miR-1301-3p promotes the proliferation and migration of lung cancer cells via direct repression of polymerase I and transcript release factor

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Abstract. Aberrant expression of microRNAs (miRNAs or miRs) is associated with a number of human diseases, including lung cancer. Although numerous differentially expressed miRNAs have been identified in lung cancer via microarray and sequencing methods, to the best of our knowledge, only a small portion of these miRNAs have been experimentally verified. In the present study, miR-1301-3p expression levels in lung tumor tissues and lung cancer cells were measured by reverse transcription-quantitative PCR (RT-qPCR) and by analyzing previously published data. Cell Counting Kit-8 and Transwell assays were used to analyze the function of miR-1301-3p in lung cancer tissues and cells. Bioinformatics analysis, RT-qPCR, western blotting and a dual-luciferase reporter assay were performed to investigate the mechanism of miR-1301-3p in lung cancer cells. It was identified that miR-1301-3p is an upregulated miRNA in lung cancer via analyzing previously published microarray and The Cancer Genome Atlas-lung squamous cell carcinoma project data, and the upregulation of miR-1301-3p was confirmed in collected clinical samples and cells. Inhibition of miR-1301-3p suppressed lung cancer cell proliferation and migration. In addition, miR-1301-3p inhibition upregulated E-cadherin, an epithelial cell maker, and downregulated vimentin, a mesenchymal cell marker. Using bioinformatics analysis, it was revealed that polymerase I and transcript release factor (PTRF) is a target of miR-1301-3p. RT-qPCR, western blotting and dual-luciferase reporter assays demonstrated that PTRF is targeted by miR-1301-3p in lung cancer cells. The rescue experiments indicated that silencing PTRF could attenuate the inhibition of cell proliferation and migration induced by miR-1301-3p inhibitor in lung cancer cells. Furthermore, a strong negative correlation between miR-1301-3p and PTRF mRNA was identified in clinical samples. In summary, the present data highlight the involvement of miR-1301-3p in the proliferation and migration of lung cancer cells, indicating that miR-1301-3p may be a promising biomarker for lung cancer.

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Introduction

Globally, lung cancer is the most prevalent cancer type in both males and females, and there were ~1,760,000 lung cancer-associated mortalities reported in 2018 (1). The majority of diagnosed lung cancer cases are histologically classified as non-small cell lung cancer (NSCLC) (2). NSCLC can be further divided into several subtypes, of which lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the most common types (3). Although the development of targeted therapy and cancer screening methods have improved the prognosis of patients with lung cancer, lung cancer remains a lethal cancer type for humans, with a 5-year overall survival rate of <20% (4). The high proliferative ability and aggressive nature of lung cancer cells is responsible for the poor prognosis of patients (5).

microRNAs (miRNAs or miRs) are short, non-coding molecules expressed in mammal cells (6). miRNAs can interact with the 3'-untranslated region (3'UTR) of target mRNAs, causing mRNA to degrade or for the translation process to stop (7). It is understood that miRNAs control the expression of key genes in multiple signaling pathways to promote normal physiological processes, such as cell differentiation and cell death (8). Dysregulation of miRNAs disrupts signaling networks and contributes to the progression of human diseases, including lung cancer (9,10). Numerous differentially expressed miRNAs have been identified in lung cancer according to several previous studies via microarray methods (11-13). Several miRNAs have been confirmed as key cancer-associated miRNAs in lung cancer via targeting oncogenes and tumor suppressors (14-16). For example, miR-19b

directly represses protein phosphatase 2 and BIM expression, activates epidermal growth factor receptor signaling, and promotes cell proliferation and apoptosis resistance in lung cancer cells (17). A previous study screened out numerous differentially expressed miRNAs in lung cancer with brain metastasis (13). However, the biological roles and expression patterns of the majority of these miRNAs have not yet been investigated.

The present study analyzed previously published microarray data (13) and identified miR-1301-3p as an upregulated miRNA in lung cancer with brain metastasis. Therefore, the aim was to investigate the expression, biological function and molecular mechanisms of miR-1301-3p in lung cancer cells.

Materials and methods

Patient samples. A total of 40 lung tumor tissue and matched normal tissue (>3 cm away from cancer) samples were obtained from patients (29 male and 11 female, age range 35-80 years; median 55 years) with lung cancer at Huadong Hospital Affiliated with Fudan University (Shanghai, China) between June 2014 and July 2017. All participants provided written informed consent. Lobectomy was performed to resect tumors and matched normal tissues from patients. None of the patients received chemotherapy or radiotherapy before the surgery and they did not have other malignancies. The protocol of the experiments was approved by the Ethical Committee of Huadong Hospital Affiliated with Fudan University (approval no. FDU201406-2). Specimens were snap-frozen in liquid nitrogen and stored in a -80˚C refrigerator.

Cell culture and cell transfection. The immortalized lung epithelial cell line BEAS-2B and lung cancer cell lines A549 and H1299 were purchased from the American Type Culture Collection. Cells were maintained in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin solution (HyClone; Cytiva) in an incubator at 37˚C with 5% CO_2. miR-1301-3p inhibitor (5'-GAAGUCACUCCC AGGCAGCUGCAA-3'), miR-1301-3p mimic (5'-UUGCAG CUGCCUGGGAGUGACUUC-3'), miR-negative control (NC) mimic (5'-UUCUCCGAACGUGUCACGUTT-3') and miR-NC inhibitor (5'-CAGUACUUUUGUGUAGUA CAA-3') were obtained from Guangzhou RiboBio Co., Ltd. A549 and H1299 cells were transfected with 50 nM miRNA mimic, inhibitor, miR-NC mimic or miR-NC inhibitor using Lipofectamine 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Polymerase I and transcript release factor (PTRF) small interfering RNA (siRNA) (5'-GAGAAGCGCAUGAACAAG CUGTT-3') and control siRNA (5'-UUCUCCGAACGUGUC ACGUTT-3') were obtained from Shanghai GenePharma Co., Ltd. Lipofectamine 3000 was used to transfect 100 nM siRNA into A549 and H1299 cells. After 48 h, the transfection efficiency was determined by western blotting and reverse transcription-quantitative PCR (RT-qPCR).

Cell proliferation and migration assays. The cell proliferation assay was performed with Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.). At 0, 24, 48 and 72 h post-transfection, CCK-8 was added to the culture medium and maintained for 2 h. Subsequently, the absorbance at 450 nM was detected by a i-Mark Microplate Reader (Bio-Rad Laboratories, Inc.) to measure the cell number.

The migration capacity of cancer cells was detected with an 8-μm pore size Boyden chamber (EMD Millipore). Cells were suspended in DMEM containing 0.5% FBS (Gibco; Thermo Fisher Scientific, Inc.) and seeded in the upper chamber, and DMEM containing 10% FBS was added to the lower chamber as the chemoattractant. Following 48 h, cells remaining in the upper side of the chamber were removed, and cells on the other side of the chamber were treated with 4% formaldehyde followed by crystal violet staining at room temperature for 15 min. Three random fields of miR-NC inhibitor + control siRNA, miR-1301-3p inhibitor + control siRNA and miR-1301-3p inhibitor + PTRF siRNA groups were examined with an inverted light microscope (magnification, x40; Nikon Corporation) and the number of migrated cells was analyzed.

RT-qPCR. RNA was collected from cells and tissues using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. First-stranded cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.) with dNTP set (Invitrogen; Thermo Fisher Scientific, Inc.) and Random Hexamers (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. qPCR was performed with a TB Green Premix Ex Taq™ II kit (Takara Bio, Inc.) on the CFX96 system (Bio-Rad Laboratories, Inc.). The thermocycling conditions were as follows: 95˚C for 10 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. mRNA and miRNA expression were normalized to GAPDH and U6. The $2^{-\Delta\Delta Cq}$ method (18) was applied to analyze the relative expression of genes. The primer sequences were as follows: E-cadherin forward, 5'-AAAGGC CCATTTCCTAAAAACCT-3' and reverse, 5'-TGCGTTCTC TATCCAGAGGCT-3'; Vimentin forward, 5'-TGCCGTTGA AGCTGCTAACTA-3' and reverse, 5'-CCAGAGGGAGTG AATCCAGATTA-3'; PTRF forward, 5'-AGGTCAGCGTCA ACGTGAAG-3' and reverse, 5'-CCGACTCTTTCAGCGATT TGC-3'; GAPDH forward, 5'-CTGGGCTACACTGAGCAC C-3' and reverse 5'-AAGTGGTCGTTGAGGGCAATG-3'; Stem-loop, 5'-CTCAACTGGTGTCGTGGAGTCGGCAAT TCAGTTGAGGAAGTC-3'; miR-1301-3p forward, 5'-GCC GAGTTGCAGCTGCCTGGGA-3' and reverse, 5'-CTCAAC TGGTGTCGTGGA-3'; and U6 forward, 5'-GCTTCGAGG CAGGTTACATG-3' and reverse, 5'-GCAACACACAACATC TCCCA-3'.

Western blot analysis. Lysates were obtained from cells using RIPA buffer (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The concentration of protein lysates was determined by a BCA protein assay kit (Thermo Fisher Scientific). PTRF (cat. no. 69036; 1:1,000), E-cadherin (cat. no. 14472; 1:1,000) and vimentin (cat. no. 5741; 1:1,000) antibodies were purchased from Cell Signaling Technology, Inc. GAPDH (cat. no. ab8245; 1:5,000) was obtained from Abcam. Horseradish peroxidase (HRP)-conjugated goat anti-mouse (cat. no. ab97040; 1:10,000) and HRP-conjugated goat anti-rabbit (cat. no. ab7090; 1:10,000) were also purchased

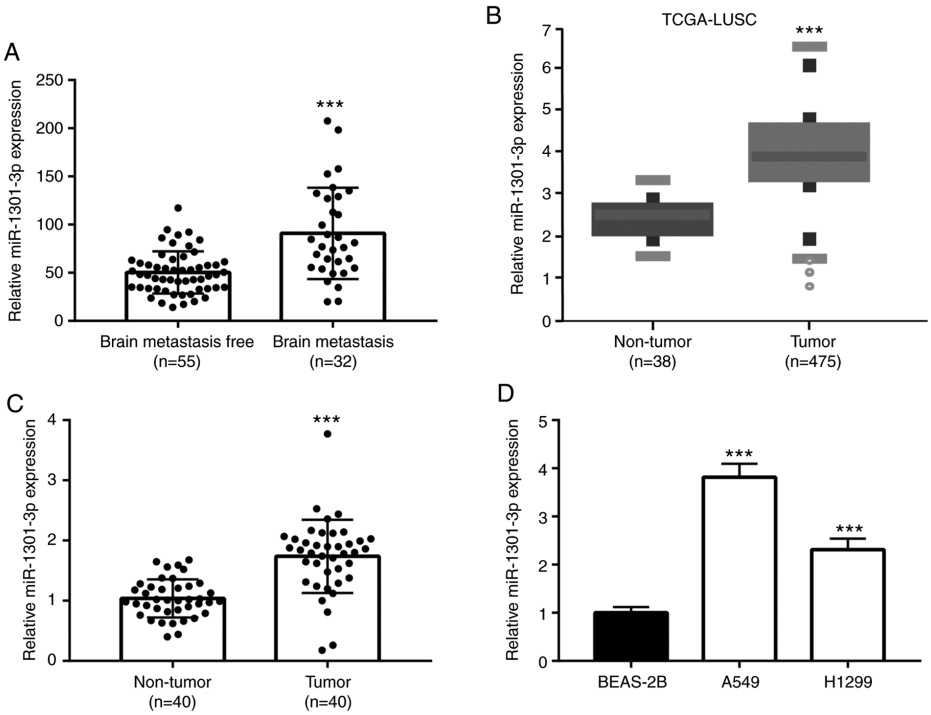

Figure 1. miR-1301-3p is increased in lung cancer. (A) Analysis of miR-1301-3p level in 32 lung tumor samples with brain metastasis and 55 lung tumor samples with non-brain metastasis from previously published microarray data. ***P<0.001 vs. non-brain metastasis. (B) The expression of miR-1301-3p in LUSC (n=475) and normal lung tissues (n=38) was analyzed in TCGA-LUSC dataset. ***P<0.001 vs. non-tumor. (C) The level of miR-1301-3p in 40 pairs of lung tumors and matched normal tissues was measured in the collected samples via RT-qPCR. ***P<0.001 vs. non-tumor. (D) The level of miR-1301-3p in BEAS-2B, A549 and H1299 cells was measured by RT-qPCR in this study. ***P<0.001 vs. BEAS-2B cells. RT-qPCR, reverse transcription-quantitative PCR; miR-1301-3p, microRNA-1301-3p; TCGA, The Cancer Genome Atlas; LUSC, lung squamous cell carcinoma.

from Abcam. Proteins (20 μg) were separated by 8% SDS-PAGE, and then transferred to a PVDF membrane. The PVDF membrane was blocked with 5% non-fat milk for 1 h at room temperature (25˚C), followed by incubation with the primary antibodies for 1 h at room temperature and secondary antibodies for 1 h at room temperature. The membrane was treated with the ECL Western Blotting Substrate (Pierce; Thermo Fisher Scientific, Inc.) to detect blot signals. The bands were quantified using ImageJ v1.52 software (National Institutes of Health).

Bioinformatics analysis. The expression data of miRNAs in lung cancer with brain metastasis (n=32) and those with non-brain metastasis (n=55) were obtained from previously published data (13). The association between the expression of miR-1301-3p and PTRF in LUAD tissues, LUSC tissues and normal lung tissues was analyzed using the Encyclopedia of RNA Interactomes (ENCORI) software (http://starbase.sysu.edu.cn/) based on The Cancer Genome Atlas (TCGA)-LUAD and TCGA-LUSC datasets (http://starbase.sysu.edu.cn/panMirDiffExp.php) (19). The ENCORI software was also used to evaluate the expression of miR-1301-3p in TCGA-LUSC dataset. The prediction of targets of miR-1301-3p and putative binding sites was performed using TargetScan version 7.2 (http://www.targetscan.org/vert_72/).

Dual-luciferase reporter assay. PTRF 3'UTR was inserted into the pmirGLO vector (Promega Corporation). pmirGLO-PTRF-Mut (mutant) was established via introducing two-point mutations into pmirGLO-PTRF-WT (wild-type). pmirGLO-PTRF-WT or pmirGLO-PTRF-WT were co-transfected with miR-1301-3p mimic or miR-NC mimic using Lipofectamine 3000 (Thermo Fisher Scientific, Inc.) into A549 cells. After 48 h, the luciferase activity was detected with a Dual-Luciferase® Reporter Assay System kit (Promega Corporation). Firefly luciferase activity was normalized to that of *Renilla* luciferase.

Statistical analysis. Data were analyzed using GraphPad Prism 5.0 (GraphPad Software, Inc.) and presented as mean ± SD, and P<0.05 was considered to indicate a statistically significant difference. The experiments were repeated in triplicate. Two groups were analyzed by unpaired Student's t-test. Multiple groups were analyzed by one-way analysis of variance with Tukey's post hoc test. The correlation between miR-1301-3p and PTRF expression levels was examined with Pearson's correlation test.

Results

miR-1301-3p is increased in lung cancer. To investigate the miRNAs involved in metastasis of lung cancer, previously published microarray data (13) were analyzed. It was identified that miR-1301-3p was one of the miRNAs expressed at a significantly higher level in lung tumors with brain metastasis (n=32) compared with those with non-brain metastasis (n=55) (Fig. 1A). Furthermore, the expression of miR-1301-3p was evaluated in TCGA-LUSC dataset, which included expression profile data of 38 normal lung tissues and 475 NSCLC tissues. The results demonstrated that miR-1301-3p was significantly

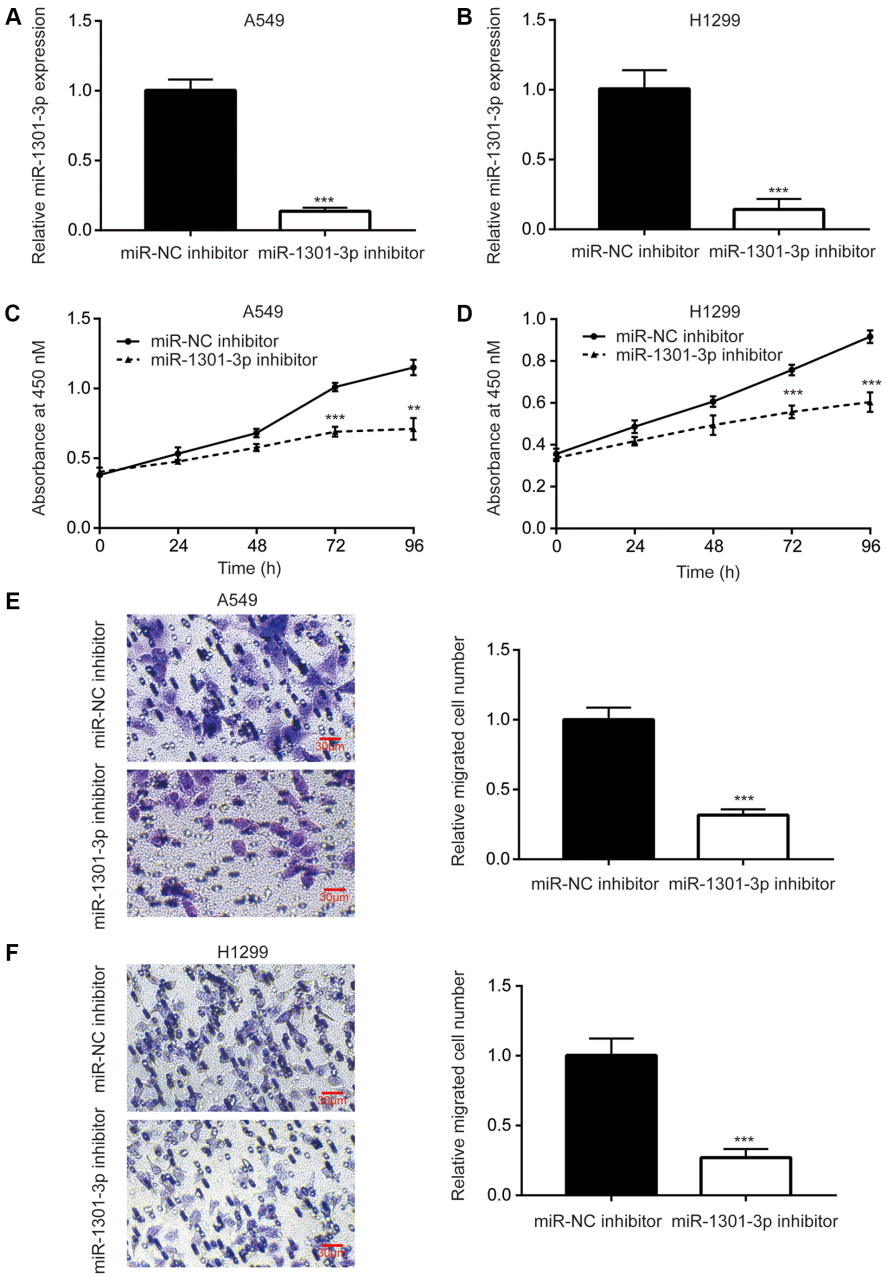

Figure 2. Downregulation of miR-1301-3p negatively affects lung cancer cell proliferation and migration. Reverse transcription-quantitative PCR was performed to measure miR-1301-3p level in (A) A549 and (B) H1299 cells after transfection with miR-1301-3p inhibitor or NC. Via Cell Counting Kit-8 assay, cell proliferation of (C) A549 and (D) H1299 cells following transfection with miR-1301-3p inhibitor or NC was detected. Cell migration of (E) A549 and (F) H1299 cells after transfection of miR-1301-3p inhibitor or NC was measured. **P<0.01, ***P<0.001 vs. miR-NC inhibitor. miR, microRNA; NC, negative control.

upregulated in lung cancer tissues compared with normal lung tissues (Fig. 1B). For validation, 40 pairs of tumors and matched normal samples were collected from patients with lung cancer. RT-qPCR revealed that miR-1301-3p was significantly higher in lung cancer samples compared with matched normal samples (Fig. 1C). Furthermore, it was observed that miR-1301-3p expression was significantly higher in A549 and H1299 cells compared with the immortalized lung epithelial cell line BEAS-2B (Fig. 1D).

Downregulation of miR-1301-3p suppresses lung cancer cell proliferation and migration. miR-1301-3p inhibitor was transfected into lung cancer cell lines A549 and H1299. RT-qPCR was then performed to confirm that miR-1301-3p inhibitor significantly reduced miR-1301-3p expression in A549 and H1299 cells (Fig. 2A and B). miR-1301-3p inhibitor significantly suppressed the proliferation of A549 and H1299 cells at 72 and 96 h (Fig. 2C and D). In addition, Transwell assay demonstrated that miR-1301-3p inhibitor significantly decreased the number of A549 and H1299 cells that migrated through the membrane (Fig. 2E and F). These data demonstrated that miR-1301-3p is associated with lung cancer cell proliferation and migration.

PTRF is suppressed by miR-1301-3p. Using TargetScan, several genes were predicted as targets of miR-1301-3p. It was identified that there was a complementary site for miR-1301-3p on the 3'UTR of PTRF (Fig. 3A). Using ENCORI software,

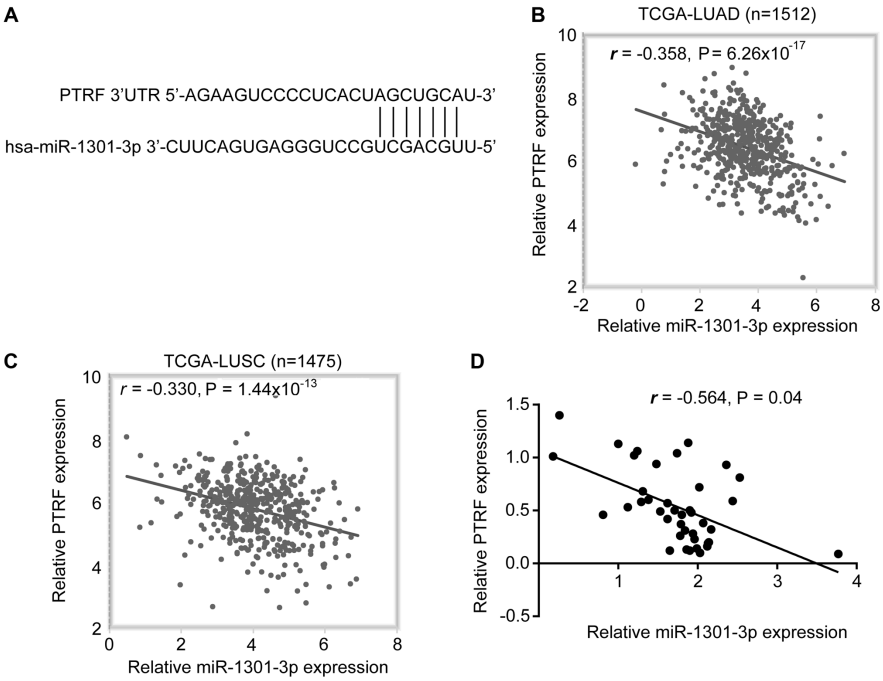

Figure 3. PTRF is a predicted target of miR-1301-3p. (A) The sequence alignment of PTRF 3'UTR and miR-1301-3p. (B) Bioinformatics analysis indicated that PTRF was inversely correlated with miR-1301-3p level in TCGA-LUAD dataset. (C) Bioinformatics analysis indicated that PTRF was inversely correlated with miR-1301-3p level in TCGA-LUSC dataset. (D) Pearson's correlation analysis was used to analyze the association between PTRF mRNA and miR-1301-3p expression in 40 lung tumors. LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; PTRF, polymerase I and transcript release factor; miR, microRNA; TCGA, The Cancer Genome Atlas; 3'UTR, 3'untranslated region.

it was further demonstrated that the miR-1301-3p expression level was significantly negatively correlated with the PTRF mRNA expression level in 1,512 LUAD samples and 1,475 LUSC samples from TCGA-LUAD and TCGA-LUSC, respectively (Fig. 3B and C). Additionally, it was identified that there was a negative correlation between miR-1301-3p and PTFR mRNA level in the collected patient samples (n=40; Fig. 3D).

Due to the higher expression of miR-1301-3p in A549 cells compared with that of H1299 cells, A549 were selected as a model to explore the molecular mechanism of miR-1301-3p in lung cancer. In A549 cells, miR-1301-3p inhibitor significantly increased PTRF mRNA expression level (Fig. 4A). Western blotting further demonstrated that miR-1301-3p inhibitor significantly increased PTRF protein level in A549 cells (Fig. 4B). Subsequently miR-1301-3p mimic was transfected into A549 cells to increase miR-1301-3p expression (Fig. 4C). Luciferase plasmids containing the 3'UTR of PTRF (PTRF 3'UTR-WT) or PTRF 3'UTR-Mut (with two-point mutations in the putative binding site) were constructed (Fig. 4D). Dual-luciferase reporter assay demonstrated that miR-1301-3p mimic significantly downregulated the luciferase activity of PTRF 3'UTR-WT, but not of PTRF 3'UTR-Mut in A549 cells (Fig. 4E).

miR-1301-3p regulates the expression of epithelial-mesenchymal transition (EMT) markers. PTRF is involved in the EMT process in lung cancer (20). In the present study, RT-qPCR demonstrated that miR-1301-3p inhibitor significantly increased E-cadherin and significantly decreased vimentin mRNA expression levels in A549 cells (Fig. 5A). Furthermore, western blotting revealed that miR-1301-3p inhibitor significantly increased E-cadherin and significantly decreased vimentin protein expression levels in A549 cells (Fig. 5B). These data indicated that miR-1301-3p regulates the EMT process in lung cancer.

Knockdown of PTRF attenuates the effect of miR-1301-3p downregulation in lung cancer. To evaluate the involvement of PTRF in miR-1301-3p-mediated cell proliferation and migration, PTRF siRNA was transfected into A549 cells. Western blotting confirmed that PTRF siRNA significantly decreased PTRF protein expression in A549 cells (Fig. 6A). By performing a CCK-8 assay, it was identified that PTRF-knockdown significantly reversed the effect of miR-1301-3p inhibitor on cell proliferation in A549 cells (Fig. 6B). Furthermore, in the migration assay, PTRF-knockdown significantly reversed the effect of miR-1301-3p on the migration of A549 cells (Fig. 6C). These data implied that miR-1301-3p facilitates cell proliferation and migration via targeting PTRF.

Discussion

A number of studies have suggested that miRNAs are involved in lung cancer progression (4,5); however, the functions of numerous miRNAs have not yet been studied. The role of miR-1301-3p in human cancer is controversial. miR-1301-3p expression is lower in serum samples of patients with colorectal cancer compared with those from healthy volunteers (21). miR-1301-3p is downregulated in pancreatic adenocarcinoma (22). However, upregulation of miR-1301-3p has been observed in prostate cancer (23). The present study re-analyzed the previously published microarray data and discovered that miR-1301-3p is increased

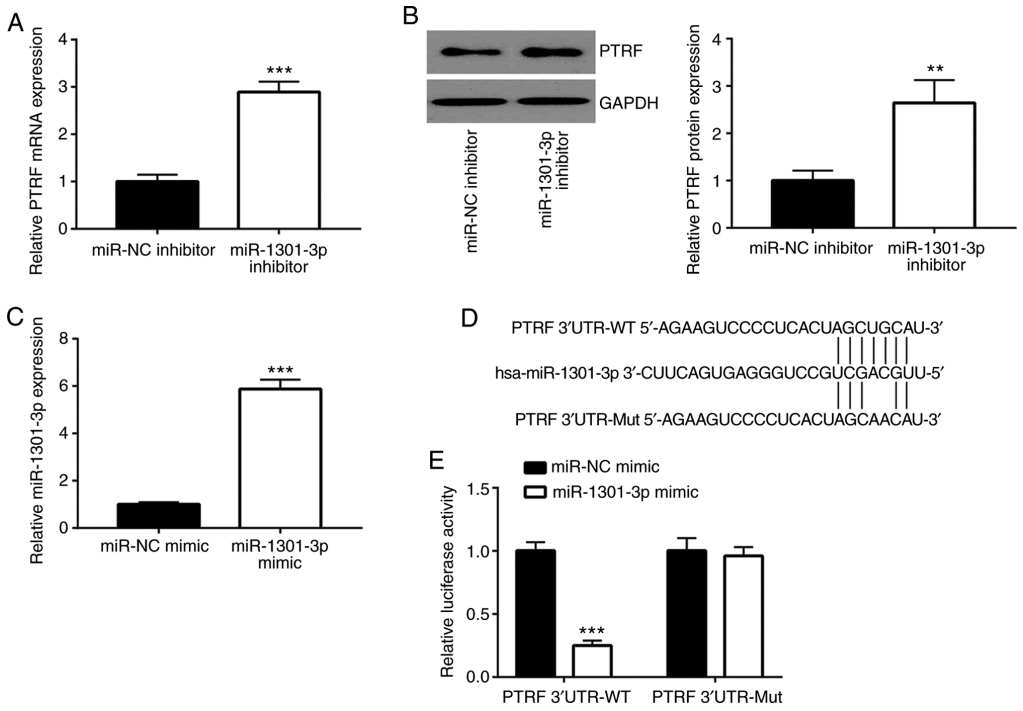

Figure 4. PTRF is directly targeted by miR-1301-3p in lung cancer cells. (A) RT-qPCR was used to measure PTRF mRNA levels in A549 cells after transfection of miR-1301-3p inhibitor or its NC. (B) Western blotting was performed to measure PTRF protein expression in A549 cells following transfection of miR-1301-3p inhibitor or its NC. (C) RT-qPCR was performed to detect miR-1301-3p expression in A549 cells transfected with miR-NC mimic or miR-1301-3p mimic. (D) Sequences of PTRF 3'UTR-WT, 3'UTR-Mut and miR-1301-3p. (E) Dual-luciferase reporter assay demonstrated that miR-1301-3p mimic repressed the relative luciferase activity of PTRF 3'UTR-WT in A549 cells. **P<0.01, ***P<0.001 vs. miR-NC inhibitor. miR, microRNA; NC, negative control; PTRF, polymerase I and transcript release factor; RT-qPCR, reverse transcription-quantitative PCR; 3'UTR, 3'-untranslated region; WT, wild-type; mut, mutant.

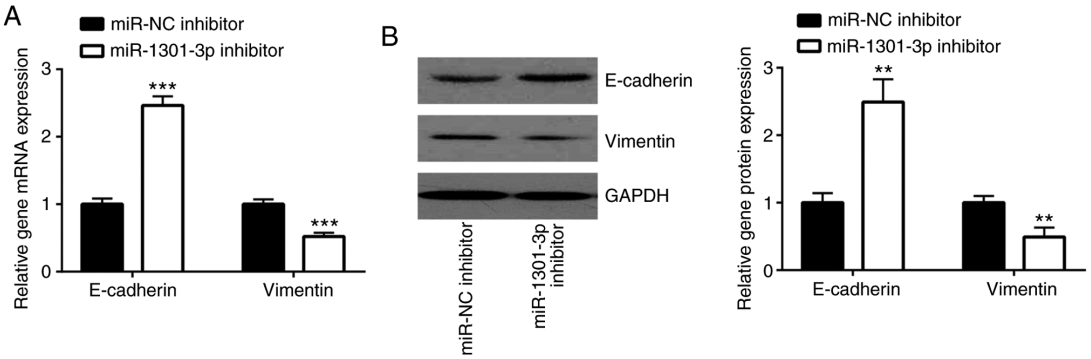

Figure 5. miR-1301-3p regulates the expression of key genes of the epithelial-mesenchymal transition. (A) Reverse transcription-quantitative PCR was performed to measure E-cadherin and Vimentin mRNA level in A549 cells following transfection with miR-1301-3p inhibitor or its NC. (B) Western blotting was performed to detect E-cadherin and Vimentin protein expression in A549 cells following transfection with miR-1301-3p inhibitor or its NC. **P<0.01, ***P<0.001 vs. miR-NC inhibitor. miR, microRNA; NC, negative control.

in lung cancer with brain metastasis compared with those with non-brain metastasis. It was further confirmed that miR-1301-3p was increased in lung tumors compared with matched normal tissues. miR-1301-3p promotes or inhibits cell growth in different cell backgrounds. In prostate cancer, miR-1301-3p targets ubiquitination factor E4B, activates the p53 signaling pathway and suppresses cancer cell metastasis (24). It has also been reported that miR-1301-3p targets PPP2R2C and promotes anchorage-dependent and independent cell proliferation in prostate cancer cells (23). Using CCK-8 and migration assays, the present study demonstrated that miR-1301-3p enhanced cell proliferation and migration of lung cancer. These data indicated that upregulation of miR-1301-3p may mediate lung cancer cell proliferation and migration. The current study evaluated the role of miR-1301-3p in lung cancer cells via *in vitro* assays. However, *in vivo* assays may provide several novel findings for investigating the function of miR-1301-3p in lung cancer. In the future, a mouse model will be used to study the involvement of miR-1301-3p in the initiation and development of lung cancer.

PTRF, also known as CAVIN1, is essential for the formation of caveolae (25). PTRF is a tumor suppressor in multiple cancer types (20,26-29). For example, PTRF overexpression restores caveolae formation, reduces matrix metalloproteinase-9 expression and inhibits the migration of

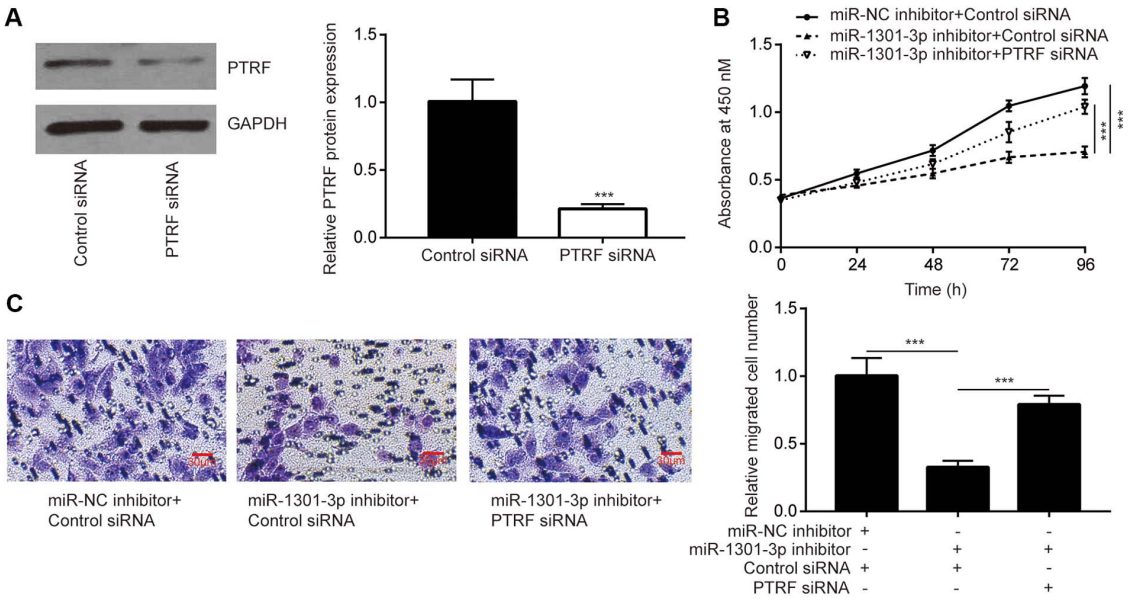

Figure 6. miR-1301-3p regulates lung cancer cell proliferation and migration via PTRF. (A) Western blotting was performed to measure PTRF protein level in A549 cells transfected with control siRNA or PTRF siRNA. ***P<0.001 vs. control siRNA. (B) The Cell Counting Kit-8 assay was performed to investigate proliferation of A549 cells after transfection with miR-1301-3p inhibitor or its NC in combination with control siRNA or PTRF siRNA. ***P<0.001. (C) The migration assay was performed to measure cell migration ability of A549 cells after transfection with miR-1301-3p inhibitor or its NC in combination with control siRNA or PTRF siRNA. ***P<0.001. miR, microRNA; NC, negative control; PTRF, polymerase I and transcript release factor; siRNA, small interfering RNA.

metastatic prostate cancer cells (29). In lung cancer, PTRF inhibits the EMT process and suppresses the cell proliferation and metastasis (20). The downregulation of PTRF is associated with dysregulation of miRNAs and DNA methylation in cancer cells (20,30). Via bioinformatics analysis, the present study identified that PTRF is a potential target of miR-1301-3p. The expression of miR-1301-3p was shown to be negatively correlated with PTRF in TCGA-LUAD and TCGA-LUSC datasets. In addition, a dual-luciferase reporter assay confirmed that miR-1301-3p targeted PTRF in A549 cells, indicating miR-1301-3p is a novel regulator of PTRF. The EMT process is essential for the metastatic potential of lung cancer cells and is associated with dysregulation of miRNAs (31-33). PTRF inhibits the EMT process in cancer cells (20). Consistently, it was identified that miR-1301-3p regulated the expression of key genes involved in the EMT process. Furthermore, silencing of PTRF attenuated the effect of miR-1301-3p downregulation on A549 cells. The results indicated that the miR-1301-3p/PTRF axis controls the EMT process and lung cancer cell metastasis. However, although the present study demonstrated a negative correlation between miR-1301-3p levels and PTRF mRNA levels, and it was experimentally revealed that miR-1301-3p targeted and repressed PTRF expression in lung cancer cells, several other mechanisms, such as post-translational modification may also regulate PTRF at the protein level. Future studies are needed to investigate the complex signaling network that regulates PTRF expression in lung cancer.

In conclusion, the current study revealed miR-1301-3p as a novel upregulated miRNA in lung cancer. miR-1301-3p was demonstrated to target PTRF and facilitate lung cancer cell proliferation and migration. Therefore, miR-1301-3p may be a promising biomarker for patients with lung cancer.

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Availability of materials and data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The results published here are in whole or part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga.

Authors' contributions

YunW, QS, XC, YueW, YN and FL performed the experiments and analyzed the data. YunW and QS collected clinical samples. QS and FL were major contributors in conception, design and writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All participants provided written informed consent. The protocol of the experiments was approved by the Ethical Committee of Huadong Hospital Affiliated with Fudan University (Shanghai, China) (approval no. FDU201406-2).

Patient consent for participation

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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