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miR-186-5p promotes cell growth, migration and invasion of lung adenocarcinoma by targeting PTEN

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ABSTRACT

Objective: To explore the expression of miR-186-5p in lung adenocarcinoma (LUAD) and its possible function associated with cancer cell proliferation, migration and invasion.

Methods: MiR-186-5p expression levels in LUAD samples, human LUAD cell lines H1299 and NCI-H1975, and normal human lung epithelial cell line BEAS-IB were assessed by quantitative real-time PCR (qRT-PCR). H1299 and NCI-H1975 cells were transfected with miR-186-5p mimic or miRNA negative control. CCK-8 assay was performed to evaluate the cell proliferation. Transwell assay and transwell-matrigel™ invasion assay were applied to assess the migration and invasion abilities of H1299 and NCI-H1975 cells.

Results: miR-186-5p expression was significantly up-regulated in LUAD tumor tissues and LUAD cell lines as compared with tumor-adjacent tissues and normal human lung epithelial cells, respectively. MiR-186-5p overexpression remarkably promoted the proliferation, migration and invasion of LUAD cells. Furthermore, phosphatase and tensin homolog (PTEN) was a direct target of miR-186-5p verified by luciferase reporter assay. Overexpression of PTEN significantly suppressed LUAD cells to proliferate, migrate and invade. MiR-186-5p overexpression-induced LUAD cell phenotype could be partially rescued by co-overexpression of miR-186-5p and PTEN.

Conclusion: This study demonstrated that miR-186-5p is up-regulated in LUAD, and functionally associated with cell proliferation, migration and invasion. MiR-186-5p promotes the proliferation, migration and invasion of LUAD cells by targeting PTEN. MiR-186-5p may be utilized as a novel molecular marker and therapeutic target of LUAD.

1. Introduction

To date, lung cancer is one of the most common malignant tumors and has been described as the most fatal type of cancers worldwide (Mutti, 2008; Bunn, 2012; Griesinger, 2017). Approximately 80% of lung cancer patients are diagnosed with non-small-cell lung cancer (NSCLC). In spite of the advances in the diagnosis and treatment of lung cancer that have been achieved in recent years, there remain great challenges due to the high recurrence and metastasis during cancer treatment (Diagnosis, 2011; Treatment of Metastatic Non-Small Cell Lung Cancer: A Systematic Review of Comparative Effectiveness and Cost-Effectiveness, 2012; Local Nonsurgical Therapies for Stage I and Symptomatic Obstructive Non–Small-Cell Lung Cancer, 2013; StatPearls, 2018). Therefore, it is extremely important to elucidate the molecular mechanisms underlying carcinogenesis and progression in NSCLC, which would contribute to providing promising therapeutic targets for lung cancer and improving the prognosis of this disease.

MicroRNAs (miRNAs) are a class of small, well-conserved non-coding RNAs (18–25 nucleotides in length) that regulate the expression of their target genes at the post transcriptional level by binding to 3′-untranslated regions (3′-UTRs) of the target genes (Bushati and Cohen, 2007; Zhang et al., 2007; Li et al., 2009). Increasing evidence indicates that miRNAs are involved in cancer development and progression and play important roles in regulating cancer-related genes (DeSano and Xu, 2009; Li et al., 2010; Kim et al., 2012; Vo et al., 2014; Zhao et al., 2018). It was found that miR-186-5p upregulation inhibited proliferation, metastasis and epithelial-to-mesenchymal transition of colorectal cancer cell by targeting ZEB1 (Li et al., 2018). It was also reported that

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Table 1
Patient characteristics.

Characteristics	Number (%)
Age (years)	
Median (range)	67.1 (48–80)
Gender	
Males	14 (70%)
Females	6 (30%)
Clinical stage	
T1	12 (60%)
T2	8 (40%)
Histological sub-classification	
Adenocarcinoma	20 (100%)
Smoking status	
Non-smoker (0 years)	12 (60%)
Previous light smoker (1–10 years)	1 (5%)
Previous heavy smoker (> 10 years)	2 (10%)
Current smoker	5 (25%)

miR-186-5p inhibition attenuated proliferation, anchorage independent growth and invasion in metastatic prostate cancer cells (Jones et al., 2018). Additionally, miR-186-5p overexpression modulated colon cancer growth by repressing the expression of the FAM134B tumor inhibitor (Islam et al., 2017). These results suggest the key functions of miR-186-5p in cancer. However, up to now, the detailed mechanisms of how miR-186-5p is regulated in lung adenocarcinoma (LUAD) and how miR-186-5p impacts the disease are largely unknown.

In the present study, our aim is to investigate the biological functions of miR-186-5p on LUAD and to explore the underlying molecular mechanisms. Here, we report that miR-186-5p is remarkably overexpressed in LUAD compared with the matching tumor-adjacent tissues, and for the first time that miR-186-5p directly targets and regulates the 3′-UTR of the human tumor suppressor gene phosphatase and tensin homolog (PTEN) mRNA, which is commonly down-regulated in many cancers. Overall, our results suggest that miR-186-5p promotes cell proliferation, migration and invasion in LUAD by directly targeting 3′-UTR of PTEN.

2. Materials and methods

2.1. Data analysis

The data analysis of miR-186-5p expression, PTEN expression and the correlation between miR-186-5p and PTEN was downloaded from starbase Pan-Cancer Analysis Platform (http://starbase.sysu.edu.cn/panCancer.php). The starbase Pan-Cancer Analysis Platform is designed for decoding Pan-cancer Networks of lncRNAs, miRNAs, pseudogenes, snoRNAs, RNA-binding proteins (RBPs) by analyzing their expression profiles across 32 cancer types integrated from TCGA project via Genomic Data Commons Data Portal.

2.2. Tumor tissue samples and cell lines

This study was approved by the human ethics and research ethics committees of China-Japan Friendship Hospital. This study included human LUAD tissues and tumor-adjacent lung tissues (> 2 cm away from the cancerous region) derived from 20 patients who underwent surgery. The clinicopathologic features of the patients are listed in Table 1. None of these patients received any chemotherapy or radiotherapy. All samples were immediately frozen in liquid nitrogen after surgery. The human LUAD cell lines H1299 and NCI-H1975 and normal human lung epithelial cell line BEAS-IS were purchased from Cell Bank, Chinese Academy of Sciences (Shanghai, China).

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from indicated cells by using TRIzol reagent (Invitrogen) according to the manufacturer's instructions, and then subjected to reverse transcription. The expression levels of genes and miRNAs were measured by the qRT-PCR kit (Life Technologies, Shanghai, China) using the QuantStudio® 6 Flex Real-Time PCR System (Life Technologies, Shanghai, China) with SYBR Green kit (TaKaRa, Tokyo, Japan). The relative expression levels of genes and miRNAs were quantified. TaqMan advanced miRNA assay kits of miR-186-5p (477940_mir) were purchased from Applied Biosystems (Carlsbad, CA,

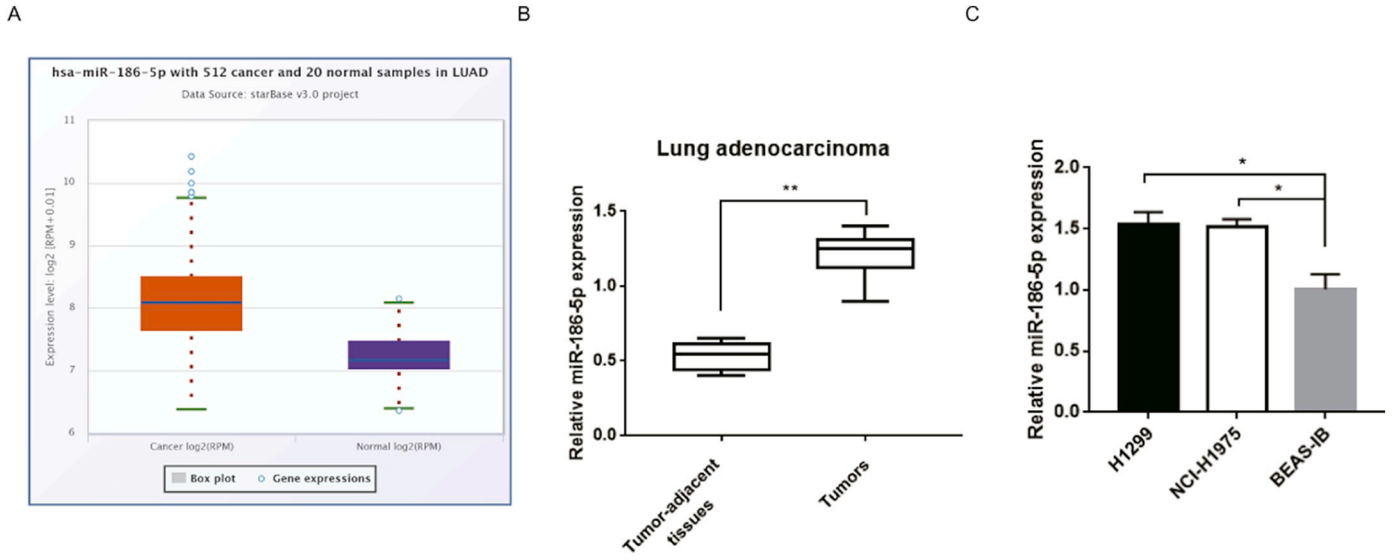

Fig. 1. The expression of miR-186-5p in LUAD tissues and cell lines. A. The expression levels of miR-186-5p in LUAD tissues and normal lung tissues obtained from starbase v3.0 project. B. qPCR quantification of miR-186-5p in LUAD tissues and tumor-adjacent lung tissues. C. qPCR examination of miR-186-5p in LUAD cell lines H1299 and NCI-H1975 and human lung epithelial cell line BEAS-IB. Assays were performed in triplicate. Data are expressed as mean ± SD. $^{*}P < .05$. $^{**}P < .01$.

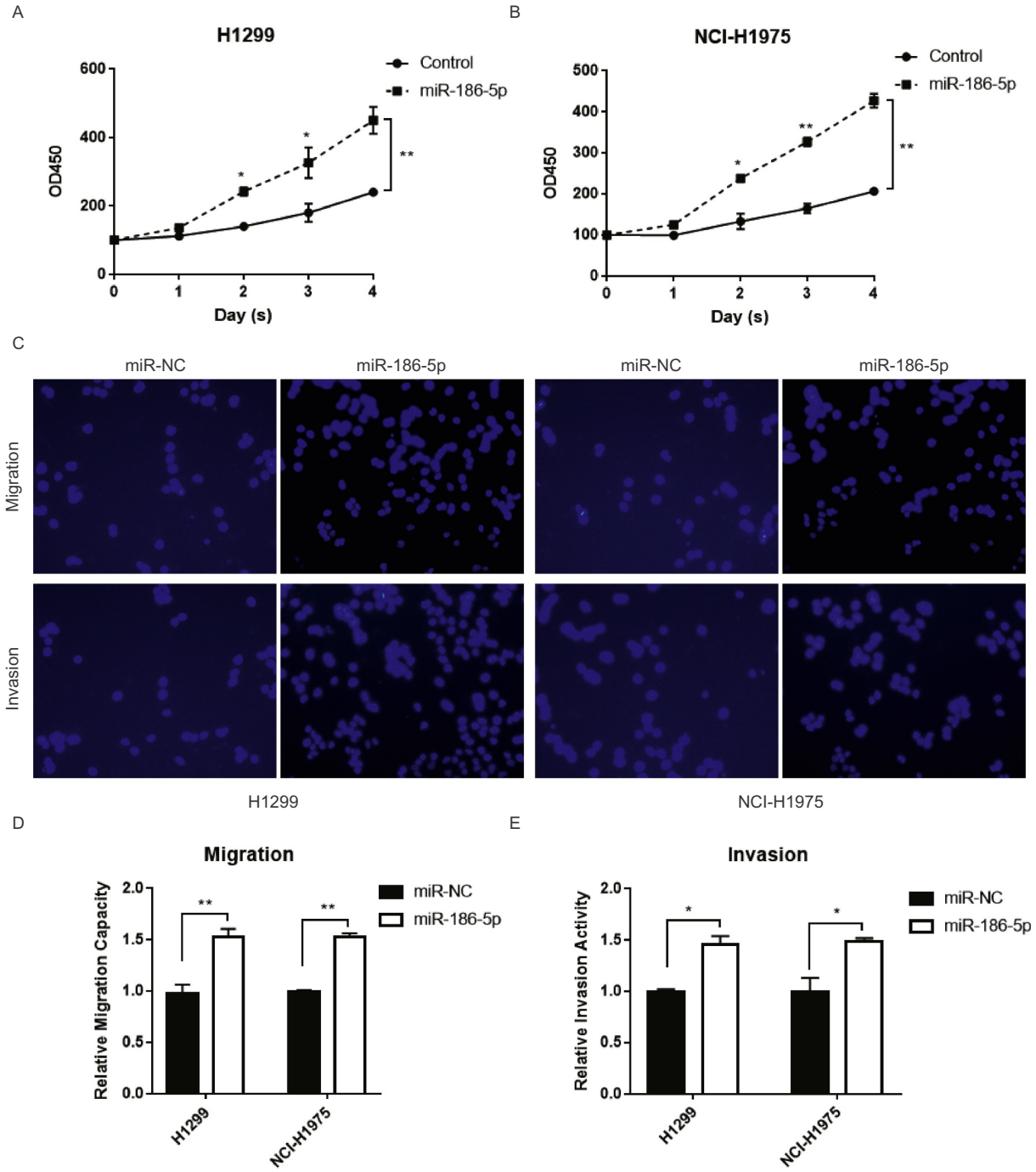

Fig. 2. miR-186-5p overexpression promotes the proliferation, migration and invasion of LUAD cells. A and B. The cell proliferation measured by CCK-8 assay in miR-186-5p transfected LUAD cells H1299 and NCI-H1975. C. Shown are representatives of transwell migration assay and transwell invasion assay after transfected with miR-186-5p mimic or control in H1299 or NCI-H1975. D. Quantitative representation of transwell migration assay and transwell invasion assay after transfected with miR-186-5p mimic or control in H1299 or NCI-H1975. Assays were performed in triplicate. Data are expressed as mean ± SD. $^{*}P < .05$, $^{**}P < .01$.

USA) to measure the expression level, and normalized using RNU48 control miRNA assay kit (P/N 001006; Applied Biosystems, Carlsbad, CA, USA), following the manufacturer's protocols.

2.4. Cell culture and transfection

Cells were cultured in DMEM (Gibco, Grand Island, NY, USA) medium supplemented with 10% fetal bovine serum (10% FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco) at 37 °C with 5% CO_2. Hsa-miR-186-5p mimic and mimic negative control were purchased from GenePharma Co., Ltd. (Shanghai, China). Cells were seeded in 6-well plates at a density of 2×10^5 cells/well and treated overnight. Cells were transfected with either miR-186-5p mimic or mimic negative control using Lipofectamine 2000 according to the

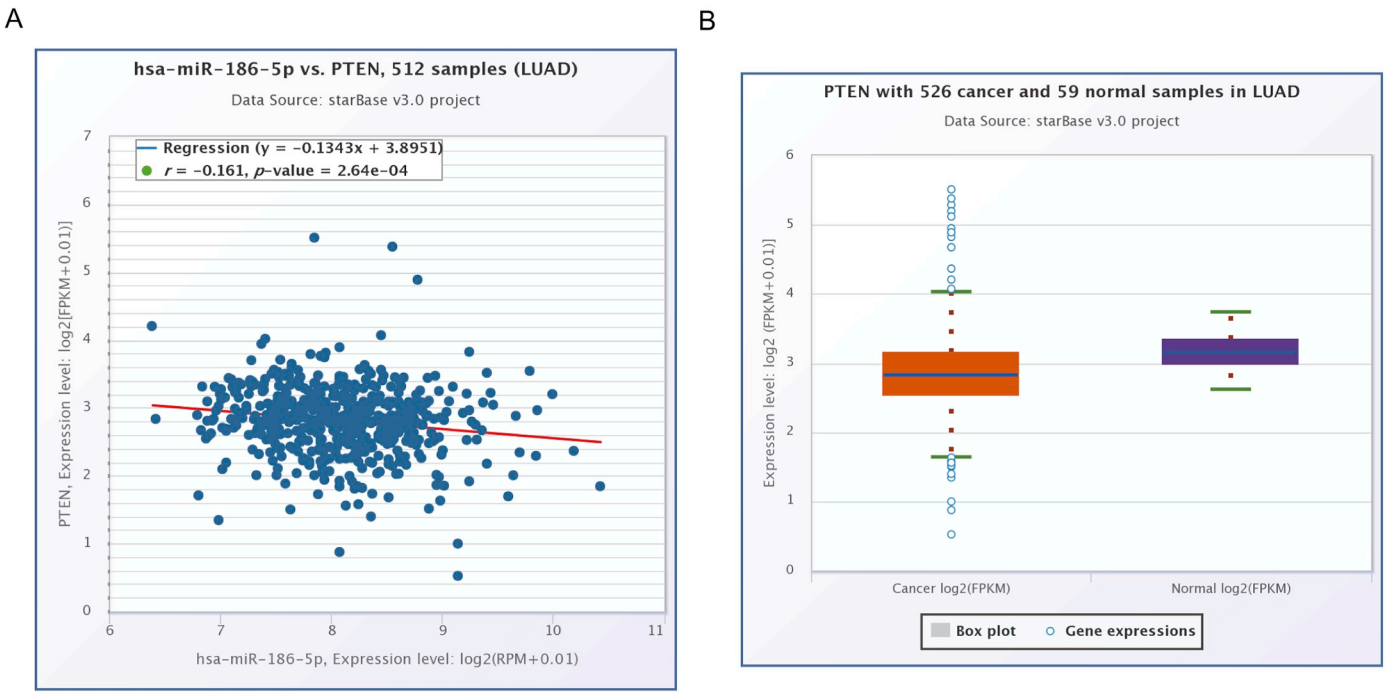

Fig. 3. PTEN is a predicted target of miR-186-5p in LUAD. A. PTEN expression has a negative correlation with miR-186-5p expression in 512 LUAD samples from Starbase v3.0. $r = -0.161$. B. The expression of PTEN is decreased in 526 LUAD tumor samples compared with 59 normal lung samples from Starbase v3.0.

manufacturer's instructions.

2.5. Cell counting kit-8 (CCK-8) assay

Proliferation of cell lines H1299 and NCI-H1975 were determined by CCK-8 kit (Dojindo, Kumamoto, Japan). The H1299 and NCI-H1975 cells were seeded in 24-well plates at a density of 5×10^5cells/well. Cells were incubated with 10% CCK-8 at the time of harvest. One hour after adding CCK-8, cellular viability was determined by measuring the absorbance of the converted dye at 450 nm. Proliferation rates of H1299 and NCI-H1975 cells were measured at 0, 1, 2, 3 and 4 days after transfection.

2.6. Transwell migration/invasion assay

H1299 and NCI-H1975 cells were grown in DMEM containing 10% FBS to about 60% confluence and transfected with miRNA mimic or gene fragment, or their corresponding negative controls. Cells were harvested by trypsinization after forty eight hours. Cell migration analysis was performed by 8-mm pore size culture inserts (Transwell; Costar, High Wycombe, UK) placed into the wells of 24-well culture plates. Cell invasion was assayed in Biocoat Matrigel invasion chambers (BD Bioscience, San Jose, CA, USA) according to the manufacturer's protocol.

2.7. Western blot analysis

Total protein was separated by 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, United States). Primary anti-PTEN (ab32199) and anti-GAPDH (ab8245) antibodies were incubated overnight at 4 °C, followed by incubation with the secondary antibody Santa Cruz Biotechnology, CA, United States) for 1 h at room temperature. Protein bands were visualized using the ECL-kit, according to the manufacturer's instructions. GAPDH was used as a loading control.

2.8. Luciferase assay

The H1299 and NCI-H1975 cells were seeded in 24-well plates at a density of5×10^5 cells/well. Then they were incubated for 24 h. In the luciferase reporter gene assay, the H1299 and NCI-H1975 cells were co-transfected with PTEN wild-type (or mutant) reporter plasmid and miR-186-5p mimic (or miR mimic NC) with Lipofectamine 2000 (Invitrogen, Shanghai, China). The activities of firefly and Renilla luciferase were measured using dual luciferase reporter assay forty-eight hours post-transfection (Promega, Shanghai, China).

2.9. Statistical analysis

All experiments were performed at least three times. Data are displayed as the means ± the standard deviations from at least three independent experiments. Differences with p-values of < 0.05 were considered statistically significant.

3. Results

3.1. The miR-186-5p is overexpressed in LUAD and LUAD cell lines

To investigate the role of miR-186-5p in LUAD, we first analyzed miR-186-5p expression in human LUAD using data from starbase v3.0.

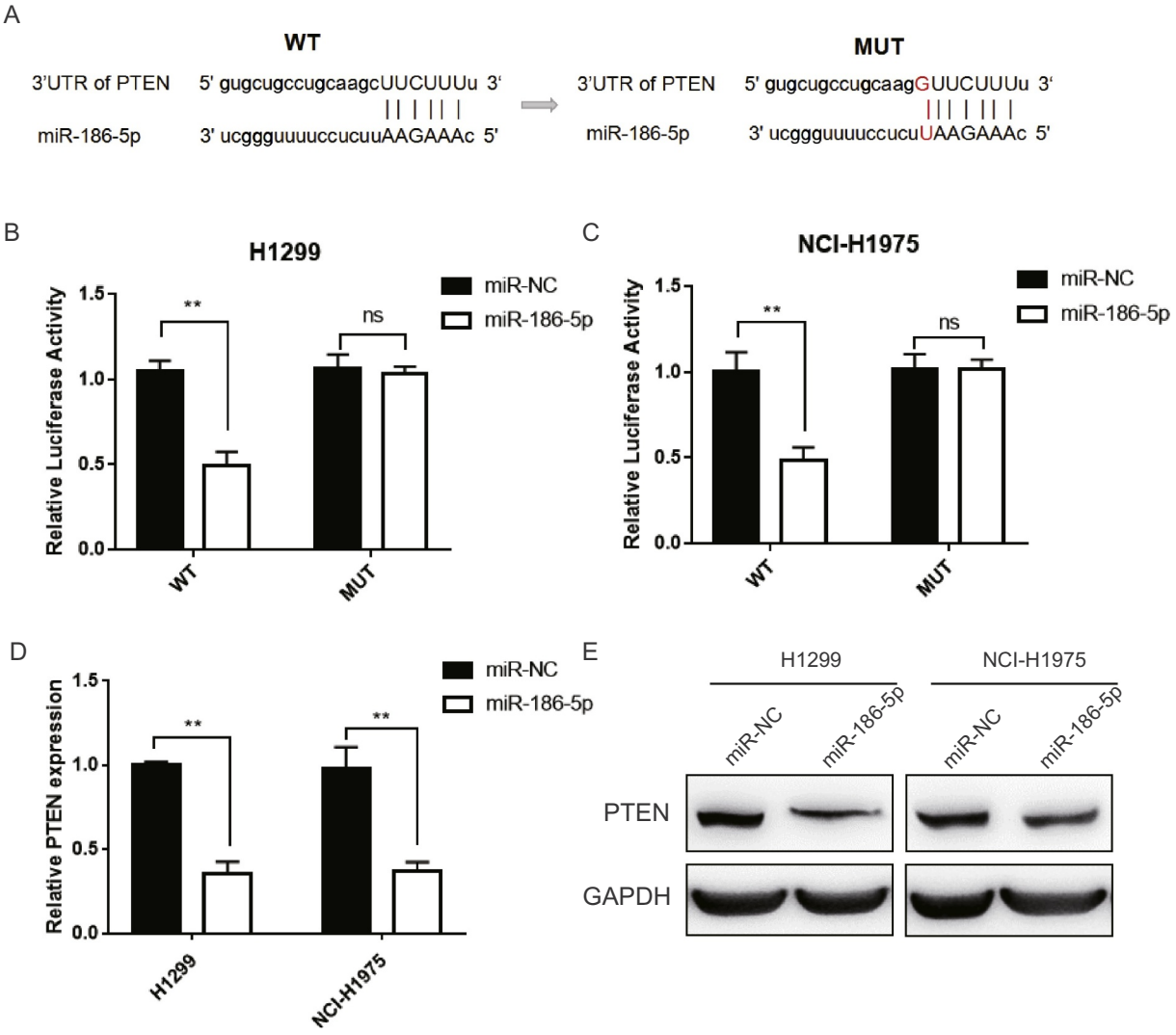

Fig. 4. miR-186-5p targets PTEN in LUAD cells. A. The predicted structure complementation between miR-186-5p and PTEN. B and C. The analysis of the relative luciferase activities of PTEN-WT, PTEN-MUT in H1299 and NCI-H1975 cells after transfection with miR-186-5p mimics. D. The mRNA level of PTEN is significantly decreased 48 h after transfection with miR-186-5p mimics in H1299 and NCI-H1975 cells by qRT-PCR. E. The protein level of PTEN is significantly decreased after transfection with miR-186-5p mimics in H1299 and NCI-H1975 cells by western blot. Assays were performed in triplicate. Data are expressed as mean ± SD.*P < .05, **P < .01.

It was found that miR-186-5p was overexpressed in LUAD samples compared to normal samples (Fig. 1A). Then, quantitative PCR was performed to examine miR-186-5p expression in primary human LUAD samples. We observed that miR-186-5p expression was significantly increased in the 20 LUAD samples we collected during surgery compared with the tumor-adjacent lung tissues (Fig. 1B). In addition, we measured the expression levels of miR-186-5p in the two LUAD cell lines. As expected, miR-186-5p was expressed at a higher level in the LUAD cell lines H1299 and NCI-H1975than in the normal human lung epithelial cell line BEAS-IB (Fig. 1C), indicating that miR-186-5p is up-regulated in LUAD.

3.2. miR-186-5p accelerates LUAD cell proliferation, migration and invasion

To further investigate the role that miR-186-5p plays in LUAD, we quantified the effect of miR-186-5p overexpression on the proliferation of LUAD cell lines using the CCK-8 assay. Our results showed that overexpression of miR-186-5p significantly facilitated the proliferation of both H1299 and NCI-H1975 cells compared to their controls (Fig. 2A and B). In addition, we also explored the role of miR-186-5p on the metastasis of LUAD cells using transwell, and the results demonstrated that miR-186-5p mimic treatment caused an increase in the migration capacity and invasive ability of H1299 and NCI-H1975 cells compared with their negative controls (Fig. 2C and D).

3.3. PTEN is a direct target of miR-186-5p

We used miRNA target prediction software (starbase v3.0) to search for the target genes ofmiR-186-5p. We found that PTEN has a negative correlation with miR-186-5p (Fig. 3A) and PTEN expression was down-regulated in LUAD tumor from the cancer genome atlas (TCGA)

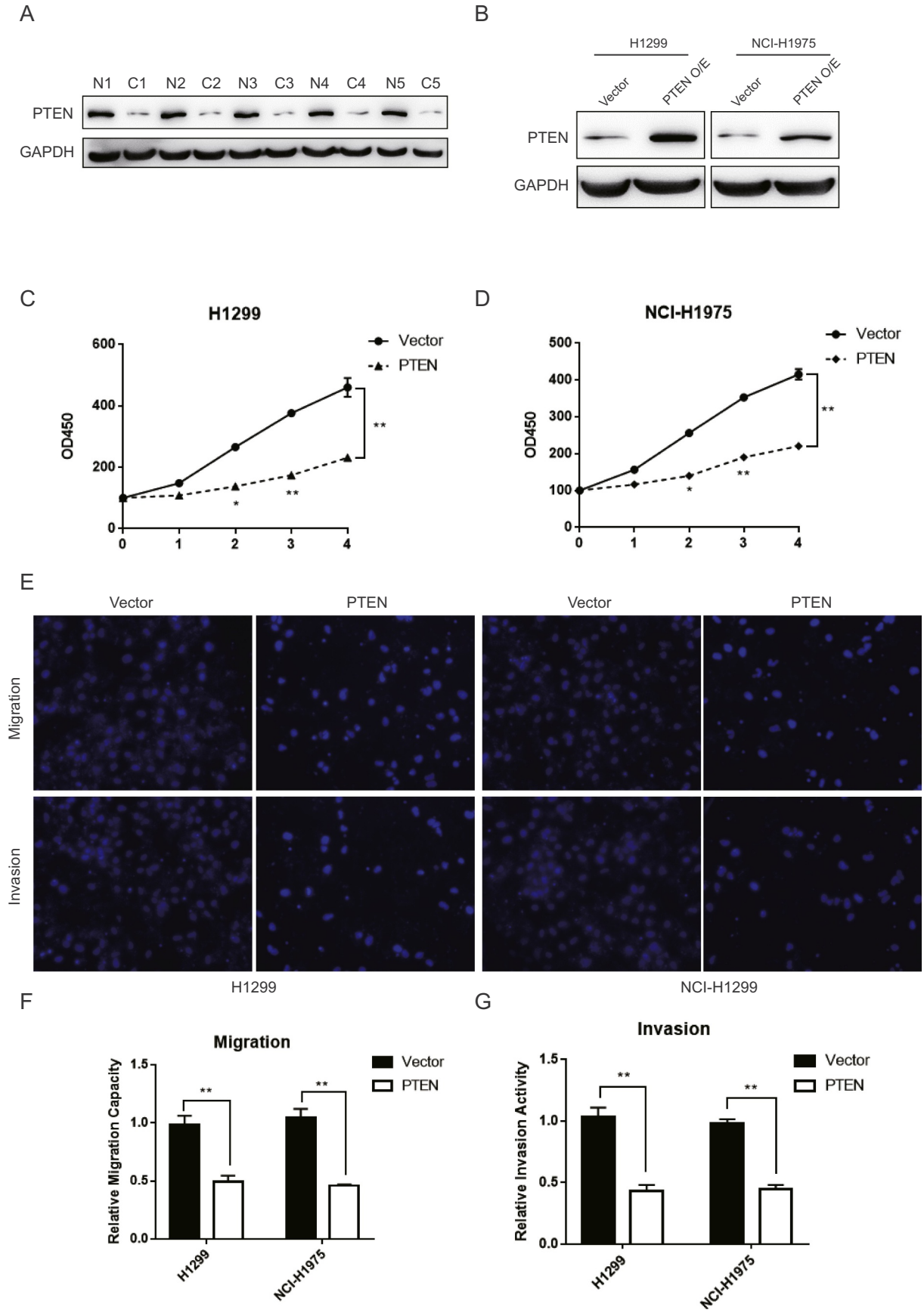

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Fig. 5. PTEN overexpression inhibits the proliferation, migration and invasion of LUAD cells. A. Western blot analysis of PTEN expression in LUAD tissues (C) and tumor-adjacent lung tissues (N). B. Western blot analysis of PTEN overexpression in human LUAD cell lines H1299 and NCI-H1975. C and D. CCK-8 assays of H1299 and NCI-H1975 cells after transfected with PTEN plasmid or vector control. E. Shown are representatives of transwell migration assay and transwell invasion assay after transfected with PTEN plasmid or vector control in H1299/NCI-H1975. F. Quantitative representation of transwell migration assay and transwell invasion assay after transfected with PTEN plasmid or vector control in H1299/NCI-H1975. Assays were performed in triplicate. Data are expressed as mean ± SD. $^{*}P < .05$, $^{**}P < .01$.

database (Fig. 3B). In addition, a complementary region of miR-186-5p was identified in the 3'-UTR of PTEN by using miRNA target prediction software (miRTarBase) (Fig. 4A). Thus, we constructed the luciferase plasmid carrying the wild-type PTEN 3'-UTR fragment (3'WT UTR) or the mutated PTEN 3'-UTR fragment (3'mutant UTR). Luciferase reporter assay showed that miR-186-5p significantly suppressed the luciferase activity compared to control miRNA in both H1299 and NCI-H1975 cells transfected with PTEN wild-type reporter plasmid (Fig. 4B). However, miR-186-5p showed no effect on the mutated PTEN 3'-UTR fragment (Fig. 4B), suggesting that PTEN is a direct target of miR-186-5p. In addition, both the mRNA and protein levels of PTEN were significantly decreased in H1299 or NCI-H1975 cells transfected with miR-186-5p mimics (Fig. 4C and D).

3.4. PTEN is required for regulating proliferation, migration and invasion of LUAD cells

We next examined the PTEN expression in human LUAD tissues using western blot. Our results showed that PTEN was significantly decreased in LUAD tissues compared to the tumor-adjacent tissues (Fig. 5A). Then, we designed PTEN overexpression plasmid to overexpress PTEN in LUAD cell lines H1299 and NCI-H1975 and verified the expression at protein level by western blot (Fig. 5B). Functional analysis revealed that overexpression of PTEN resulted in proliferation inhibition both in H1299 and NCI-H1975 cells (Fig. 5C and D). In addition, both the migration and invasion abilities of H1299 and NCI-H1975 cells that were transfected with PTEN plasmid were significantly impaired (Fig. 5E-G), which was opposite to the phenotypes that resulted from miR-186-5p overexpression (Fig. 2C and D). These results suggested that miR-186-5p promotes the proliferation, migration and invasion of LUAD cells possibly by directly targeting PTEN.

3.5. MiR-186-5p promotes LUAD cell growth, migration and invasion by targeting PTEN

To validate our hypothesis that miR-186-5p functions by directly targeting PTEN, we co-overexpressed PTEN together with miR-186-5p in H1299 and NCI-H1975 cells. The CCK-8 assay showed that overexpression of miR-186-5p significantly accelerated LUAD cell proliferation while co-overexpression of miR-186-5p and PTEN partially impaired the cell growth of LUAD cells induced by miR-186-5p overexpression (Fig. 6A and B), suggesting that miR-186-5p functions directly through PTEN. Moreover, we examined the cell migration capacity and invasion ability of cells with ectopic expression of miR-186-5p and PTEN. Our data showed that the enhanced migration and invasion abilities of LUAD cells caused by miR-186-5p overexpression could be attenuated by co-overexpression of miR-186-5p and PTEN (Fig. 6C and D). Western blot revealed that overexpression of miR-186-5p significantly impaired the expression of PTEN while co-overexpression of miR-186-5p and PTEN restored the PTEN protein levels in H1299 and NCI-H1975 cells (Fig. 6F). Taken together, the results indicate that miR-186-5p promotes the growth, migration and invasion of LUAD cells by directly targeting the expression of PTEN.

4. Discussion

miRNAs have diagnostic, prognostic and therapeutic potential in LUAD (Vansteenkiste et al., 2012; Giordano et al., 2018). A series of studies reported that altered expression of miRNAs was involved in the initiation and progression of LUAD (Mou and Liu, 2016; Su et al., 2016; Liu et al., 2017a; Yang et al., 2018). For example, Duan and coauthors reported that direct interaction between miR-203 and ZEB2 suppressed the epithelial-mesenchymal transition signaling and reduced LUAD chemoresistance (Duan et al., 2016). Yuan et al. found that overexpression of miR-30a in LUAD A549 cell line inhibited the migration and invasion via targeting EYA2 (Yuan et al., 2016). Li et al. demonstrated that miR-1304 suppressed human non-small cell lung cancer cell growth in vitro by targeting heme oxygenase-1 (Li et al., 2017). In the current study, our data suggests that the overexpression of miR-186-5p promotes the development, migration and invasion of LUAD, indicating an oncogene role of miR-186-5p that mediates through directly targeting PTEN.

No special studies had been performed to examine the mechanistic function of miR-186-5p in LUAD. Thus, it was of great importance to investigate the expression levels and regulatory molecular mechanisms of miR-186-5p in LUAD to better understand the role of miR-186-5p in LUAD. Our results showed that miR-186-5p is not only upregulated in the two LUAD cell lines H1299 and NCI-H1975, but is also increased in human LUAD tissues, suggesting that miR-186-5p might accelerate LUAD tumorigenesis. Besides the stimulative role of miR-186-5p in LUAD proliferation, our results indicated that miR-186-5p overexpression promotes the migration and invasion of H1299 and NCI-H1975 cells, suggesting a promotional effect of miR-186-5p in cancer cell migration and invasion. In vivo studies on the function of miR-186-5p in LUAD metastasis should be performed in the future to validate these findings.

We also used luciferase reporter assay to demonstrate that miR-186-5p functions by repressing PTEN. Our data revealed that PTEN expression is reduced in human LUAD tissues, which is in line with the previous reports, in which PTEN was found to be down-regulated in many types of human cancers including lung carcinoma, colon cancer and prostate cancer (Bai et al., 2009; Liang et al., 2015; Ye et al., 2015; Liu et al., 2017b). Furthermore, restoration of PTEN expression rescued the cell growth accumulation that resulted from miR-186-5p overexpression. Indeed, evidence seemed to indicate that PTEN acts as a tumor suppressor in tumor initiation, progression and dissemination (Cao et al., 2007; Shearn and Petersen, 2015; Joshi and Ellenson, 2017). Our results showed that downregulation of PTEN is associated with the growth, migration and invasion of LUAD cells, further supporting this idea. For the first time, our data showed that PTEN inhibition through miR-186-5p overexpression leads to increased migration and invasion of LUAD cells.

In conclusion, our study demonstrates that miR-186-5p is up-regulated in LUAD, and promotes LUAD cell proliferation, migration and invasion via inhibiting PTEN. This novel miR-186-5p/PTEN axis deepens our understanding of the mechanisms underlying LUAD, and brings new genetic insights into lung cancer. Besides, since miR-186-5p overexpression facilitates the proliferation, migration and invasion of LUAD cells, indicating that miR-186-5p might play an "oncogene" role in LUAD progression, it might offer potential markers for the early diagnosis of LUAD. In addition, miR-186-5p stimulates the progression of LUAD by targeting PTEN, and therefore, both miR-186-5p and PTEN may serve as putative candidates for future therapeutic approaches in this disease. *Further* carefully designed *studies involving large number of subjects are needed to confirm the diagnostic, prognostic and therapeutic roles of these agents.*

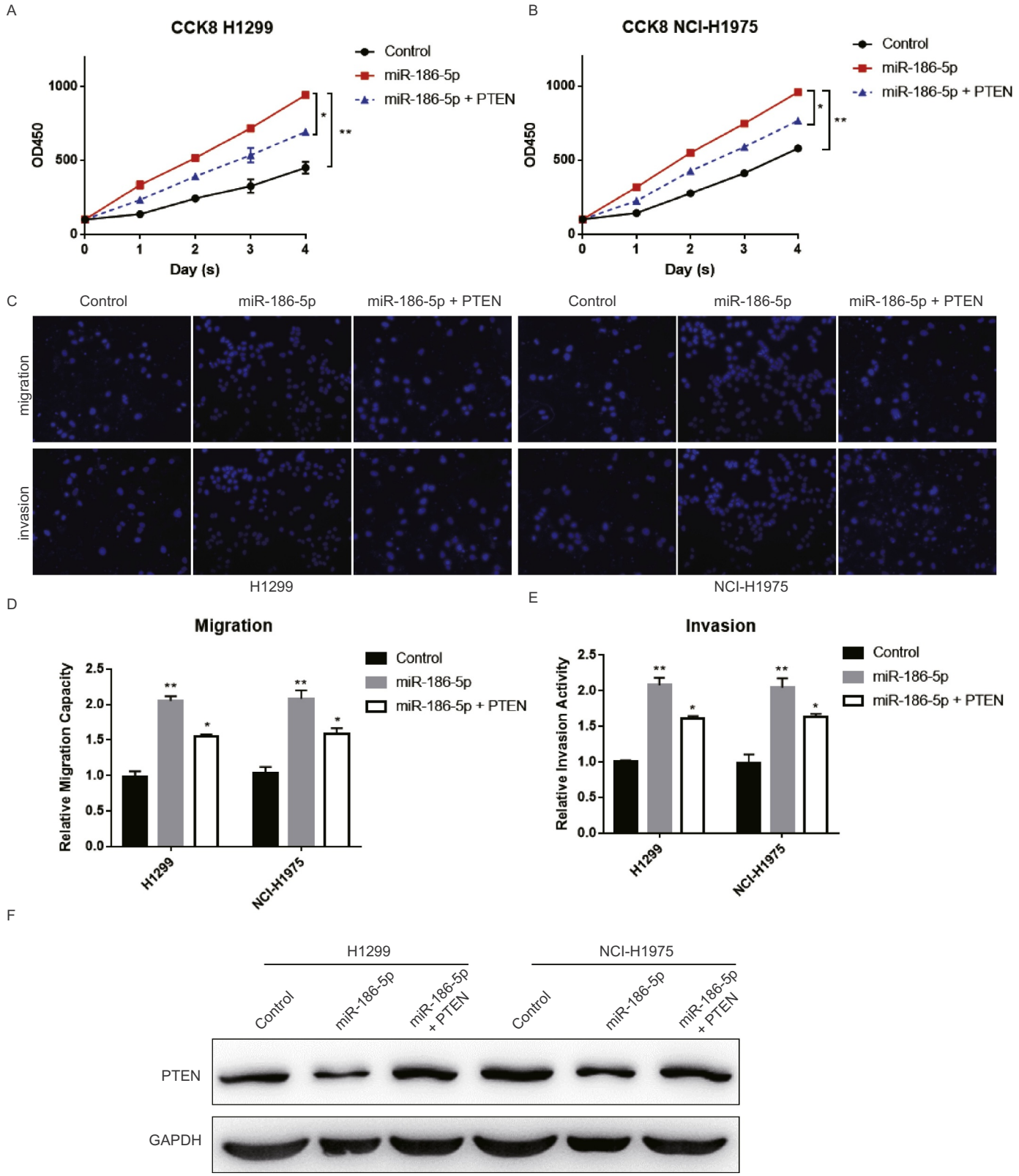

Fig. 6. Effect of PTEN overexpression on the oncogene role of miR-186-5p. A and B. CCK-8 analysis of cell viability of H1299/NCI-H1975 cells with empty vector, H1299/NCI-H1975 cells overexpressing miR-186-5p, and H1299/NCI-H1975 cells co-overexpressing miR-186-5p and PTEN. C. Cell migration and cell invasion analysis of H1299/NCI-H1975 cells with empty vector, H1299/NCI-H1975 cells overexpressing miR-186-5p, and H1299/NCI-H1975 cells co-overexpressing miR-186-5p and PTEN. D and E. Quantitative representation of transwell migration assay and transwell invasion assay after transfected with empty vector, miR-186-5p mimic, miR-186-5p mimic and PTEN in H1299/NCI-H1975. F. Western blot analysis of PTEN expression in empty vector control, miR-186-5p-overexpressing, PTEN and miR-186-5p co-overexpressing H1299/NCI-H1975 cells. Assays were performed in triplicate. Data are expressed as mean ± SD.*P < .05, **P < .01.

Conflict of interest

Authors have declared that no competing interest exists.

References

Bai, W., Wang, L., Ji, W., Gao, H., 2009. Expression profiling of supraglottic carcinoma: PTEN and thrombospondin 2 are associated with inhibition of lymphatic metastasis. Acta Otolaryngol. 129, 569–574.

Bunn, P.A., 2012. Worldwide overview of the current status of lung cancer diagnosis and treatment. Arch. Pathol. Lab. Med. 136, 1478–1481.

Bushati, N., Cohen, S.M., 2007. microRNA functions. Annu. Rev. Cell Dev. Biol. 23, 175–205.

Cao, L.Q., Chen, X.L., Wang, Q., Huang, X.H., Zhen, M.C., Zhang, L.J., Li, W., Bi, J., 2007. Upregulation of PTEN involved in rosiglitazone-induced apoptosis in human hepatocellular carcinoma cells. Acta Pharmacol. Sin. 28, 879–887.

DeSano, J.T., Xu, L., 2009. MicroRNA regulation of cancer stem cells and therapeutic implications. AAPS J 11, 682–692.

Duan, X., Fu, Z., Gao, L., Zhou, J., Deng, X., Luo, X., Fang, W., Luo, R., 2016. Direct interaction between miR-203 and ZEB2 suppresses epithelial-mesenchymal transition signaling and reduces lung adenocarcinoma chemoresistance. Acta Biochim. Biophys. Sin. Shanghai 48, 1042–1049.

Giordano, M., Boldrini, L., Servadio, A., Niccoli, C., Melfi, F., Lucchi, M., Mussi, A., Fontanini, G., 2018. Differential microRNA expression profiles between young and old lung adenocarcinoma patients , am. J Transl Res 10, 892–900.

Griesinger, F., 2017. Current aspects of Diagnosis and treatment of lung Cancer. Dtsch. Med. Wochenschr. 142, 1808–1812.

Islam, F., Gopalan, V., Vider, J., Wahab, R., Ebrahimi, F., Lu, C.T., Kasem, K., AKY, L., 2017. MicroRNA-186-5p overexpression modulates colon cancer growth by repressing the expression of the FAM134B tumour inhibitor. Exp. Cell Res. 357, 260–270.

Jones, D.Z., Schmidt, M.L., Suman, S., Hobbing, K.R., Barve, S.S., Gobejishvili, L., Brock, G., Klinge, C.M., Rai, S.N., Park, J., Clark, G.J., Agarwal, R., Kidd, L.R., 2018. MicroRNA-186-5p inhibition attenuates proliferation, anchorage independent growth and invasion in metastatic prostate cancer cells. BMC Cancer 18, 421.

Joshi, A., Ellenson, L.H., 2017. PI3K/PTEN/AKT genetic mouse models of endometrial carcinoma. Adv. Exp. Med. Biol. 943, 261–273.

Kim, E., Yang, J., Park, J., Kim, S., Kim, N.H., Yook, J.I., Suh, J.S., Haam, S., Huh, Y.M., 2012. Consecutive targetable smart nanoprobe for molecular recognition of cytoplasmic microRNA in metastatic breast cancer. ACS Nano 6, 8525–8535.

Li, C., Feng, Y., Coukos, G., Zhang, L., 2009. Therapeutic microRNA strategies in human cancer. AAPS J 11, 747–757.

Li, M., Li, J., Ding, X., He, M., Cheng, S.Y., 2010. microRNA and cancer. AAPS J. 12, 309–317.

Li, C.G., Pu, M.F., Li, C.Z., Gao, M., Liu, M.X., Yu, C.Z., Yan, H., Peng, C., Zhao, Y., Li, Y., Ma, Z.L., Qi, X.M., Wang, Y.Z., Miao, L.L., Ren, J., 2017. MicroRNA-1304 suppresses human non-small cell lung cancer cell growth in vitro by targeting heme oxygenase-1. Acta Pharmacol. Sin. 38, 110–119.

Li, J., Xia, L., Zhou, Z., Zuo, Z., Xu, C., Song, H., Cai, J., 2018. MiR-186-5p upregulation inhibits proliferation, metastasis and epithelial-to-mesenchymal transition of colorectal cancer cell by targeting ZEB1. Arch. Biochem. Biophys. 640, 53–60.

Liang, N., Zhou, X., Zhao, M., Zhao, D., Zhu, Z., Li, S., Yang, H., 2015. Down-regulation of microRNA-26b modulates non-small cell lung cancer cells chemoresistance and migration through the association of PTEN. Acta Biochim. Biophys. Sin. Shanghai 47, 530–538.

Liu, Y., Wang, F., Xu, P., 2017a. miR-590 accelerates lung adenocarcinoma migration and invasion through directly suppressing functional target OLFM4. Biomed. Pharmacother. 86, 466–474.

Liu, T., Chen, G., Sun, D., Lei, M., Li, Y., Zhou, C., Li, X., Xue, W., Wang, H., Liu, C., Xu, J., 2017b. Exosomes containing miR-21 transfer the characteristic of cisplatin resistance by targeting PTEN and PDCD4 in oral squamous cell carcinoma. Acta Biochim. Biophys. Sin. Shanghai 49, 808–816.

Local Nonsurgical Therapies for Stage I and Symptomatic Obstructive Non–Small-Cell Lung Cancer. Agency for Healthcare Research and Quality (US), Rockville (MD).

Mou, X., Liu, S., 2016. MiR-485 inhibits metastasis and EMT of lung adenocarcinoma by targeting Flot2. Biochem. Biophys. Res. Commun. 477, 521–526.

Mutti, A., 2008. Molecular diagnosis of lung cancer: an overview of recent developments. Acta Biomed 79 (Suppl. 1), 11–23.

Shearn, C.T., Petersen, D.R., 2015. Understanding the tumor suppressor PTEN in chronic alcoholism and hepatocellular carcinoma. Adv. Exp. Med. Biol. 815, 173–184.

StatPearls, StatPearls Publishing, Treasure Island (FL), 2018.

Su, T.J., Ku, W.H., Chen, H.Y., Hsu, Y.C., Hong, Q.S., Chang, G.C., Yu, S.L., Chen, J.J., 2016. Oncogenic miR-137 contributes to cisplatin resistance via repressing CASP3 in lung adenocarcinoma. Am. J Cancer Res 6, 1317–1330.

The Diagnosis and Treatment of Lung Cancer (Update), National Collaborating Centre for Cancer (UK), Cardiff (UK), 2011.

Treatment of Metastatic Non-Small Cell Lung Cancer: A Systematic Review of Comparative Effectiveness and Cost-Effectiveness. Department of Veterans Affairs (US), Washington (DC).

Vansteenkiste, J., Dooms, C., Mascaux, C., Nackaerts, K., 2012. Screening and early detection of lung cancer. Ann. Oncol. 23 (Suppl. 10), x320–x327.

Vo, D.D., Staedel, C., Zehnacker, L., Benhida, R., Darfeuille, F., Duca, M., 2014. Targeting the production of oncogenic microRNAs with multimodal synthetic small molecules. ACS Chem. Biol. 9, 711–721.

Yang, L., Luo, P., Song, Q., Fei, X., 2018. DNMT1/miR-200a/GOLM1 signaling pathway regulates lung adenocarcinoma cells proliferation. Biomed. Pharmacother. 99, 839–847.

Ye, Z.L., Huang, Y., Li, L.F., Zhu, H.L., Gao, H.X., Liu, H., Lv, S.Q., Xu, Z.H., Zheng, L.N., Liu, T., Zhang, J.L., Jin, H.J., Qian, Q.J., 2015. Argonaute 2 promotes angiogenesis via the PTEN/VEGF signaling pathway in human hepatocellular carcinoma. Acta. Pharmacol. Sin. 36, 1237–1245.

Yuan, Y., Zheng, S., Li, Q., Xiang, X., Gao, T., Ran, P., Sun, L., Huang, Q., Xie, F., Du, J., Xiao, C., 2016. Overexpression of miR-30a in lung adenocarcinoma A549 cell line inhibits migration and invasion via targeting EYA2. Acta Biochim. Biophys. Sin. (Shanghai) 48, 220–228.

Zhang, W., Dahlberg, J.E., Tam, W., 2007. MicroRNAs in tumorigenesis: a primer. Am. J. Pathol. 171, 728–738.

Zhao, L., Li, R., Xu, S., Li, Y., Zhao, P., Dong, W., Liu, Z., Zhao, Q., Tan, B., 2018. Tumor suppressor miR-128-3p inhibits metastasis and epithelial-mesenchymal transition by targeting ZEB1 in esophageal squamous-cell cancer. Acta Biochim. Biophys. Sin. Shanghai 50, 171–180.