

# Downregulation of microRNA-182 inhibits cell growth and invasion by targeting programmed cell death 4 in human lung adenocarcinoma cells

Min Wang · Yuanyuan Wang · Wenqiao Zang · Huaqi Wang · Heying Chu · Ping Li · Min Li · Guojun Zhang · Guoqiang Zhao

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**Abstract** Lung cancer is a major cause of cancer death worldwide. Programmed cell death 4 (PDCD4), an important tumor suppressor, influences transcription and translation of multiple genes and modulates different signal transduction pathways. However, the upstream regulation of this gene is largely unknown. In our study, we found that microRNA-182 (miR-182) was upregulated, whereas PDCD4 was downregulated in lung cancer cell lines. We performed methyl thiazolyl tetrazolium and colony formation assays to study the influence of miR-182 on proliferation of the lung cancer cell lines A549 and SPC-A-1. We also carried out Transwell and wound healing assays to investigate the effect of miR-182 on invasion and migration of A549 and SPC-A-1. Finally, using the luciferase reporter assay and restore assay, we demonstrated that PDCD4 is a direct target of miR-182. These results suggest that in lung adenocarcinoma cells, miR-182 plays an oncogenic role as a direct negative regulator of PDCD4.

**Keywords** miR-182 · PDCD4 · Nonsmall cell lung cancer (NSCLC) · Cell growth · Invasion

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Min Wang and Yuanyuan Wang contributed equally to this work.

M. Wang  
Department of Respiratory Medicine, People's Hospital of Zhengzhou, Zhengzhou 450053, China

Y. Wang · W. Zang · M. Li · G. Zhao (✉)  
College of Basic Medical Sciences, Zhengzhou University,  
No.100 Kexue Road, Zhengzhou 450001, China  
e-mail: zhaogq@zzu.edu.cn

H. Wang · H. Chu · P. Li · G. Zhang  
Department of Respiratory Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

## Introduction

MicroRNAs (miRNAs) are a recently discovered class of small (approximately 18–24 nucleotides in length), noncoding regulatory RNAs that negatively regulate gene expression at the posttranscriptional and/or translational level. miRNAs can trigger cleavage of target mRNAs or inhibit protein translation through sequence-specific interactions with the 3'-untranslated regions (3'-UTRs) of the target mRNAs [1–6]. Although the full extent of the biological functionalities of miRNAs has yet to be identified, they have been suggested to act as intrinsic regulators of many cellular processes including cell invasion, differentiation, proliferation, and apoptosis [7–12]. Furthermore, aberrant expression of miRNAs has been linked to the development and progression of cancer and has been shown to have prognostic significance in several tumor types, including lung and esophageal cancer, neuroblastoma, and lymphocytic leukemia [13–16].

Lung cancer is the primary cancer killer worldwide with a 5-year survival rate of approximately 15 %. According to its pathological classification, lung cancer can be roughly divided into two groups: small cell lung cancer and nonsmall cell lung cancer (NSCLC). The latter, NSCLC, is the predominant form of lung cancer, accounting for approximately 80 % of all incidences [17–19].

Programmed cell death 4 (PDCD4), a 64-kDa protein, is a novel tumor suppressor inhibiting TPA-induced neoplastic transformation [20] and tumor promotion and progression [21]. PDCD4 interacts with the translation initiation factors eIF4A and eIF4G to inhibit translation [22, 23]. Relatively little is known about the mechanisms regulating PDCD4 expression in cancer. Until now, the only miRNA that has been identified to directly regulate PDCD4 is microRNA-21 (miR-21). MiR-21 has been found to negatively regulate PDCD4 expression in ovarian cancer [24], breast cancer

[25], colorectal cancer [26], and malignant peripheral nerve sheath tumors [27]. However, other potential PDCD4-targeting miRNAs remain to be defined.

In this study, we used a variety of assays to study the influence of miR-182 on proliferation and invasion of A549 and SPC-A-1 cells to investigate whether PDCD4 is a direct target of miR-182.

## Materials and methods

### Cell lines and cell culture

The lung cancer cell lines A549 (ATCC), NCI-H1299 (ATCC), LTEP-a-2 (lung adenoma, Cell Bank in Shanghai no. TCHu 33), and SPC-A-1 (lung adenoma, Cell Bank in Shanghai no. TCHu 53) were cultured in DMEM containing 10 % fetal bovine serum (Gibco<sup>®</sup>, Invitrogen, Carlsbad, CA), 100 units/mL penicillin, and 100 µg/mL streptomycin, at 37 °C in a 5 % CO<sub>2</sub> humidified incubator to the log phase of proliferation before harvesting the cells. Normal human bronchial epithelial cells (NHBE) (Clonetics<sup>™</sup>) were maintained in a culture medium according to the protocol provided by Clonetics.

### RNA extraction and quantitative real-time PCR

Using the Qiagen RNeasy kit (Valencia, CA) according to the manufacturer's protocol, we isolated total RNA from the human lung cancer cell lines A549, NCI-H1299, LTEP-a-2, and SPC-A-1 and from NHBE cells.

To verify mature miRNA expression, quantitative real-time (qRT)-PCR was carried out in triplicate using a high-specificity miR-182 qRT-PCR detection kit (Stratagene Corp, La Jolla, CA), performed on an ABI 7500 fast real-time PCR system according to the manufacturer's protocols. U6 snRNA served as an endogenous control for normalization. Real-time qRT-PCR results are expressed as relative miR-182 threshold cycle ( $C_t$ ) values, which were then converted to  $x$ -fold changes ( $2^{-\Delta\Delta C_t}$ ).

### Western blotting

Total protein was extracted from cultured cells using RIPA buffer containing PMSF. A BCA protein assay kit (Beyotime, Haimen, China) was used to determine total protein concentration. Proteins were electrophoresed by SDS-PAGE and transferred onto PVDF membranes. After blocking, the membranes were washed four times with TBST at room temperature, then incubated overnight at 4 °C with diluted primary antibody (rabbit anti-PDCD4 antibody, 1:1,000, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Following extensive washing, the membranes were incubated with the secondary

antibody (HRP-conjugated goat antirabbit IgG, 1:3,000, Santa Cruz Biotechnology). Signals were visualized using a chemiluminescence detection kit (Amersham Pharmacia Biotech, Piscataway, NJ). An antibody against GAPDH (Santa Cruz Biotechnology) served as an endogenous reference.

### Construction of 3'-UTR-luciferase plasmid and reporter assays

The 3'-UTR of the PDCD4 fragment was PCR amplified from human genomic DNA and inserted into the pGL3 control vector (Promega, Madison, WI) at the XbaI site 3' to the luciferase gene. The primer sequences used for PCR amplification were as follows: forward 5'-GGGTCTAGAGACA TTTT ATAAACCTACAT-3', reverse 5'-GGGTCTAGAA ATCAATACTGCTTCAC ATG-3'. The full-length 3' UTR of PDCD4 was checked by sequencing and named pGL-3-wt 3'-UTR. Site-directed mutagenesis of the miR-182 target site in the PDCD4-3'-UTR was carried out using a Quikchange site-directed mutagenesis kit (Promega), with pGL-3-wt 3'-UTR as the template. For the luciferase reporter assay, A549 and SPC-A-1 cells were cultured in 96-well plates; then, using Lipofectamine<sup>™</sup> 2000 (Invitrogen), they were each cotransfected with wild-type or the mutant-type reporter plasmid (100 nM), Renilla vector (pRL-TK; 25 nM), and microRNA (100 nM). At 48 h after transfection, luciferase activity was measured using the dual-luciferase assay system (Promega).

### Methyl thiazolyl tetrazolium assay for cell proliferation

Three test groups (blank, untransfected cells; inhibitor-NC, cells transfected with miR-182 inhibitor negative control; inhibitor miR-182, cells transfected with miR-182 inhibitor) of the A549 and SPC-A-1 cell lines were seeded into 96-well plates at a density of  $1 \times 10^4$  cells/well with five replicate wells of each condition. The methyl thiazolyl tetrazolium (MTT) (Sigma-Aldrich, St Louis, MO) reagent (5 mg/mL) was added to the maintenance cell medium, and cultures were incubated at 37 °C for an additional 4 h before being measured. The reaction was terminated by adding 150 µL dimethylsulfoxide (Sigma-Aldrich) per well. Absorbance values were measured daily for six consecutive days at a wavelength of 490 nm.

### Colony formation assay

To measure colony-forming activity, the three groups of A549 and SPC-A-1 cells were counted and seeded into 12-well plates at 100 cells per well. The culture medium was replaced every 3 days. Twelve days after seeding, the numbers of colonies containing more than 50 cells were counted.

### Wound healing assay

The three groups of A549 and SPC-A-1 cells were cultured to confluence or near confluence (>90 %) in six-well plates. A sterile 200- $\mu$ L pipette tip was used to scratch a straight line through the cell layer. The medium was removed and replaced with fresh medium, and the cultures were photographed 24 h after scratching. Assays were repeated three times for each clone.

### Transwell invasion assay

Transwell filters (Costar) were coated with 40  $\mu$ L Matrigel (BD, Franklin Lakes, NJ) at a concentration of 3.9  $\mu$ g/ $\mu$ L on the upper surface of the polycarbonic membrane (6.5-mm diameter, 8- $\mu$ m pore size). The Matrigel, after solidifying at 37 °C for 30 min, served as the extracellular matrix for analysis of tumor cell invasion. Cells ( $2 \times 10^5$ ) were resuspended in 200  $\mu$ L of serum-free medium and seeded into the upper chamber of the Transwell filters. The remaining medium (500  $\mu$ L) containing 10 % FBS (used as a chemoattractant) was added to the bottom chamber. The cells were allowed to migrate for 24 h at 37 °C in a humidified incubator with 5 % CO<sub>2</sub>; then, the upper surface of the membrane was wiped with a cotton tip to mechanically remove noninvasive cells, and the invasive cells attached to the lower surface of the membrane were stained with crystal violet for 20 min. The membranes were cut out and mounted under cover slips with the cells on the upper surface. Under an inverted microscope at  $\times 200$  magnification, the number of cells invading the Matrigel was counted in three randomly selected visual fields from the central and peripheral portions of the filter. Each type of cell was assayed in triplicate.

### Statistical analysis

Statistical analysis was performed using the SPSS software version 15.0. All data are shown as the mean  $\pm$  SE where applicable. Differences were evaluated using Student's *t* test. A probability of <0.05 was considered statistically significant.

## Results

miR-182 is upregulated, whereas PDCD4 protein is downregulated, in lung cancer cell lines

We first determined the expression levels of miR-182 in four different lung cancer cell lines (A549, NCI-H1299, LTP-a-2, SPC-A-1) and in normal human bronchial epithelial cells by qRT-PCR. The results showed that miR-182 levels in the four lung cancer cell lines were all notably higher than in

normal cells (Fig. 1a) ( $P < 0.05$ ). We then analyzed PDCD4 protein levels in the five different cell types by western blot, and our results showed that PDCD4 was lower in lung cancer cell lines than in normal cells (Fig. 1b) ( $P < 0.05$ ). These data indicate that miR-182 expression is higher, whereas PDCD4 is lower in lung cancer cell lines.

### Downregulation of miR-182 inhibits cell proliferation in A549 and SPC-A-1 cells

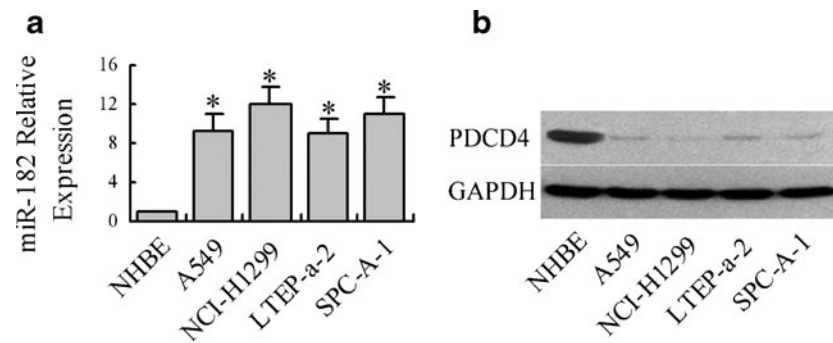
We performed MTT and colony formation assays in lung cancer cells to further evaluate the effect of miR-182 on cell growth. In the MTT assay, we observed that, compared with the blank and inhibitor-NC groups, the viability of the inhibitor miR-182 group decreased from the third day onwards in A549 (Fig. 2a) and SPC-A-1 (Fig. 2b) cells ( $P < 0.05$ ). In contrast, we found no significant difference between the blank and the inhibitor-NC groups ( $P > 0.05$ ). In the colony formation assay, the colony-forming activity of the inhibitor miR-182 group was lower than that of the blank and inhibitor-NC groups in both A549 (Fig. 2c) and SPC-A-1 (Fig. 2d) cells ( $P < 0.05$ ), while there was no significant difference between the blank and the inhibitor-NC groups ( $P > 0.05$ ). Based on these results, we concluded that the downregulation of miR-182, as a result of transfection with miR-182 inhibitor, could slow the proliferation of A549 and SPC-A-1 cells.

### Downregulation of miR-182 restricts cell invasion and migration ability of A549 and SPC-A-1 cells

We performed a Transwell assay and a wound healing assay to evaluate the role of miR-182 in regulating the invasion and migration activity of lung cancer cells. Panels a and b of Fig. 3 show the average numbers of cells penetrating the Transwell membrane in the three groups of A549 and SPC-A-1 cells, respectively. There was no significant difference ( $P > 0.05$ ) between the blank and the inhibitor-NC groups, but compared with both blank and inhibitor-NC groups, the average number of cells penetrating the Transwell membrane was significantly lower in the inhibitor miR-182 group ( $P < 0.05$ ). Figure 3c shows the migration ability of all three groups of A549 and SPC-A-1 cells. Both blank and inhibitor-NC groups had reached a higher cell density at 24 h postwounding compared to the inhibitor miR-182 group in both cell types. These data indicate that inhibiting miR-182 restricts the migration and invasion capacity of A549 and SPC-A-1 cells.

### PDCD4 is a direct target of miR-182

Bioinformatics analysis by TargetScan and miRanda indicated that the 3'-UTR of PDCD4 contains a predicted binding

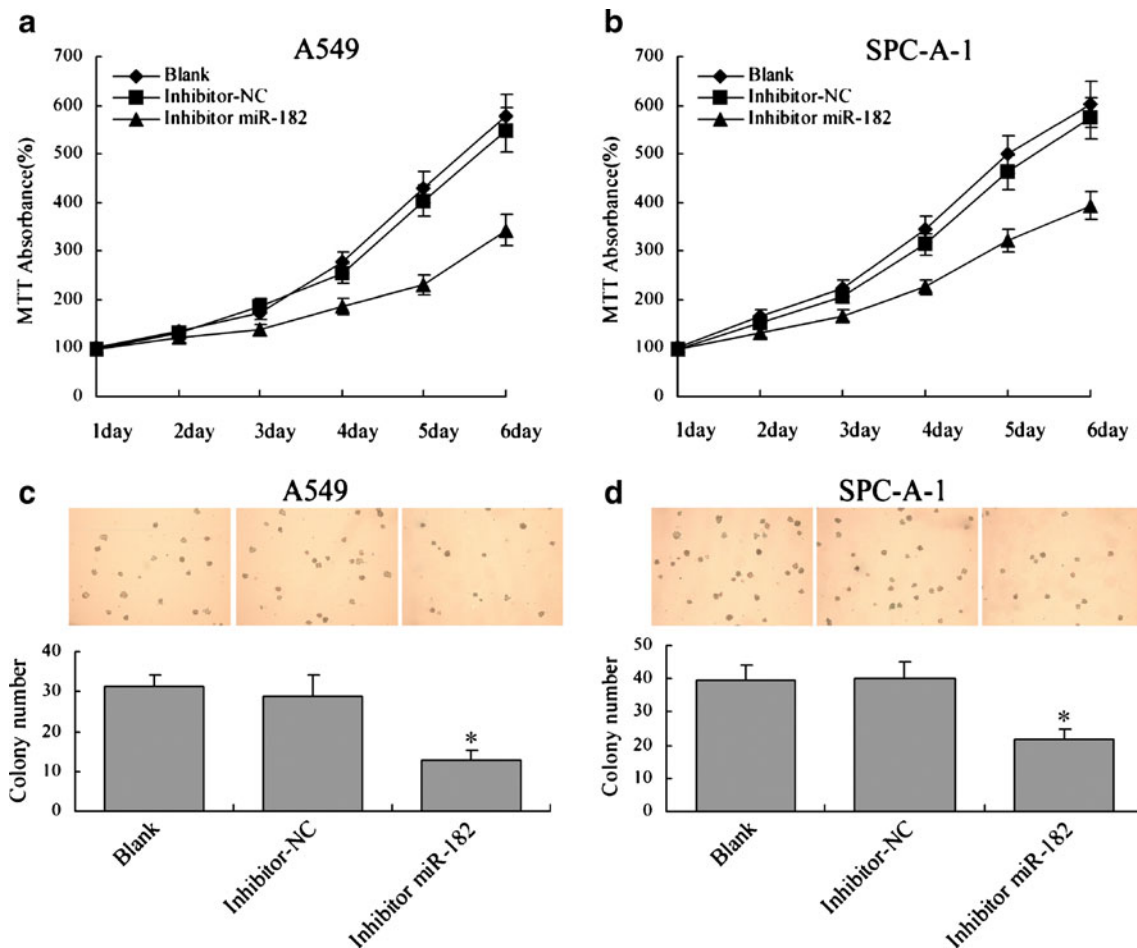


**Fig. 1** Expression of miR-182 and of PDCD4 protein in four lung cancer cell lines (A549, NCI-H1299, LTEP-a-2, SPC-A-1) and in NHBE. **a** Relative expression of miR-182, measured by qRT-PCR. U6 snRNA served as an endogenous control for normalization. The expression of miR-182 differed significantly between lung cancer cells

and normal cells ( $*P < 0.05$ ). **b** PDCD4 protein level, measured by western blot, in the five different cell types. GAPDH served as an endogenous reference. PDCD4 expression in the four lung cancer cell lines was significantly lower than in normal cells

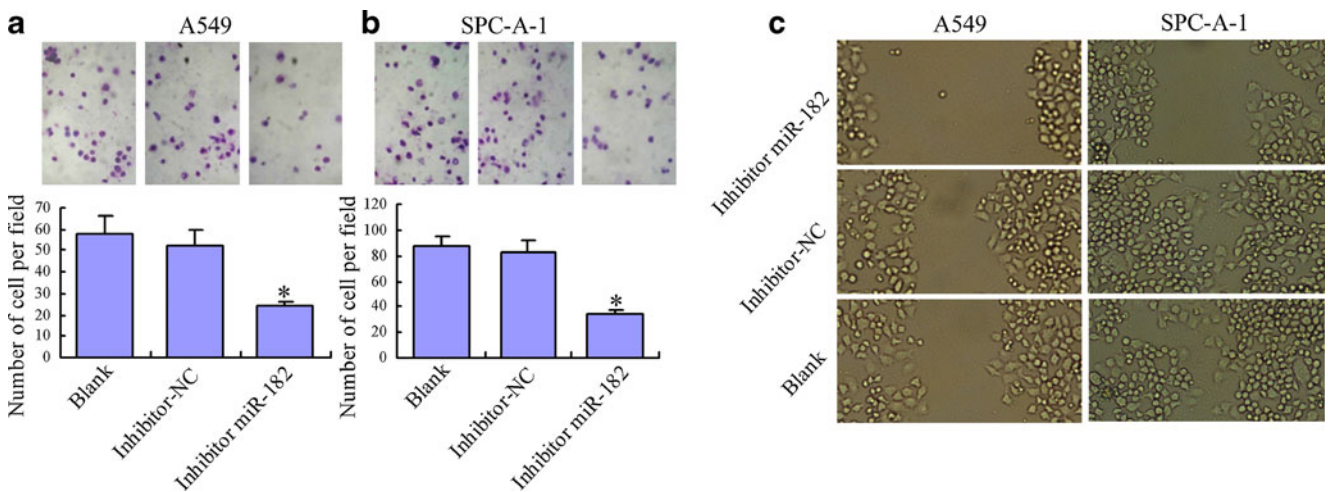
site for miR-182 (Fig. 4a). To determine whether PDCD4 is regulated by miR-182, we conducted western blot analysis

and luciferase reporter assays. Western blot analysis showed that PDCD4 expression was upregulated in A549 and SPC-



**Fig. 2** To analyze the biologic function of miR-182 inhibitor on cell proliferation of the lung cancer cell lines A549 and SPC-A-1, MTT and colony formation assays were performed. **a**, **b** MTT assay. A statistically significant decrease in A549 (**a**) and SPC-A-1 (**b**) proliferation was observed in the inhibitor miR-182 group compared with the inhibitor-NC and the blank groups ( $*P < 0.05$ ). No difference was found between the blank

group and the inhibitor-NC group. **c**, **d** colony forming assay. A statistically significant reduction in the number of colonies in A549 (**c**) and SPC-A-1 (**d**) cultures was observed in the inhibitor miR-182 group compared with the inhibitor-NC and blank groups ( $*P < 0.05$ ). *Blank* untransfected cells, *Inhibitor-NC* cells transfected with miR-182 inhibitor negative control, *Inhibitor miR-182* cells transfected with miR-182 inhibitor

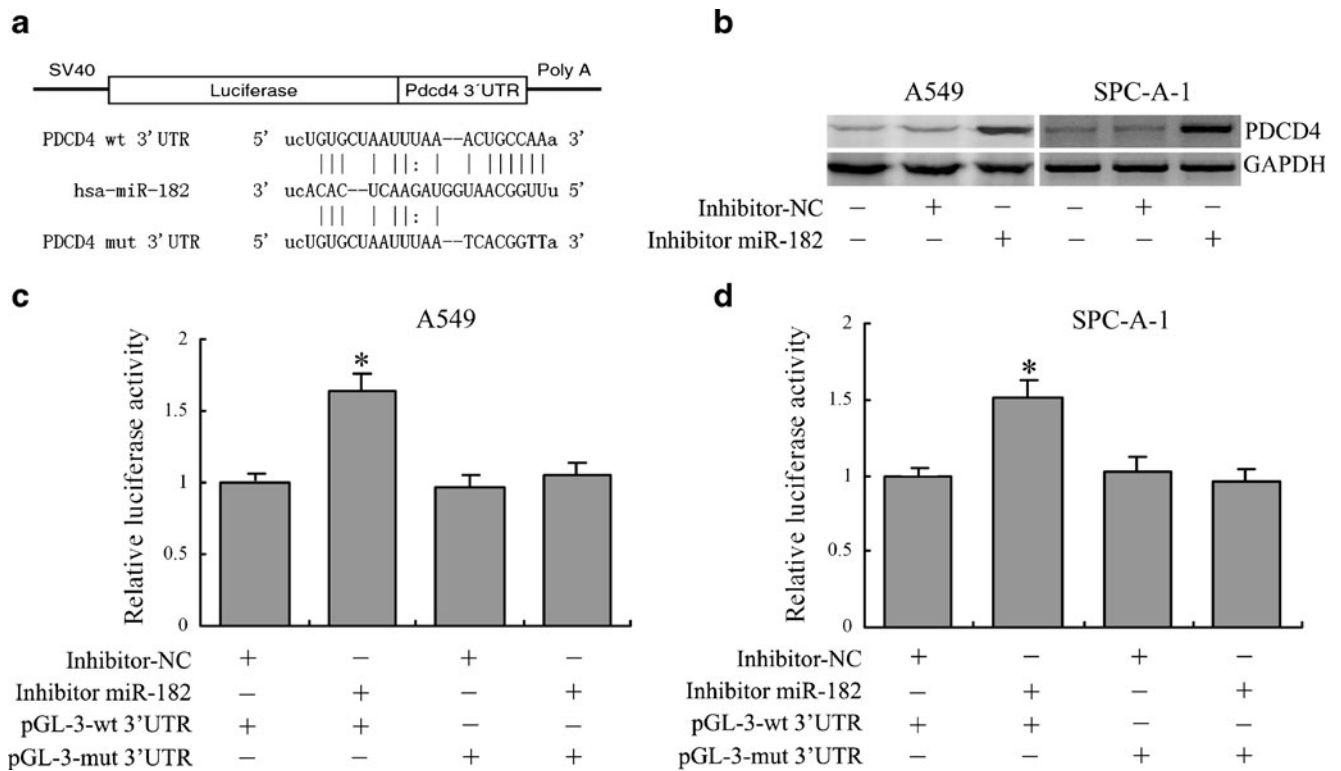


**Fig. 3** The invasive ability of A549 and SPC-A-1 lung cancer cells after transfection with miR-182 was assessed by Transwell assay and wound healing assay. **a** The number of A549 cells passing through the Matrigel was much lower in the inhibitor miR-182 group than in either the blank or the inhibitor-NC group ( $*P<0.05$ ). **b** The number of SPC-A-1 cells passing through the Matrigel was significantly lower in the

inhibitor miR-182 group than in either the blank or the inhibitor-NC group ( $*P<0.05$ ). **c** Both the blank and the inhibitor-NC groups contained a higher density of cells at 24 h after wounding compared to the inhibitor miR-182 group. *Blank* untransfected cells, *Inhibitor-NC* cells transfected with miR-182 inhibitor negative control, *Inhibitor miR-182* cells transfected with miR-182 inhibitor

A-1 cells after transfection with miR-182 inhibitor (Fig. 4b). To verify whether PDCD4 is a direct target of miR-182, we

first employed a dual-luciferase reporter system with luciferase reporter vectors containing either the wild-type or the



**Fig. 4** PDCD4 identified as a target gene of miR-182 in the human lung cancer cell lines A549 and SPC-A-1. **a** The putative PDCD4 wt 3'-UTR and PDCD4 mut 3'-UTR binding sequences in miR-182. **b** Western blot analysis of PDCD4 expression in transfected cells. GAPDH was used as the endogenous reference. miR-182 inhibitor can significantly increase expression of PDCD4 in both A549 and SPC-A-1 cells. **c, d** Luciferase reporter assay. Luciferase reporter vectors that contained wild-type (or

mutant-type) 3'UTR segments of PDCD4 were constructed and cotransfected into A549 (**c**) or SPC-A-1 (**d**) cells together with inhibitor-NC or inhibitor miR-182. Cotransfection of miR-182 inhibitor significantly increased the luciferase activity of the reporter containing pGL-3-wt 3'-UTR but did not increase that of the pGL-3-mut 3'-UTR reporter ( $*P<0.05$ ). *Inhibitor-NC* cells transfected with miR-182 inhibitor negative control, *Inhibitor miR-182* cells transfected with miR-182 inhibitor

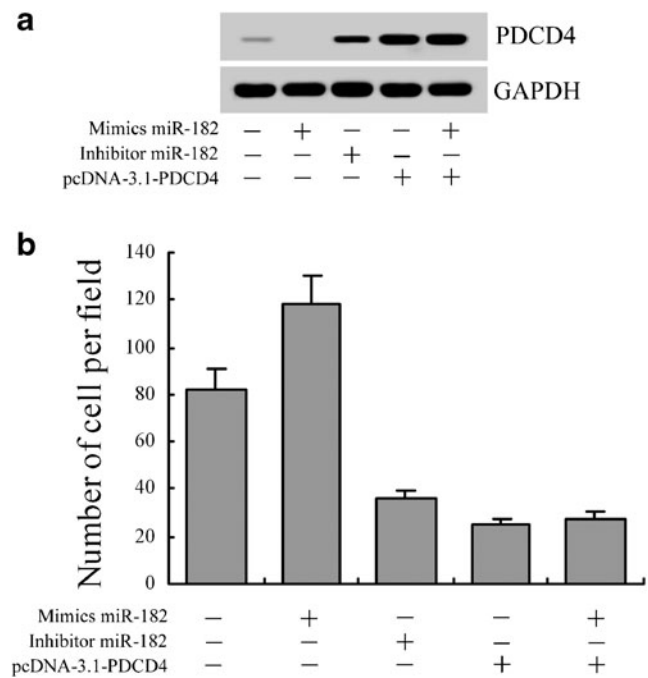
mutant-type 3'-UTR of PDCD4. While cotransfection with miR-182 inhibitor significantly increased the luciferase activity of the reporter containing wild-type 3'-UTR, it did not suppress that of the mutant-type reporter in A549 (Fig. 4c) or SPC-A-1 (Fig. 4d) cells. Taken together, these data strongly suggest that miR-182 negatively regulates PDCD4 expression by directly binding to its putative binding site in the 3'-UTR sequence. This finding also reveals that PDCD4 is a direct functional target of miR-182.

#### Expression of PDCD4 restores miR-182 antimigration function

We next performed western blots and Transwell assays in order to confirm that PDCD4 is a direct target of miR-182. The PDCD4 level in cells was lower after transfection with miR-182 mimics and was higher after transfection with vector containing PDCD4 lacking the 3'UTR (pcDNA3.1-PDCD4) into A549 cells. Cotransfection of miR-182 mimics and pcDNA3.1-PDCD4 led to increased expression of PDCD4 and abrogated the PDCD4 expression-reducing effect of miR-182 mimics (Fig. 5a). For Transwell assays, the mean number of cells penetrating the Transwell membrane increased after transfection with miR-182 mimics and decreased after transfection with pcDNA3.1-PDCD4. However, cotransfection with pcDNA3.1-PDCD4 and miR-182 mimics reduced the average number of Transwell cells, abrogating the effect of miR-182 mimics on increasing cell numbers. These results suggest that PDCD4 is a major target of miR-182 (Fig. 5b).

#### Discussion

miRNAs have been estimated to regulate up to 30 % of human genes and to control a variety of cellular processes [28, 29]. Recent studies have shown that miRNAs are dysregulated in various cancers, and their expression is relevant to a diverse array of tumors [30, 31]. Furthermore, information emerging about the role of miR-182 in cancers indicates that miR-182 acts as an oncogenic miRNA, which is frequently overexpressed in many solitary tumors [32–35]. These previous research findings correlate with our results, demonstrating that miR-182 is markedly upregulated in human lung cancer cells. However, it has been reported that miR-182 is downregulated in human gastric adenocarcinoma and negatively regulates cAMP-response element-binding protein 1 [36]. In this study, we conducted MTT and colony formation assays to further evaluate the effect of miR-182 on cell growth, and we performed Transwell and wound healing assays to evaluate its role in regulating invasion and migration activity. Our results demonstrate that miR-182 acts as an oncogene. The expression level of miR-182 varies among



**Fig. 5** Expression of PDCD4 abrogates miR-182 antimigration function. **a** The PDCD4 level of A549 cells was lower after transfection with miR-182 mimics and was higher after transfection with miR-182 inhibitor. However, transfection with PDCD4 vector lacking 3'UTR (pcDNA3.1-PDCD4) together with miR-182 mimics increased PDCD4 expression, abrogating the inhibitory effect of miR-182 mimics on PDCD4 expression. **b** The average numbers of cells penetrating the Transwell membrane increased after transfection with miR-182 mimics and decreased after transfection with miR-182 inhibitor. However, transfection with the PDCD4 vector lacking the 3'UTR (pcDNA3.1-PDCD4) together with miR-182 mimics decreased the average number of Transwell cells, abrogating the effect of miR-182 mimics on increasing cell numbers. These results suggest that PDCD4 is a major target of miR-182. *Inhibitor-NC* cells transfected with miR-182 inhibitor negative control, *Inhibitor miR-182* cells transfected with miR-182 inhibitor, *pcDNA3.1-PDCD4* cells transfected with vector of PDCD4 lacking 3' UTR

different cancers, and its function differs depending on context. The reported variability in miRNA-182 function may be due to the fact that miRNA can downregulate numerous targets, including both oncogenes and oncosuppressor genes, and that different genes take effect in different cancers.

Based on the results of bioinformatics analysis by TargetScan and miRanda, we hypothesized that PDCD4 was among the targets of miR-182. The PDCD4 gene was first isolated from a human glioma cell cDNA library as a tumor-related gene [37, 38] and originally identified as a transcript upregulated in apoptotic cells [39]. Accumulating evidence indicates that PDCD4 is a novel tumor suppressor gene. Loss or reduction of PDCD4 expression has been found in several types of human primary tumors such as colorectal cancer [40], glioma [41], and lung cancer.

In cancer cells, PDCD4 regulates multiple proteins which are involved in tumor progression, cell cycle, and differentiation

[42]. In colon cancer cells, PDCD4 inhibits the expression of a kinase upstream of JNK, known as MAPK kinase (MAPKKK), and suppresses cell invasion [43]. In breast cancer, PDCD4 increases tissue inhibitor of metalloproteinase 2 and thereby inhibits cell invasion [44]. These data indicated that PDCD4 might act as a cancer suppressor. In the current study, we found that downregulation of miR-182 expression positively regulated the expression of PDCD4 and simultaneously restricted cell proliferation and reduced the metastasis and invasiveness of A549 and SPC-A-1 lung cancer cells. Moreover, we showed, by western blot and luciferase reporter assay, that PDCD4 is a target of miR-182, and we confirmed these findings using an override assay. These results suggest that miR-182 might inhibit proliferation and suppress invasiveness by suppressing the molecule Bcl-2.

In conclusion, we have demonstrated that miR-182 is upregulated in lung adenocarcinoma cells. We have also shown that in lung cancer cell lines (A549 and SPC-A-1), by targeting PDCD4, downregulation of miR-182 inhibits proliferation and invasion by these cells. Our results expand knowledge of the mechanism of action of miRNA in regulating cancer cells and may also aid the development of new therapeutic strategies to target lung adenocarcinoma.

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**Conflicts of interest** None

## References

- Ambros V. The functions of animal microRNAs. *Nature*. 2004; 431(7006):350–5.
- Kim VN, Nam JW. Genomics of microRNA. *Trends Genet*. 2006; 22(3):165–73.
- Kwak PB, Iwasaki S, Tomari Y. The microRNA pathway and cancer. *Cancer Sci*. 2010;101(11):2309–15.
- Farazi TA, Spitzer JI, Morozov P, Tuschl T. miRNAs in human cancer. *J Pathol*. 2011;223(2):102–15.
- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281–97.
- Zamore PD, Haley B. Ribo-gnome: the big world of small RNAs. *Science*. 2005;309(5740):1519–24.
- Brennecke J, Cohen SM. Towards a complete description of the microRNA complement of animal genomes. *Genome Biol*. 2003; 4(9):228.
- Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res*. 2005;65(14):6029–33.
- Jensen RH, Tiirikainen M, You L, Ginzinger D, He B, Uematsu K, et al. Genomic alterations in human mesothelioma including high resolution mapping of common regions of DNA loss in chromosome arm 6q. *Anticancer Res*. 2003;23(3B):2281–9.
- Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*. 1993;75(5):843–54.
- Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell*. 1993;75(5):855–62.
- He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, et al. The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci U S A*. 2005;102(52):19075–80.
- Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA*. 2008;299(4):425–36.
- Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell*. 2006;9(3):189–98.
- Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res*. 2005;65(16):7065–70.
- Iorio MV, Visone R, Di LG, Donati V, Petrocca F, Casalini P, et al. MicroRNA signatures in human ovarian cancer. *Cancer Res*. 2007; 67(18):8699–707.
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin*. 2009;59(4):225–49.
- Spira A, Ettinger DS. Multidisciplinary management of lung cancer. *N Engl J Med*. 2004;350(4):379–92.
- Jemal A, Siegel R, Xu J, Ward E. Cancer statistics. *CA Cancer J Clin*. 2010;60(5):277–300.
- Cmarik J, Min H, Hegamyer G, Zhan S, Kulesz-Martin M, Yoshinaga H, et al. Differentially expressed protein Pcd4 inhibits tumor promoter-induced neoplastic transformation. *Proc Natl Acad Sci*. 1999;96:14037–42.
- Jansen AP, Camalier C, Colburn NH. Epidermal expression of the translation inhibitor programmed cell death 4 suppresses tumorigenesis. *Cancer Res*. 2005;65:6034–41.
- Yang H, Cho M, Zakowicz H, Hegamyer G, Sonenberg N, Colburn NH. A novel function of the MA-3 domains in transformation and translation suppressor Pcd4 is essential for its binding to eukaryotic translation initiation factor 4A. *Mol Cell Biol*. 2004;24:3894–906.
- Zakowicz H, Yang H, Stark C, Wlodawer A, Laronde-Leblanc N, Colburn NH. Mutational analysis of the DEAD-box RNA helicase eIF4AII characterizes its interaction with transformation suppressor Pcd4 and eIF4GI. *RNA*. 2005;11:261–74.
- Wang Y-Q, Guo R-D, Guo R-M, Sheng W, Yin L-R. MicroRNA-182 promotes cell growth, invasion, and chemoresistance by targeting programmed cell death 4 (PDCD4) in human ovarian carcinomas. *J Cell Biochem*. 2013;114:1464–73.
- Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *J Biol Chem*. 2008;283:1026–33.
- Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*. 2008; 27:2128–36.
- Itani S, Kunisada T, Morimoto Y, Yoshida A, Sasaki T, Ito S, et al. MicroRNA-21 correlates with tumorigenesis in malignant peripheral nerve sheath tumor (MPNST) via programmed cell death protein 4 (PDCD4). *J Cancer Res Clin Oncol*. 2012;138: 1501–9.
- Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. 2005;120(1):15–20.
- Schickel R, Boyerinas B, Park SM, Peter ME. MicroRNAs: key players in the immune system, differentiation, tumorigenesis and cell death. *Oncogene*. 2008;27(45):5959–74.

30. Guo Y, Chen Z, Zhang L, Zhou F, Shi S, Feng X, et al. Distinctive microRNA profiles relating to patient survival in esophageal squamous cell carcinoma. *Cancer Res.* 2008;68(1):26–33.
31. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer.* 2006;6(11):857–66.
32. Liu Z, Liu J, Segura MF, Shao C, Lee P, Gong Y, et al. MiR-182 overexpression in tumorigenesis of high-grade serous ovarian carcinoma. *J Pathol.* 2012;228:204–15.
33. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S, et al. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. *Proc Natl Acad Sci.* 2009;106:1814–9.
34. Stittrich AB, Haftmann C, Sgouroudis E, Kuhl AA, Hegazy AN, Panse I, et al. The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. *Nat Immunol.* 2010;11:1057–62.
35. Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S, Miyazono K. Modulation of microRNA processing by p53. *Nature.* 2009;460:529–33.
36. Kong WQ, Bai R, Liu T, Cai CL, Liu M, Li X, et al. MicroRNA-182 targets cAMP-responsive element-binding protein 1 and suppresses cell growth in human gastric adenocarcinoma. *FEBS J.* 2012;279:1252–60.
37. Matsushashi S, Yoshinaga H, Yatsuki H, Tsugita A, Hori K. Isolation of a novel gene from a human cell line with Pr-28 MAb which recognizes a nuclear antigen involved in the cell cycle. *Res Commun Biochem Cell Mol Biol.* 1997;1:109–20.
38. Yoshinaga H, Matsushashi S, Fujiyama C, Masaki Z. Novel human PDCD4 (H731) gene expressed in proliferative cells is expressed in the small duct epithelial cells of the breast as revealed by an anti-H731 antibody. *Pathol Int.* 1999;49:1067–77.
39. Shibahara K, Asano M, Ishida Y, Aoki T, Koike T, Honjo T. Isolation of a novel mouse gene MA-3 that is induced upon programmed cell death. *Gene.* 1995;166:297–301.
40. Wang Q, Sun Z, Yang H. Downregulation of tumor suppressor Pcdcd4 promotes invasion and activates both b-catenin/Tcf and AP-1-dependent transcription in colon carcinoma cells. *Oncogene.* 2007;27:1527–35.
41. Gao F, Wang X, Zhu F, Wang Q, Zhang X, Guo C, et al. PDCD4 gene silencing in gliomas is associated with 5′CpG island methylation and unfavourable prognosis. *J Cell Mol Med.* 2009;13:4257–67.
42. Lankat-Buttgereit B, Goke R. The tumour suppressor Pcdcd4: recent advances in the elucidation of function and regulation. *Biol Cell.* 2009;101:309–17.
43. Yang HS, Matthews CP, Clair T, Wang Q, Baker AR, Li CC, et al. Tumorigenesis suppressor Pcdcd4 down-regulates mitogen-activated protein kinase kinase kinase 1 expression to suppress colon carcinoma cell invasion. *Mol Cell Biol.* 2006;26:1297–306.
44. Nieves-Alicea R, Colburn NH, Simeone AM, Tari AM. Programmed cell death 4 inhibits breast cancer cell invasion by increasing tissue inhibitor of metalloproteinases-2 expression. *Breast Cancer Res Treat.* 2009;114:203–9.