated in miR-7-5p-inhibited A549 cells (Figure 6b,c). Also, miR-7-5p
overexpression decreased the luciferase activity of the PFN2 3’UTR WT reporter in NCI-H460 and A549 cells (Figure 6d, e). There was a prominent enrichment of PFN2 and miR-7-5p in the immunoprecipitates pulled down by the Ago2 antibody (Figure 6f, g). As expected, the levels of PFN2 mRNA and protein were upregulated in NSCLC tissues (Figure 6h, i). The expression of PFN2 mRNA was also negatively correlated with miR-7-5p and positively correlated with circATXN7 in NSCLC tissues (Figure 6j, k). In addition, the levels of PFN2 mRNA and protein were also elevated in NCI-H460 and A549 cells (Figure 6l, m). Furthermore, the level of PFN2 protein was elevated in circATXN7-transfected NCI-H460 cells and reduced in sh-circATXN7-transfected A549 cells, but these tendencies were restored after miR-7-5p overexpression or silencing (Figure 6n, o). Together, these results suggested that PFN2 was a downstream target of miR-7-5p.

**MiR-7-5p repressed NSCLC cell malignancy by targeting PFN2**

Because miR-7-5p targeted PFN2, we further explored whether miR-7-5p regulated malignant behaviors of NSCLC cells by targeting PFN2. The knockdown efficiencies of si-PFN2 and in-miR-7-5p in NCI-H460 cells and the overexpression efficiencies of PFN2 and miR-7-5p in A549 cells are shown in Figure 7a, b. Moreover, miR-7-5p inhibition facilitated NCI-H460 cell proliferation and constrained NCI-H460 cell apoptosis, but these impacts were restored after si-PFN2 introduction. However, miR-7-5p elevation curbed A549 cell proliferation and accelerated A549 cell apoptosis, whereas these trends were reversed after PFN2 overexpression (Figure 7c–j). Furthermore, PFN2 silencing counteracted the promoting effect of miR-7-5p inhibitor on NCI-H460 cell metastasis and invasion, while PFN2 upregulation offset the suppressive influence of miR-7-5p mimic on A549 cell metastasis and invasion (Figure 7k–n). As expected, miR-7-5p inhibitor reduced E-cadherin protein level and elevated N-cadherin and vimentin protein levels in NCI-H460 cells, but these trends were overturned by PFN2 knockdown (Figure 7o). Additionally, miR-7-5p mimic elevated E-cadherin protein level and decreased N-cadherin and vimentin protein levels in A549 cells, whereas these tendencies were reversed after PFN2 upregulation (Figure 7p). These results manifested that miR-7-5p repressed cell malignancy by targeting PFN2 in NSCLC cells.

**CircATXN7 knockdown repressed tumor growth in vivo**

To verify the influence of circATXN7 knockdown in tumor growth in vivo, A549 cells stably transfected with sh-circATXN7 or sh-NC were injected into nude mice. The results revealed that tumor volume and weight derived from
nude mice injected with A549 cells carrying sh-circATXN7 were smaller and lighter compared to the control group (Figure 8a–c). RT-qPCR exhibited that circATXN7 and PFN2 mRNA were downregulated in mice tumors in the sh-circATXN7 group in contrast to the control group, while miR-7-5p expression had the opposite trend (Figure 8d). WB and IHC analysis showed that the level of PFN2 protein was also downregulated in mice tumors in the sh-circATXN7 group (Figure 8e,f). In addition, the level of E-cadherin was upregulated in mice tumors in the sh-circATXN7 group, but the levels of N-cadherin and vimentin protein were downregulated (Figure 8g). Collectively, these results suggested that circATXN7 knockdown curbed NSCLC growth in vivo.
CircATXN7 sponged miR-7-5p to regulate NSCLC cell malignancy. (a and b) RT-qPCR verified the overexpression efficiency of miR-7-5p in NCI-H460 cells and the knockdown efficiency of in-miR-7-5p in A549 cells. (c–n) The proliferation, apoptosis, metastasis, and invasion of NCI-H460 cells transfected with vector, circATXN7, circATXN7 + miR-NC, or circATXN7 + miR-7-5p and A549 cells transfected with sh-NC, sh-circATXN7, sh-circATXN7 + in-miR-NC, or sh-circATXN7 + in-miR-7-5p were analyzed by CCK-8, colony formation, EdU, flow cytometry, and transwell assays. (o, p) Protein levels of E-cadherin, N-cadherin, and Vimentin in NCI-H460 cells transfected with vector, circATXN7, circATXN7 + miR-NC, or circATXN7 + miR-7-5p and A549 cells transfected with sh-NC, sh-circATXN7, sh-circATXN7 + in-miR-NC, or sh-circATXN7 + in-miR-7-5p were detected by WB.

*p < 0.05, **p < 0.01, and ***p < 0.001
DISCUSSION

Studies have revealed that differential circRNA expression is observably related to cancer TNM staging, metastasis, and other clinical characteristics of cancer.\(^{28}\) Also, circRNAs are promising therapeutic targets for cancer, including NSCLC.\(^{29}\) Herein, we validated that circATXN7 functioned as a miR-7-5p sponge and facilitated NSCLC growth through upregulating PFN2.

It is worth mentioning that our results offer new evidence that circATXN7 facilitates the progression of NSCLC. The inference is based on the following points: (1) circATXN7 was highly expressed in NSCLC; (2) the silence of circATXN7 inhibited NSCLC cell proliferation,
FIGURE 7 MiR-7-5p targeted PFN2 to repress the malignancy of NSCLC cells. (a and b) WB and RT-qPCR validated the knockdown efficiencies of si-PFN2 and in-miR-7-5p in NCI-H460 cells and the overexpression efficiencies of PFN2 and miR-7-5p in A549 cells. (c–n) The proliferation, apoptosis, metastasis, and invasion of NCI-H460 cells transfected with in-miR-NC, in-miR-7-5p, in-miR-7-5p + si-con, or in-miR-7-5p + si-PFN2 and A549 cells transfected with miR-NC, miR-7-5p, miR-7-5p + pcDNA, or miR-7-5p + PFN2 were determined by CCK-8, colony formation, EdU, flow cytometry, and transwell assays. (o and p) Protein levels of E-cadherin, N-cadherin, vimentin in NCI-H460 cells transfected with in-miR-NC, in-miR-7-5p, in-miR-7-5p + si-PFN2 and A549 cells transfected with miR-NC, miR-7-5p, miR-7-5p + pcDNA, or miR-7-5p + PFN2 were analyzed by WB. *p < 0.05, **p < 0.01, and ***p < 0.001.
metastasis, invasion, and EMT in vitro, whereas circATXN7 overexpression exerted the opposing function; (3) circATXN7 silencing repressed NSCLC cell growth and EMT in xenograft mouse models. A previous study revealed that the parent gene ATXN7 of circATXN7 was an oncogene. Moreover, circATXN7 could elevate ENTPD4 expression through functioning as an miR-4319 sponge, resulting in promoting GC cell growth and metastasis. Also, hsa_circ_0007761 (hsa_circATXN7_007) had been exposed to be regulated in NSCLC, and hsa_circ_0007761 silencing curbed invasion and proliferation of NSCLC cells. Our results are consistent with these reports and offer evidence to support that circATXN7 exerts a promoting effect on NSCLC progression.

Recent studies have demonstrated that circRNAs can adsorb miRs through miR response elements to modulate gene expression, thus affecting the progression of NSCLC. Thus, we verified circATXN7 as a miR-7-5p decoy. MiR-7-5p could repress Dox-induced homologous recombination repair via targeting PARP1, thus elevating the sensitivity of small cell LC cells to Dox. In contrast, miR-7-5p targeted Bcl-2 and PARP1 to promote autophagy and decrease DNA
Herein, forced miR-7-5p expression overturned the promoting influence of circATXN7 upregulation on NSCLC cell malignant behaviors, whereas miR-7-5p inhibitor counteracted circATXN7 silencing-mediated repressive influence on NSCLC cell malignancy. These data illustrated that circATXN7 regulated NSCLC cell malignant behaviors through adsorbing miR-7-5p. Notably, miR-7-5p could repress NSCLC cell metastasis by targeting NOVA2.\(^{20}\) Our data exhibited that circATXN7 overexpression elevated the NOVA2 protein level in NSCLC cells, but circATXN7 silencing reduced the NOVA2 protein level (Figure \(S1\)). These results manifested that circATXN7 might regulate NOVA2 expression by sequestering miR-7-5p. In the future, the relationship between NOVA2 and circATXN7 should be discussed in depth.

Accumulated studies have revealed that PFN2 upregulation is associated with the advancement of a variety of cancers. Researchers have found that PFN2 activated PI3K/AKT/β-catenin pathway, thus accelerating head and neck cancer cell metastasis and proliferation.\(^{24}\) Also, hnRNP2A2/B1 repressed breast cancer cell metastasis via binding PFN2 at least partly.\(^{32}\) Moreover, PFN2 constrained the recruitment of HDAC1 to the Smad2 and Smad3 promoters, thus facilitating metastasis and growth of LC.\(^{33}\) MiR-30a-5p targeted PFN2 to curb cell EMT in high invasive NSCLC.\(^{25}\) Herein, PFN2 was verified as a miR-7-5p target. Furthermore, PFN2 silencing restored miR-7-5p inhibitor-mediated promoting effect on NSCLC cell malignant behaviors, but PFN2 elevation offset the repressive impact of miR-7-5p mimic on NSCLC cell malignancy, manifesting that miR-7-5p constrained NSCLC cell malignancy through downregulating PFN2. Importantly, circATXN7 modulated PFN2 expression by adsorbing miR-7-5p in NSCLC cells. Thus, we concluded that circATXN7 promoted the growth of NSCLC via increasing PFN2 expression through sponging miR-7-5p.

In conclusion, circATXN7 exerted a promoting impact on NSCLC progression. Mechanically, circATXN7 adsorbed miR-7-5p to elevate PFN2 expression, thus promoting NSCLC cell proliferation, metastasis, invasion, and EMT (Figure \(9\)). The study offered evidence to support the carcinogenic effect of circATXN7 on NSCLC.

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

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How to cite this article: Li D, Fu Z, Dong C, Song Y. Downregulation of circATXN7 represses non-small cell lung cancer growth by releasing miR-7-5p. Thorac Cancer. 2022;13(11):1597–610. https://doi.org/10.1111/1759-7714.14426