Long non-coding RNA PITPNA-AS1 silencing suppresses proliferation, metastasis and epithelial-mesenchymal transition in non-small cell lung cancer cells by targeting microRNA-32-5p

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Abstract. Lung cancer is one of the most common types of cancer and has a high mortality rate, worldwide. The major histopathological subtype is non-small cell lung cancer (NSCLC). The aim of the present study was to investigate the role of long non-coding (lnc) RNA PITPNA antisense RNA 1 (PITPNA-AS1) in NSCLC and elucidate its potential mechanisms. The expression of PITPNA-AS1 was determined in several NSCLC cell lines. Following PITPNA-AS1-silencing, cell proliferation, invasion and migration were evaluated using Cell Counting Kit-8, colony formation, Transwell assay and wound healing assays, respectively. The expression levels of proliferation-, migration- and epithelial-mesenchymal transition (EMT)-associated proteins were examined using immunofluorescence assay or western blot analysis. A luciferase reporter assay was conducted to verify the potential interaction between PITPNA-AS1 and microRNA(miR)-32-5p. Subsequently, rescue assays were performed to investigate the effects of PITPNA-AS1 and miR-32-5p on NSCLC progression. The results demonstrated that PITPNA-AS1 was highly expressed in NSCLC tissues and cell lines. It was found that PITPNA-AS1 silencing inhibited the proliferation, invasion and migration of NSCLC cells. Furthermore, the protein expression of E-cadherin was upregulated, while the expression levels N-cadherin and vimentin were downregulated. The luciferase reporter assay confirmed that miR-32-5p was a direct target of PITPNA-AS1. The rescue experiments suggested that a miR-32-5p inhibitor significantly reversed the inhibitory effects of PITPNA-AS1 silencing on proliferation, invasion, migration and EMT in NSCLC cells. Collectively, the present results demonstrated that PITPNA-AS1 silencing could suppress the progression of NSCLC by targeting miR-32-5p, suggesting a promising biomarker in NSCLC diagnosis and treatment.

Introduction

Lung cancer is considered to be one of the most frequent malignancies, with a high mortality rate, as there are 1.6 million tumor-related mortalities annually worldwide (1,2). Non-small cell lung cancer (NSCLC) is the predominant type of lung cancer and accounts for >80% of cases with a low 5-year survival rate (3,4). Despite significant advances in the diagnosis and treatment of lung cancer in recent decades, the 5-year survival rate still remains <20% (5). Poor prognosis in patients with NSCLC is associated with the lack of early diagnostic biomarkers, as well as high potentials of invasion and metastasis (6). Therefore, elucidating the detailed molecular mechanism underlying the progression of NSCLC and identifying novel biomarkers is urgently required for early diagnosis, prevention and treatment.

Long non-coding (lnc) RNAs are functional non-protein coding transcripts, with a length of >200 nucleotides (7).

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Based on emerging research, lncRNAs exert a diverse set of functions in numerous biological processes, such as cell proliferation, invasion, differentiation, apoptosis and metastasis (8,9). In addition, the expression profiles of lncRNA have been associated with a number of diseases, including cancer, suggesting that they may serve as potential mediators in carcinogenesis (10). PITPNA antisense RNA 1 (PITPNA-AS1) is a newly reported lncRNA, which is located on chromosome 17p13.3 (11). It has been reported that PITPNA-AS1 facilitates hepatocellular carcinoma progression via regulation of the microRNA(miRNA/miR)-876-5p/WNT5A signaling pathway (11). Moreover, emerging evidence supports the hypothesis that PITPNA-AS1 accelerates cervical cancer progression via regulating cellular proliferation and apoptosis by targeting the miR-876-5p/c-MET axis (12). Therefore, PITPNA-AS1 may exert oncogenic effects. These findings indicated that PITPNA-AS1 may serve as a potential diagnostic and therapeutic target for NSCLC.

The aims of the present study were to investigate the role of lncRNA PITPNA-AS1 in NSCLC development and to identify the underlying molecular mechanisms. These data may provide a novel diagnostic and therapeutic target for the control of NSCLC progression.

Materials and methods

The Cancer Genome Atlas (TCGA) database analysis. Human RNA-sequencing data from NSCLC projects, which included 1,038 patients with NSCLC and 322 normal tissues were obtained from TCGA data in the UALCAN database (ualcan. path.uab.edu). A Mann-Whitney test was used by UALCAN to determine the statistical differences in the PITPNA-AS1 expression levels between normal and tumor samples.

Cells and cell culture. A total of four human NSCLC cell lines (HCC827, NCI-H1299, A549 and NCI-H1650) and one normal human bronchial epithelioid cell line (BEAS-2B), were purchased from the Cell Bank of the Chinese Academy of Sciences. The cell lines were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 μg/ml penicillin and 0.1 μg/ml streptomycin at 37˚C in a humidified incubator with 5% CO_2.

Cell transfection. Cells were plated into 6-well plates (1x10^6 cells per well), and transfection was performed when cells in the logarithmic growth phase reached 80% confluence. pIRES vectors containing (sh)RNAs targeting PITPNA-AS1 (shRNA-PITPNA-AS1-1 or shRNA-PITPNA-AS1-2; 1 μg) or a scrambled shRNA [shRNA-negative control (NC); 1 μg] were synthesized by Shanghai GenePharma Co., Ltd. miR-32-5p inhibitor (5'-UGCAACUUAGUAAUGUGCAAUA-3'; 50 nM), miR-32-5p inhibitor NC (inhibitor-NC; 5'-CAGUACUUUU GUGUAGUACAA-3'; 50 nM), miR-32-5p mimic (forward, 5'-UAUUGCACAUUACUAAGUUGCA-3' and reverse, 5'-CAACUUAGUAAUGUGCAAUAUU-3'; 50 nM) and miR-32-5p mimic NC (mimic NC; 5'-UUCUCCGAACG UGUCACGUU-3'; 50 nM) were designed and synthesized by Shanghai GenePharma Co., Ltd. Transfection experiments were performed using Lipofectamine® 2000 (Invitrogen;

Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Cells were harvested 48 h following transfection and the transfection efficiency was determined using reverse transcription-quantitative PCR (RT-qPCR).

Cell Counting Kit-8 (CCK-8) assay. A549 cells were maintained in the exponential phase and then seeded in 96-well plates (2x10^4 cells/well). Cell proliferation was detected using a CCK-8 assay (Shanghai YiSheng Biotechnology Co., Ltd.) according to the manufacturer's instructions. At the indicated times of 24, 48 and 72 h, 10 μl CCK-8 solution was added to each well and the cells were incubated for an additional 4 h at 37˚C. The optical density was measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Inc.).

Colony formation assay. Cells were exposed to the indicated transfections accordingly and were plated into 6-cm dishes (1,000 cells per well), followed by incubation for 14 days at 37˚C, until the colonies could be observed under a fluorescence microscope (magnification, x10; Olympus Corporation). Subsequently, cells were fixed with 4% paraformaldehyde for 10 min at room temperature and stained with 0.2% crystal violet for 5 min at room temperature.

Immunofluorescence assay. Cells (5x10^4 cells/well) were plated on coverslips in 24-well plates following transfection and were cultured until 80% confluence was reached. Subsequently, cells were fixed in 4% paraformaldehyde at room temperature for 10 min and permeabilized with 0.05% Triton X-100. After blocking with 5% normal goat serum (Boster Biological Technology) for 1 h at room temperature, cells were incubated with a primary antibody against Ki67 (cat. no. 12075S; 1:1,000; Cell Signaling Technology, Inc.) overnight at 4˚C. The cells were washed three times with PBS, incubated with DyLight™ 488-conjugated secondary antibody (cat. no. 35553; 1:500; Invitrogen; Thermo Fisher Scientific, Inc.) for 2 h at 37˚C and stained with DAPI (Sigma-Aldrich; Merck KGaA) at room temperature for 15 min. The stained cells were subsequently imaged under a fluorescence microscope (magnification, x200; Olympus Corporation).

Transwell assay. The invasive ability of A549 cells was detected using an 8-μm pore insert precoated with Matrigel (BD Biosciences) overnight at 37˚C. A total of 200 μl serum-free medium containing cells was added to the upper chamber following 48 h of transfection. RPMI-1640 containing 10% FBS was added to the lower compartment as a chemoattractant. After 24 h of incubation, cells invading the lower chamber were fixed with 4% formaldehyde for 20 min at 37˚C and stained with 0.1% crystal violet for 30 min at 37˚C. Images were captured using an inverted light microscope (magnification, x100; Olympus Corporation) and the number of invasive cells were counted from five randomly selected fields.

Wound healing assay. For the wound healing assay, cells (3x10^5 cells/well) were incubated in a 6-well culture plate to achieve 80% confluence. Subsequently, cells were incubated with serum-free medium overnight at 37˚C prior to the experiment. Then, the cell monolayer was scratched using a sterilized 200-μl pipette tip. The migrated cells were observed

at 0 and 24 h following the creation of the wound, using a phase contrast microscope (magnification, x100; Olympus Corporation). Quantitative analysis of the wound healing area was performed using ImageJ software (version 1.52r; National Institutes of Health).

Luciferase activity reporter assay. The TargetScan database 7.1 (http://www.targetscan.org/vert_71) was used to predict the targets of PITPNA-AS1. The wild-type (WT) and mutant (MUT) sequences of PITPNA-AS1 3'UTR were amplified by Shanghai GenePharma Co., Ltd., cloned into a pGL3 luciferase vector (Promega Corporation) and respectively named pGL3-PITPNA-AS1-WT and pGL3-PITPNA-AS1-MUT. Cells were co-transfected with miR-32-5p mimic (Shanghai GenePharma Co., Ltd.; 50 nM) or mimic-NC (Shanghai GenePharma Co., Ltd.; 50 nM) and 50 ng pGL3-PITPNA-AS1-WT or pGL3-PITPNA-AS1-MUT. Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used for transfection. Following 48 h of transfection at 37°C, a Dual Luciferase Reporter Assay kit (Promega Corporation) was used to evaluate the relative luciferase signals, and firefly luciferase activity was normalized to *Renilla* luciferase activity.

RT-qPCR. Total RNA was extracted from A549 cells using TRIzol® (Thermo Fisher Scientific, Inc) following the manufacturer's instructions. Subsequently, cDNA was synthesized at 42°C for 30 min using a reverse transcription kit (PrimeScript™ RT Reagent kit; Takara Bio, Inc.). RT-qPCR was then performed with 2 μg cDNA, as the template using iTaq™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, Inc.) on an ABI 7500 instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used: Initial denaturation at 95°C for 10 min; followed by 40 cycles of denaturation at 95°C for 15 sec and annealing at 60°C for 1 min; and a final extension of 10 min at 72°C. The following primers pairs were used: PITPNA-AS1 forward, 5'-GCAGGGTGGATAAAGAGGA-3' and reverse, 5'-CCTACTGACAGGATGTCCT-3'; GAPDH forward, 5'-ACAACTTTGGTATCGTGGAAGG-3' and reverse, 5'-GCCATCACGCCACAGTTTC-3'; miR-32-5p forward, 5'-CGGTATTGCACATTACTAAGTTGCA-3' and reverse, 5'-CTCGCTTCGGCAGCACA-3'; U6 forward, 5'-AGGGGC CATCCACAGTCTTC-3' and reverse, 5'-AACGCTTCACGA ATTTGCGT-3'. GAPDH or U6 was used as the internal reference gene. The $2^{-\Delta\Delta Cq}$ method was used to compare relative expression levels (13).

Western blot analysis. Protein samples were extracted from cells using RIPA lysis buffer (Beyotime Institute of Biotechnology) for western blot analysis. The concentration of the protein samples was determined using the BCA protein assay kit (Beyotime Institute of Biotechnology). The protein samples (40 μg/lane) were separated using 10% SDS-PAGE and transferred onto PVDF membranes. After blocking with 5% skimmed milk for 1.5 h at room temperature, the blots were incubated with specific primary antibodies at 4°C overnight. Subsequently the membranes were washed three times with TBS-0.2% Tween-20, then incubated with a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:3,000; cat. no. 7074S; Cell Signaling Technology, Inc.) or horse anti-mouse HRP-conjugated secondary antibody (1:3,000; cat. no. 7076S; Cell Signaling Technology, Inc.) for 1.5 h at room temperature. The immunoreactive protein bands on the membranes were visualized using an enhanced chemiluminescence assay (EMD Millipore). The intensity of the bands was semi-quantified using ImageJ software (version 1.52r; National Institutes of Health). The following primary antibodies were used: Anti-Ki67 (cat. no. sc-23900; 1:1,000; Santa Cruz Biotechnology, Inc.), anti-proliferating cell nuclear antigen (PCNA; cat. no. 13110T; 1:1,000; Cell Signaling Technology, Inc.), anti-MMP2 (cat. no. 40994S; 1:1,000; Cell Signaling Technology, Inc.), anti-MMP9 (cat. no. 13667T; 1:1,000; Cell Signaling Technology, Inc.), anti-E-cadherin (E-cad; cat. no. 3195T; 1:1,000; Cell Signaling Technology, Inc.), anti-N-cadherin (N-cad; cat. no. 13116T; 1:1,000; Cell Signaling Technology, Inc.), anti-Vimentin (Vim; cat. no. 5741T; 1:1,000; Cell Signaling Technology, Inc.) and anti-GAPDH (cat. no. 5174T; 1:1,000; Cell Signaling Technology, Inc.). GAPDH was used as an internal control.

Statistical analysis. All experiments were repeated independently in triplicate. Data are presented as the mean ± standard deviation. Statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Software, Inc.). Statistical comparisons between two groups were performed using an unpaired Student's t-test. One-way ANOVA followed by Tukey's post hoc test was conducted for comparison among multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

PITPNA-AS1 is highly expressed in NSCLC cell lines. To investigate the role of PITPNA-AS1 in NSCLC, The Cancer Genome Atlas (TCGA) database was used to analyze the expression of PITPNA-AS1 in NSCLC tumor tissues (Tumor; n=1,038) and adjacent non-tumor tissues (healthy; n=322). The mRNA expression level of PITPNA-AS1 was increased in NSCLC tissues compared with that in adjacent non-tumor tissue (Fig. 1A). Subsequently, the mRNA expression level of PITPNA-AS1 was detected in several NSCLC cells using RT-qPCR. The expression level of PITPNA-AS1 was increased in the NSCLC cell lines, particularly in A549 cells, compared with that in the BEAS-2B cells (Fig. 1B). Therefore, the A549 cell line was selected for further experimentation, as it exhibited the highest PITPNA-AS1 mRNA expression level.

PITPNA-AS1 silencing inhibits the proliferation of NSCLC cells. To evaluate the effect of PITPNA-AS1 on the proliferation of NSCLC cells, the mRNA expression level of PITPNA-AS1 was silenced via transfection with shRNA-PITPNA-AS1-1 or shRNA-PITPNA-AS1-2. The results demonstrated that A549 cells transfected with shRNA-PITPNA-AS1-1 displayed the lowest mRNA expression levels of PITPNA-AS1 compared with the shRNA-NC (Fig. 1C). Therefore, shRNA-PITPNA-AS1-1 was used in the following experiments.

PITPNA-AS1 silencing significantly inhibited the proliferation and colony formation of A549 cells compared with the
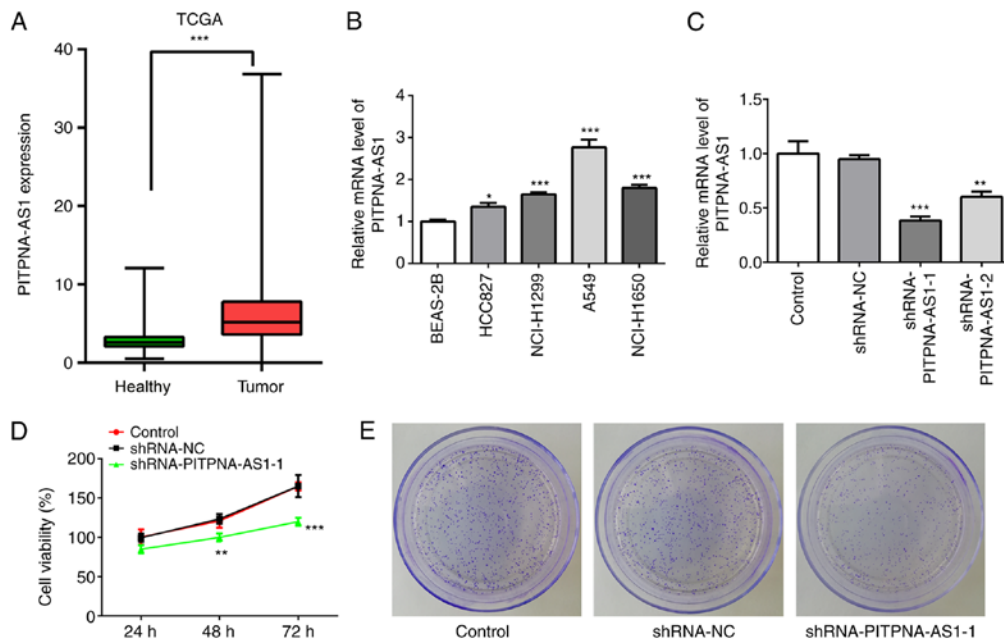
Figure 1. PITPNA-AS1 is highly expressed in NSCLC cell lines and PITPNA-AS1 silencing inhibits the proliferation of A549 cells. (A) TCGA database was used to analyze the mRNA expression of PITPNA-AS1 in NSCLC tumor tissues and adjacent non-tumor tissues. ***$P<0.001$ vs. healthy. (B) mRNA expression of PITPNA-AS1 in several NSCLC cell lines and one normal human bronchial epithelioid cell line (BEAS-2B) was examined using RT-qPCR. *$P<0.05$, ***$P<0.001$ vs. BEAS-2B. (C) mRNA expression of PITPNA-AS1 was measured using RT-qPCR following transfection with shRNA-PITPNA-AS1. Proliferation of A549 cells was detected using (D) Cell Counting Kit-8 and (E) colony formation assays. **$P<0.01$, ***$P<0.001$ vs. shRNA-NC. NC, negative control; sh, short inhibiting; RT-qPCR, reverse transcription-quantitative PCR; NSCLC, non-small cell lung cancer; PITPNA-AS1, PITPNA antisense RNA 1; TCGA, The Cancer Genome Atlas.
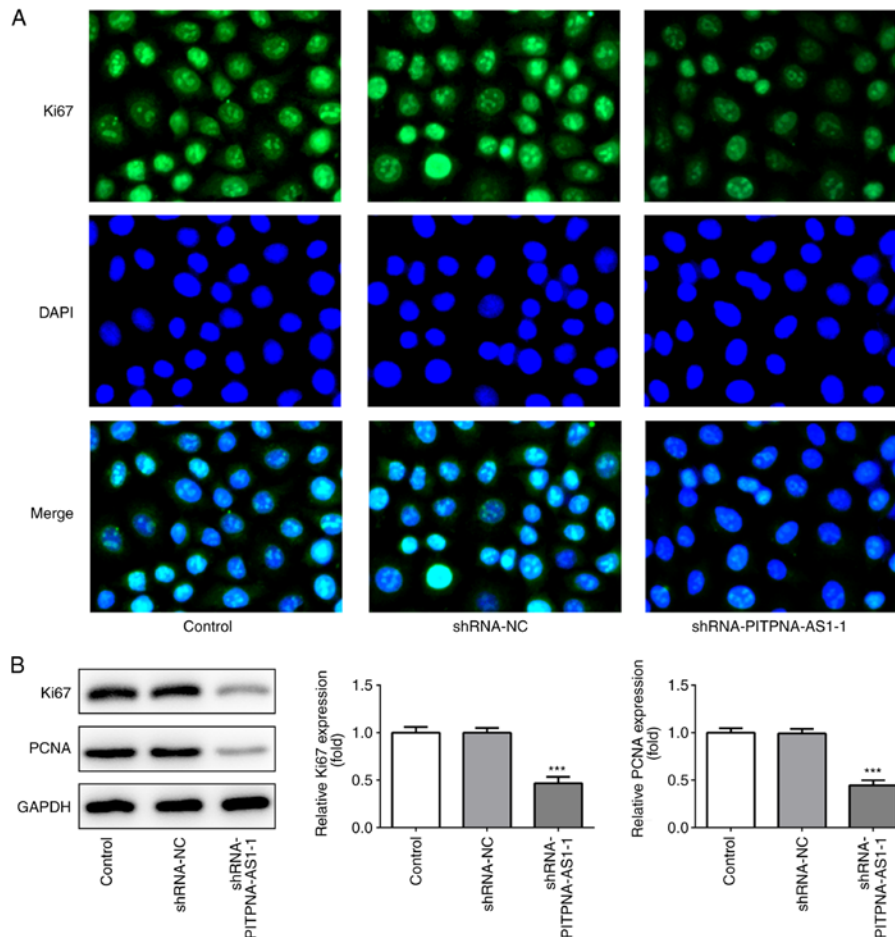

Figure 2. PITPNA-AS1-silencing decreases the expression levels of proliferation-associated proteins in A549 cells. (A) Immunofluorescence assay was used to evaluate the expression of Ki67. Magnification, x200. (B) Expression levels of Ki67 and PCNA were examined using western blot analysis. ***$P<0.001$ vs. shRNA-NC. NC, negative control; sh, short inhibiting; PCNA, proliferating cell nuclear antigen; PITPNA-AS1, PITPNA antisense RNA 1.
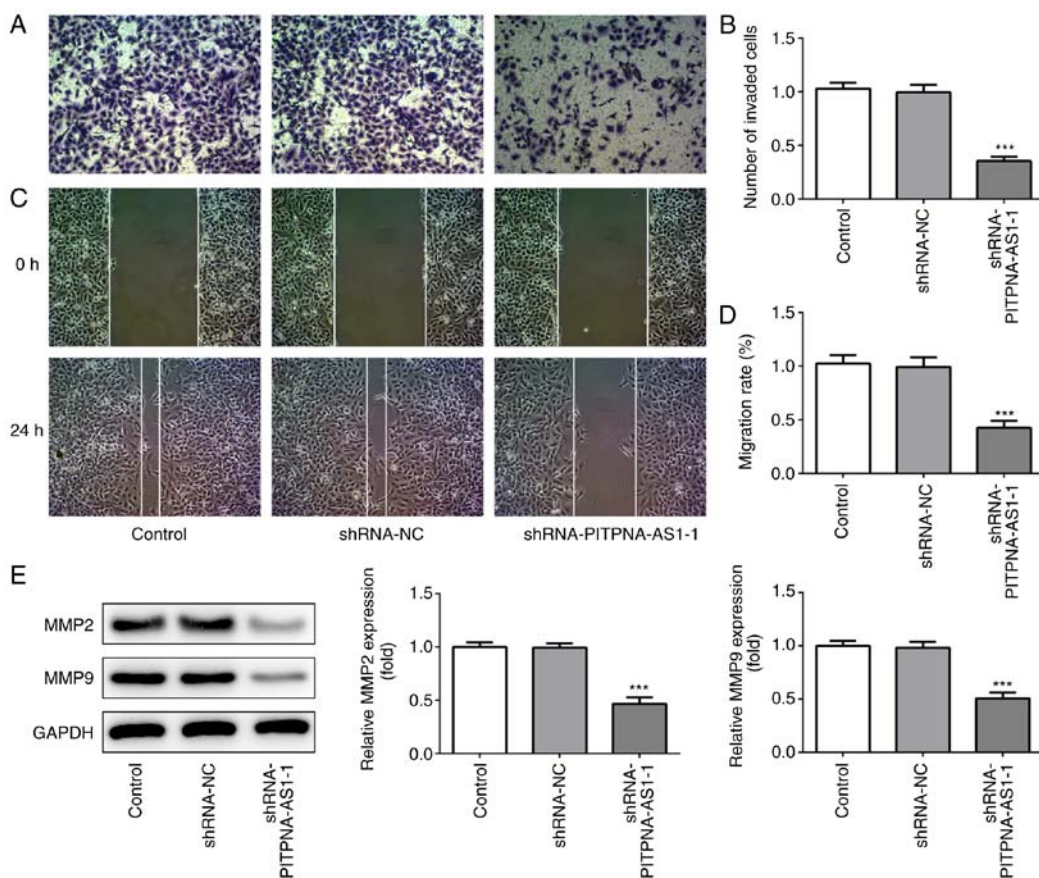
Figure 3. PITPNA-AS1 silencing suppresses the invasion and migration of A549 cells. (A) Invasive activity of A549 cells was determined using Transwell assay and the (B) number of invaded cells was quantified. Magnification, x100. (C) Representative images and (D) relative quantification of cell migration, as measured using a scratch assay at different time points. Magnification, x100. (E) Expression levels of migration-associated proteins were detected using western blot analysis. ***P<0.001 vs. shRNA-NC. NC, negative control; sh, short inhibiting; PITPNA-AS1, PITPNA antisense RNA 1.

shRNA-NC group (Fig. 1D and E). Subsequently, the expression levels of the proliferation-associated proteins, Ki67 and PCNA, were detected using immunofluorescence assay (Fig. 2A) and western blot analysis (Fig. 2B). PITPNA-AS1-knockdown significantly downregulated the expression levels of Ki67 and PCNA. These results suggested that PITPNA-AS1 silencing suppressed the proliferation of A549 cells.

PITPNA-AS1 silencing reduces invasion, migration and epithelial-mesenchymal transition (EMT) in NSCLC cells. The effects of PITPNA-AS1 silencing on the invasion and migration of A549 cells were detected using Transwell and wound healing assays, respectively. The invasive ability of A549 cells following transfection with shRNA-PITPNA-AS1-1 was significantly alleviated compared with that in the shRNA-NC group (Fig. 3A and B). Moreover, the migratory ability of A549 cells was suppressed (Fig. 3C and D), along with a significant decrease in the expression levels of MMP2 and MMP9 (Fig. 3E).

The protein expression levels of EMT-related proteins were determined using western blot analysis. Silencing of PITPNA-AS1 significantly decreased the protein expression levels of N-cadherin (N-cad) and vimentin (vim), which are important mesenchymal marker genes (14), while it enhanced the expression of E-cadherin (E-cad), which is a crucial epithelial marker (Fig. 4) (15). These results provided evidence

that PITPNA-AS1-silencing inhibited the invasion, migration and EMT of NSCLC cells.

miR-32-5p is a direct target of PITPNA-AS1. The results from the TargetScan analysis identified that miR-32-5p was one of the potential targets of PITPNA-AS1 (Fig. 5A). It was found that the expression of miR-32-5p was decreased in NSCLC cells compared with that in the BEAS-2B cells (Fig. 5B). The transfection efficiency of the miR-32-5p mimic was assessed via RT-qPCR (Fig. 5C). Subsequently, a luciferase activity reporter assay was utilized to verify the potential association. Following transfection with miR-32-5p mimic and PITPNA-AS1-WT, the luciferase activity was significantly decreased compared with that in cells co-transfected with mimic-NC and PITPNA-AS1-WT (Fig. 5D). However, co-transfection of miR-32-5p mimic and PITPNA-AS1-MUT resulted in no change in the luciferase activity, suggesting that miR-32-5p could bind to the PITPNA-AS1 3'UTR. Moreover, significantly elevated miR-32-5p expression was identified following transfection with shRNA-PITPNA-AS1-1 (Fig. 5E). Thus, these results suggested that miR-32-5p was a direct target of PITPNA-AS1.

miR-32-5p inhibitor restores the effects of PITPNA-AS1-silencing on the proliferation of NSCLC cells. To investigate the regulatory association between PITPNA-AS1 and miR-32-5p in NSCLC,
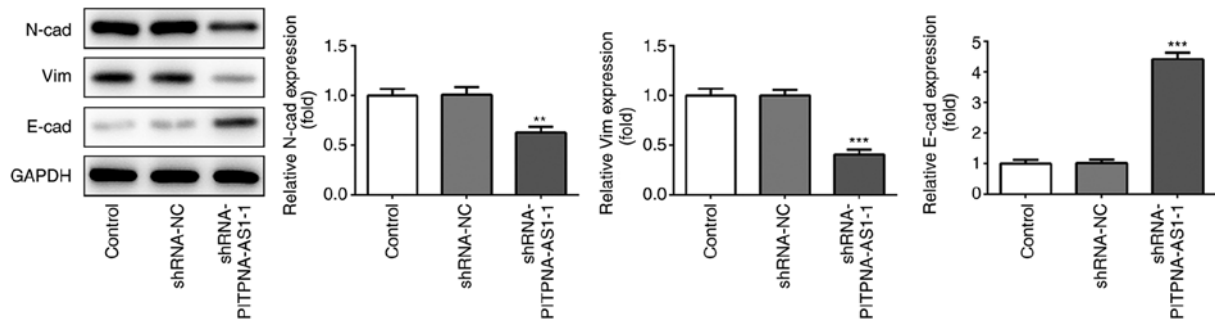
Figure 4. PITPNA-AS1 silencing suppresses EMT of A549 cells. Western blot analysis was performed to examine the expression levels of EMT-related proteins, including N-cad, E-cad and Vim. **$P<0.01$, ***$P<0.001$ vs. shRNA-NC. NC, negative control; sh, short inhibiting; PITPNA-AS1, PITPNA antisense RNA 1. EMT, epithelial-to-mesenchymal transition; N-cad, N-cadherin; E-cad, E-cadherin; Vim, Vimentin.
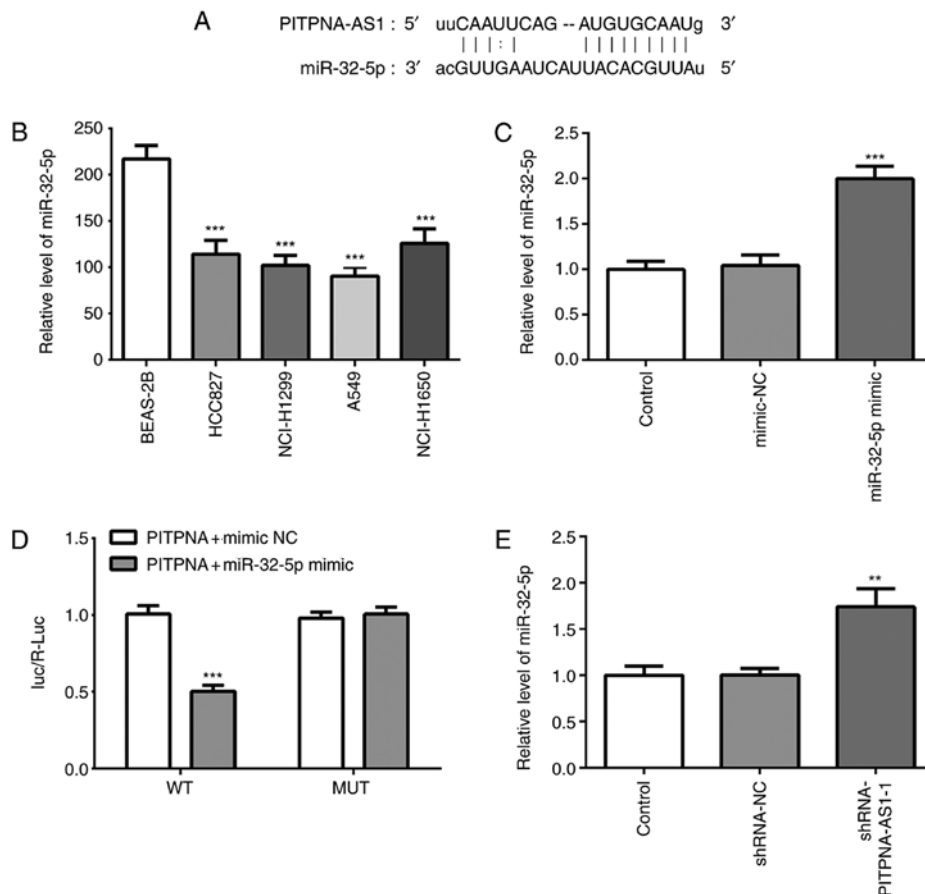

Figure 5. miR-32-5p is a direct target of PITPNA-AS1. (A) Binding region between PITPNA-AS1 and miR-32-5p. (B) Expression of miR-32-5p was detected using RT-qPCR in several NSCLC cell line and one normal human bronchial epithelioid cell line (BEAS-2B). ***$P<0.001$ vs. BEAS-2B. (C) Expression of miR-32-5p was measured using RT-qPCR after transfection with miR-32-5p mimic. ***$P<0.001$ vs. mimic-NC. (D) Relative luciferase activities were detected in A549 cells. ***$P<0.001$ vs. PITPNA-AS1 + mimic-NC. (E) RT-qPCR was employed to examine the expression of miR-32-5p in A549 cells transfected with shRNA-PITPNA-AS1-1. **$P<0.01$ vs. shRNA-NC. NC, negative control; sh, short inhibiting; PITPNA-AS1, PITPNA antisense RNA 1; RT-qPCR, reverse transcription-quantitative PCR; WT, wild-type; MUT, mutant; Luc, luciferase; miR, microRNA.

the mRNA expression of miR-32-5p was silenced using transfection with miR-211-5p inhibitor (Fig. 6A). Co-transfection with miR-32-5p inhibitor and shRNA-PITPNA-AS1-1 increased the proliferative ability of A549 cells compared with cells transfected with shRNA-PITPNA-AS1-1 alone, which was accompanied by an increase in Ki67 and PCNA expression levels (Fig. 6B and C). Collectively, it was indicated that PITPNA-AS1 could regulate the proliferation of NSCLC cells by targeting miR-32-5p.

miR-32-5p inhibitor counteracts the inhibitory effects of PITPNA-AS1-silencing on invasion, migration and EMT in NSCLC cells. It was found that the miR-32-5p inhibitor reversed the effect of PITPNA-AS1-silencing on the invasion (Fig. 6D and E) and migration (Fig. 6F and G) of A549 cells, which was also coupled with an increase in the protein expression levels of MMP2 and MMP9 (Fig. 6H). Consistently, the expression levels of EMT-related proteins, including N-cad, Vim and E-cad, in cells transfected with
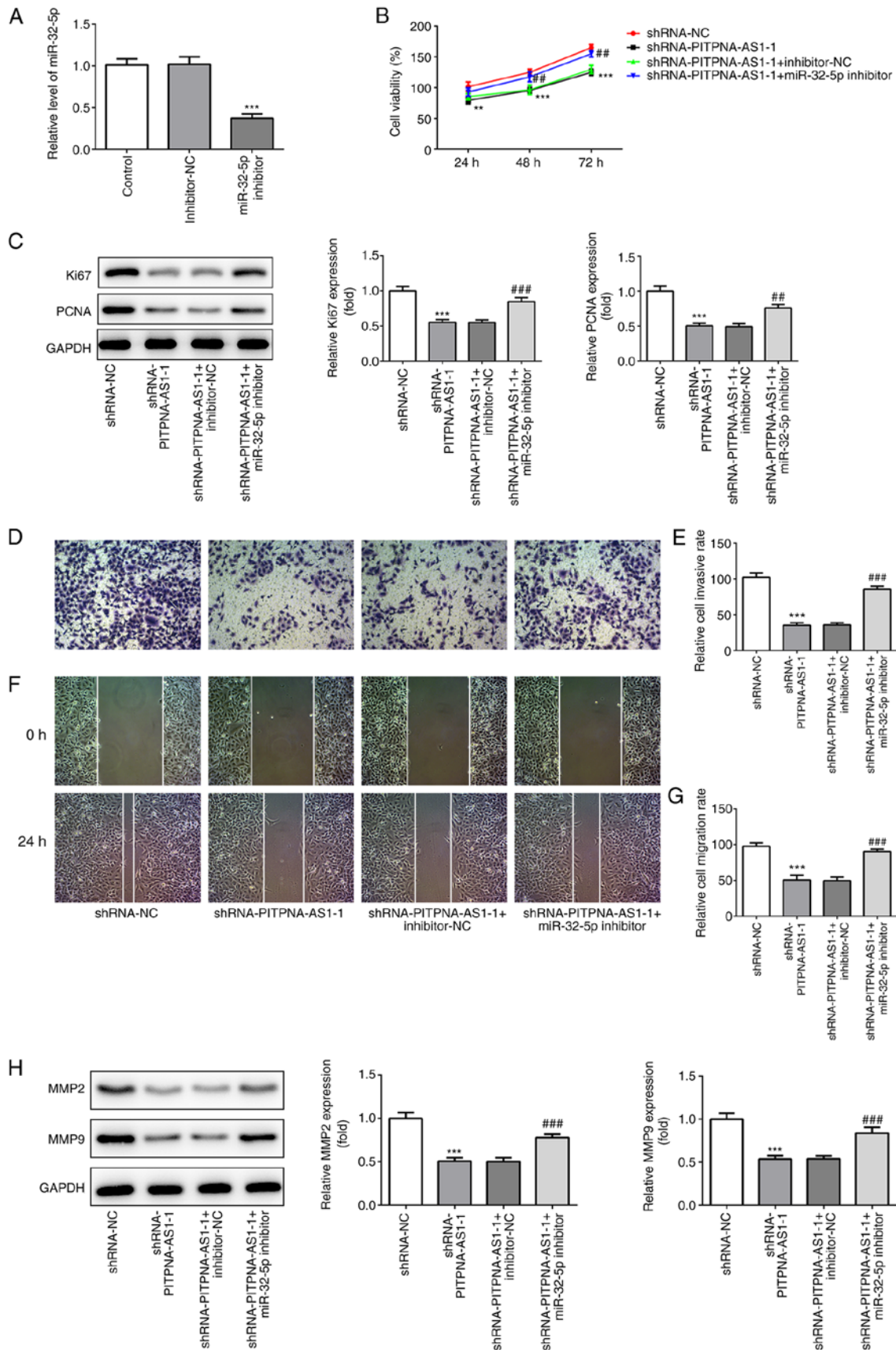
Figure 6. miR-32-5p inhibitor restores the effects of PITPNA-AS1-silencing on the proliferation, invasion and migration of A549 cells. (A) Expression of miR-32-5p was evaluated using RT-qPCR. ***P<0.001 vs. inhibitor-NC. The proliferative ability of A549 cells was measured using (B) Cell Counting Kit-8 assay and (C) the expression levels of proliferation-related proteins were tested using western blot analysis. (D) Transwell assay was performed to determine invasion of A549 cells and the (E) number of invaded cells was quantified. Magnification, x100. (F) Representative images and (G) relative quantification of cell migration, as examined using a scratch assay. Magnification, x100. (H) Western blot analysis was executed for measuring the expression of migration-associated proteins in A549 cells. **P<0.01, ***P<0.001 vs. shRNA-NC; ##P<0.01, ###P<0.001 vs. shRNA-PITPNA-AS1-1 + inhibitor-NC. NC, negative control; sh, short inhibiting; PITPNA-AS1, PITPNA antisense RNA 1; RT-qPCR, reverse transcription-quantitative PCR.
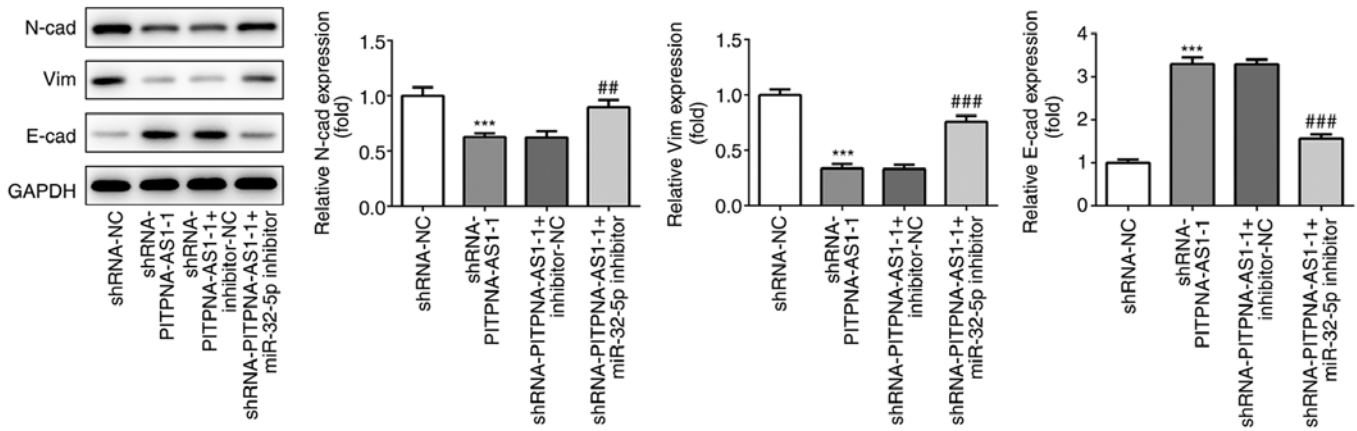
Figure 7. miR-32-5p inhibitor reverses the inhibitory impact of PITPNA-AS1-knockdown on EMT of A549 cells. Western blot analysis was performed to determine the expression levels of EMT-related proteins including N-cad, E-cad and Vim. ***P<0.001 vs. shRNA-NC; ##P<0.01, ###P<0.001 vs. shRNA-PITPNA-AS1-1+inhibitor-NC. NC, negative control; sh, short inhibiting; PITPNA-AS1, PITPNA antisense RNA 1; EMT, epithelial-to-mesenchymal transition; N-cad, N-cadherin; E-cad, E-cadherin; Vim, Vimentin.

shRNA-PITPNA-AS1-1 + miR-32-5p inhibitor demonstrated the opposite trends compared with those in cells transfected with shRNA-PITPNA-AS1-1 + inhibitor-NC (Fig. 7). Taken together, the results suggested that the miR-32-5p inhibitor attenuated the effects of PITPNA-AS1-silencing on the invasion, migration and EMT of NSCLC cells.

Discussion

Continuing advances in the development of next-generation sequencing and transcriptomics suggest that the investigation of lncRNAs in the regulation of cancer is a potential research field (16). Worldwide research has focused on identifying potential diagnostic and prognostic markers, which may accurately predict survival outcomes. Previous studies have reported that aberrant expression levels of lncRNAs are associated with the development and progression of different types of cancer, including NSCLC (17,18). In the present study, significantly upregulated PITPNA-AS1 expression was observed in NSCLC tissues and cell lines, and the TargetScan database predicted that miR-32-5p possesses potential binding sites with PITPNA-AS1. Therefore, the role of PITPNA-AS1 and miR-32-5p in the progression of NSCLC was further investigated.

PITPNA-AS1 is a newly reported lncRNA, which is located on chromosome 17p13.3 (11). A previous study revealed that PITPNA-AS1 could facilitate the progression of hepatocellular carcinoma via the regulation of the miR-876-5p/WNT5A signaling pathway (11). Analyzing data from TCGA database, the present results demonstrated that the expression of PITPNA-AS1 in NSCLC tissues was significantly increased compared with that in the adjacent non-tumor tissues. In addition, PITPNA-AS1 mRNA expression levels in NSCLC cell lines exhibited a similar trend compared with that in NSCLC tissues, suggesting the involvement of PITPNA-AS1 in NSCLC.

It is well-known that abnormal and uncontrolled proliferation is a characteristic of cancer cells (19). Furthermore, invasion and migration are considered as two dominant processes for tumor metastasis, which leads to

cancer-associated mortality (20). In this regard, interruption of the aforementioned processes is an effective method for resistance of cancer metastasis. The present study indicated that PITPNA-AS1-silencing reduced the proliferation, invasion and migration of A549 cells, suggesting the inhibitory effects of PITPNA-AS1 silencing on NSCLC.

EMT, a crucial driver of tumor progression, has been proposed as the classical tumor metastasis theory in previous years (21). Cells undergoing EMT lose the typical epithelial phenotype and are converted into a mesenchymal phenotype, with an increased migratory ability and invasiveness, which contributes to cancer metastasis (22). Aberrant expression of a large number of genes is observed during the activation of EMT. For example, the protein expression of E-cad, a crucial epithelial marker, is decreased, while there is an increase in the expression levels of N-cad and Vim, which are significant mesenchymal marker genes (23,24). An increasing number of studies have revealed that lncRNAs could modulate the invasion and metastasis of NSCLC cells via the regulation of the EMT process. For example, lncRNA FBXL19-AS1 accelerates the proliferation and metastasis of NSCLC by mediating EMT (25), while lncRNA double homeobox A pseudogene 8-downregulation suppresses metastasis via inhibition of EMT in NSCLC (26). In the present study, increased E-cad protein expression and decreased N-cad and vim expression levels were found following PITPNA-AS1-knockdown, suggesting an inhibitory effect of PITPNA-AS1 silencing on the EMT process.

An increasing number of studies have highlighted the importance of lncRNAs, acting as competing endogenous RNAs in tumor biology (27). Previous studies have reported that lncRNAs could modulate gene expression by sponging specific miRNAs, a class of endogenous conserved non-coding RNAs, ~18-25 nucleotides in length, by indirectly mediating gene expression at the post-transcriptional level (28). In the present study, the TargetScan database predicted that miR-32-5p was a potential target of PITPNA-AS1, which was verified using a luciferase activity reporter assay. It has been revealed that miR-32-5p inhibits the proliferation and metastasis of cervical cancer, colorectal cancer and

clear cell renal cell carcinoma (29-31). Furthermore, down-regulated miR-32-5p expression is observed in colorectal cancer cell lines, and SNHG14 can regulate proliferation, metastasis and EMT in colorectal cancer progression by targeting miR-32-5p (32). These findings prompted further investigation of the role of PITPNA-AS1 and miR-32-5p in the tumorigenesis and progression of NSCLC. The present study demonstrated that the effect of PITPNA-AS1-silencing on cell proliferation, metastasis and EMT was rescued by a miR-32-5p inhibitor. These observations indicated that PITPNA-AS1 could regulate the progression of NSCLC by targeting miR-32-5p.

In conclusion, to the best of our knowledge, the present study demonstrated for the first time, that PITPNA-AS1 was highly expressed in NSCLC tissues and cell lines. It was found that PITPNA-AS1 regulated the proliferation, invasion, migration and EMT of A549 cells, at least partially by targeting miR-32-5p, suggesting the novelty of this study, which presents a new promising target for the clinical diagnosis and therapeutic interventions of NSCLC. However, the lack of studies verifying the clinical value and prognostic value of PITPNA-AS1 and miR-32-5p in clinical tissue samples, as well as the relationship of PITPNA-AS1 and miR-32-5p to the clinical prognosis data of patients are limitations of the present research. Moreover, further experiments are required to elucidate the specific signaling pathway or specific signaling protein regulated by PITPNA-AS1 and miR-32-5p; therefore, comprehensive analysis is required in the future.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

GC, ZZ and JL searched the literature, designed the experiments and performed the experiments. PZ, ZW, SG and HL analyzed and interpreted the data. JM and JS searched the literature, performed the experiments and wrote the manuscript. HL revised the manuscript. GC confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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