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Original Article

Overexpression of PTEN suppresses non-small-cell lung carcinoma metastasis through inhibition of integrin αVβ6 signaling

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Abstract: Studies have demonstrated that the abnormal expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is associated with multiple malignancies, but its functional role in non-small-cell lung carcinoma (NSCLC) metastasis remains to be elucidated. In the present study, we investigated the role of PTEN in regulating proliferation, migration, and invasion of NSCLC cells by establishing NSCLC cell strains with constitutively silenced or elevated PTEN expression. We demonstrated that ectopic expression of PTEN inhibits migration and invasion of NSCLC cells in vitro through wound healing and Transwell invasion assays. Furthermore, PTEN overexpression in NSCLC cells greatly inhibits cell viability and colony formation, which was confirmed by MTT and colony formation assays. Conversely, further analysis indicated that suppression of PTEN expression via shRNA promotes metastasis and growth of NSCLC cells. Finally, our findings demonstrate that PTEN promotes invasion and migration of NSCLC cells through the integrin αVβ6 signaling pathway. Overall, this study provides novel insights into the role of PTEN as a crucial regulator of NSCLC cell metastasis, and suggests that targeted treatment of PTEN-expressing tumors serves as a new therapeutic target for NSCLC.

Keywords: PTEN, NSCLC, metastasis, integrin αVβ6

Introduction

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which is encoded by the PTEN gene, is a modulator of cell survival and cell cycle progression. In normal physiological conditions, PTEN plays an essential role in smooth muscle differentiation, maintains regulatory T cell stability, mediates angiogenesis, and coordinates intraretinal neurogenesis [1]. However, PTEN has been identified as a tumor suppressor that is downregulated and mutated at high frequency in many types of cancer, including non-small-cell lung carcinoma (NSCLC). Tumor progression and metastasis are the major causes of cancer-related death, especially in human NSCLC [2]. NSCLC is one of the most aggressive malignant tumors, with poor prognosis largely due to distant metastasis and post-surgical recurrence. Indeed, inactivation of PTEN can augment invasiveness and anchorage-independent growth in several cancer cell types [3]. In addition, PTEN inactivation has been shown to accelerate tumorigenesis in some animal models. On the other hand, exogenously expressed PTEN suppresses breast and liver tumorigenesis. Similarly, PTEN upregulation inhibits tumor cell growth and promotes partial apoptosis. Tumor suppression by PTEN depends on its negative regulation of the phosphatidylinositol 3-kinase-AKT-mammalian target of rapamycin (PI3K-Akt-mTOR) signaling pathway [4]. Recent investigation into the role of PTEN in tumor development indicates the involvement of certain intracellular signaling cascades, including V-Ki-ras2 kirsten rat sarcoma viral oncogene homolog (K-RAS) and mitogen-activated protein kinase (MAPK). Findings on the role of PTEN in cancer therefore seem to be inconsistent between studies [5]. Despite these studies, the underlying mechanism of PTEN dysregulated expression in NSCLC growth and metastasis remain elusive.

Integrins are a family of transmembrane adhesion receptors, which are composed of 18α and 8β subunits that interact non-covalently to form at least 25 different heterodimeric receptors [6]. Integrins are expressed in almost every cell type, and are mediators of attachment to the extracellular matrix (ECM) and critical regulators of tumor cell physiology, including cell migration and proliferation. During the process of tumor cell invasion and metastasis, integrins act as crucial transducers of bidirectional cell signaling, regulating cell adhesion, migration, and tissue remodeling [7]. In addition to establishing a physical bridge from the ECM to the actin cytoskeleton, integrins control the activation of a variety of intracellular signaling pathways, including the control of activation of actin nucleation, polymerization, and cross-linking proteins, as well as pro-survival and mitogenic signaling. Integrin αVβ6 is predominantly expressed in squamous epithelial cells, endothelial cells, immature thymocytes, Schwann cells, and fibroblasts of the peripheral nervous system [8]. It interacts with growth factor receptors, including those of the epidermal growth factor receptor family, to activate signaling pathways involved in tumorigenesis and metastasis, including PI3K, AKT, and MAPK signaling [9].

Given the likely involvement of PTEN in cancer development, we investigated the effects of targeting the PTEN gene in NSCLC metastasis, and showed that reducing the expression of PTEN promotes proliferation and accelerates cell invasion and migration in NSCLC cancer cells, whereas ectopic expression of endogenous PTEN has the opposite effect. Furthermore, we showed that PTEN negatively regulates NSCLC cell metastasis by inhibiting integrin αVβ6 signaling. Together, our results provide new evidence that reduced PTEN expression promotes the progression of NSCLC, and that PTEN might therefore represent a novel therapeutic target for NSCLC.

Materials and methods

Cell lines and cell viability

The human non-small cell lung carcinoma cell lines H1975, A549, HCC827, and H1650, and the control human bronchial epithelial cell line BEAS-2B were purchased from the Chinese Academy of Sciences Cell Bank of Type Culture Collection (CBTCCCAS, Shanghai, China). The NSCLC cell lines were maintained in RPMI-1640 or DMEM medium supplemented with 10% FBS, streptomycin (100 μg/mL), and penicillin (100 μg/mL). BEAS-2B cells were cultured in LHC-8 medium supplemented with 10% FBS. All cells were maintained in an incubator with a humidified atmosphere containing 5% CO_2 at 37°C. The number of viable cells was determined using the trypanblue dyeexclusion test.

Oncomine analysis and patient survival assay

The expression level of the PTEN gene in the selected cancers was analyzed using the Oncomine database. Cancer specimens were compared against data from normal controls. In order to reduce the false discovery rate, we selected $P<0.01$ as a threshold. We analyzed the results for their *p*-values and fold change [10]. Kaplan-Meier curves for non-small-cell lung carcinoma patients with high versus low PTEN mRNA levels were generated using Kaplan-Meier Plotter (http://kmplot.com).

RNA interference

PTENshRNA was synthesized by Biomics Biotechnologies Co. Ltd (Shanghai, China). Cells were transfected with PTENshRNA (sense, 5'-GACCAACUCUCUCCUGUAUTT-3' and antisense, 5'-AUACAGGAGAGAGUUGGUCTT-3') or non-specific shRNA (0.5 μg/well for 96-well plates and 2 μg/well for 6-well plates) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. Full-length human PTEN complementary DNA (cDNA) was amplified by PCR and cloned into the pcDNA3.1 (+) expression vector (Invitrogen) before being transfected into NSCLC cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. At 48 h post-transfection, cells were collected for analysis by western blot and clonogenicity, invasion, and migration assays.

Colony formation assay

Colony formation assay was performed to assess the ability of single H1975 or A549 cells to grow into colonies in vitro. Briefly, after transfection with PTENshRNA or plasmid for 48 h, 2×10^4 cells were seeded in complete RPMI-1640 medium in 60 mm plates containing a top layer of 1% agar and a bottom layer of 10%

agar. The plates were incubated for 4 weeks at 37°C and then stained with 0.1% crystal violet. Colonies with greater than 50 cells were counted manually [11].

Wound healing analysis

The migration ability of PTEN-silenced or PTEN-overexpressing NSCLC cells was assessed using the scratch assay. After PTENshRNA transfection, cells were seeded in 6-well plastic plates at a density of 3×10^5 cells/well. After 24 h, cells reached 90-100% monolayer confluence. A straight scratch was artificially created in the cell monolayers with a sterile 100-μl pipette tip. Resulting cell debris was removed with phosphate-buffered saline (PBS) and cultures were then supplemented with fresh RPMI-1640 medium for 48 h at 37°C. Migration images were captured using an inverted microscope (CarlZeiss, Oberkochen, Germany). Scratch wound widths were measured under the microscope and the relative percentage of wound closure was determined by comparing to control cells [12].

Transwell invasion assay

The effect of PTENshRNA or overexpression on NSCLC cell migration was evaluated by Transwell invasion assay. After shRNAor PTEN plasmid transfection for 48 h, 200 μl 5×10^3 cells were plated into the Transwell upper chamber with Matrigel-coated (1 mg/ml, BD Matrigel™) polycarbonate membrane (8.0 μm, Corning, Corning, NY, USA). A total of 600 μl RPMI-1640 medium containing 10% FBS was added to the Transwell lower chamber. After incubation for 6 h, cells on the lower surface of the polycarbonate membrane were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The non-migrated cells on the upperside of the polycarbonate membrane were removed with a cotton swab. Images of cells that had migrated to the under-surface of the membrane were captured using five different microscopic fields. The number of migrated cells was counted from five randomly selected fields per polycarbonate membrane [13].

Western blot analysis

Cells were transfected with PTENshRNA or plasmid for 48 h. Whole-cell lysates were prepared with RIPA buffer containing protease and phosphatase inhibitors. Equal amounts of cell lysates (30 μg) were separated on 10% SDS-PAGE gels and transferred onto PVDF membranes. After membranes were blocked, they were incubated with monoclonal antibodies against PTEN (1:1000, Signalway Antibody, College Park, MD, USA), integrin αVβ6 (1:1000, Signalway Antibody), and β-actin (1:5000, Bioworld Technology, St Louis Park, MN, USA) followed by incubation with goat anti-rabbit IgG (H&L) HRP (1:10000, BioworldTechnology). All target proteins were detected using the ECL Gel system (Millipore, Braunschweig, Germany) and visualized using the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA).

Quantitative real-time PCR

Total RNA was isolated using the TRIzol reagent (Invitrogen) according to the manufacturer's recommendations. cDNA was then synthesized from 1 μg total RNA using the PrimeScript RT reagent kit (TakaraBio, Kusatsu, Japan) according to the manufacturer's protocol. qRT-PCR was performed using IQTM SYBR Green supermix and the iQ5 real-time detection system (Bio-Rad). The $2^{-\Delta\Delta Ct}$ method was used to quantify the expression levels. The primers used for PCR were as follows (sense and antisense, respectively): GAPDH: 5'-TGTGGGCATCAATGGATTTGG-3' (forward) and 5'-ACACCATGTATTCCGGGTCAAT-3' (reverse); PTEN: 5'-TGGATTCGACTTAGACTTGACCT-3' (forward) and 5'-GGTGGGTTATGGTCTTCAAAAGG-3' (reverse). GAPDH RNA expression was used to normalize the target genes levels.

Microarray analysis

In order to screen for PTEN target genes in human NSCLC cells, microarray experimental procedures were carried out following the manufacturer's protocols. Total RNA (1 μg) extracted from parental H1975 and PTEN-knockdown cells was labeled with either Cy5 or Cy3 during the transcription process. Approximately 0.8 μg of Cy-labeled cRNA was cleaved to an average size of around 50-100 nucleotides by incubation with fragmentation buffer (Agilent Technologies, CA, USA) at 60°C for 30 min. Equal Cy-labeled cRNA was pooled and hybridized to SurePrint G3 Human Gene Expression 8 × 60 K v2 arrays (Agilent Technologies) at 65°C for 17 h. After washing, followed by drying using a nitrogen gun, microarrays were scanned with

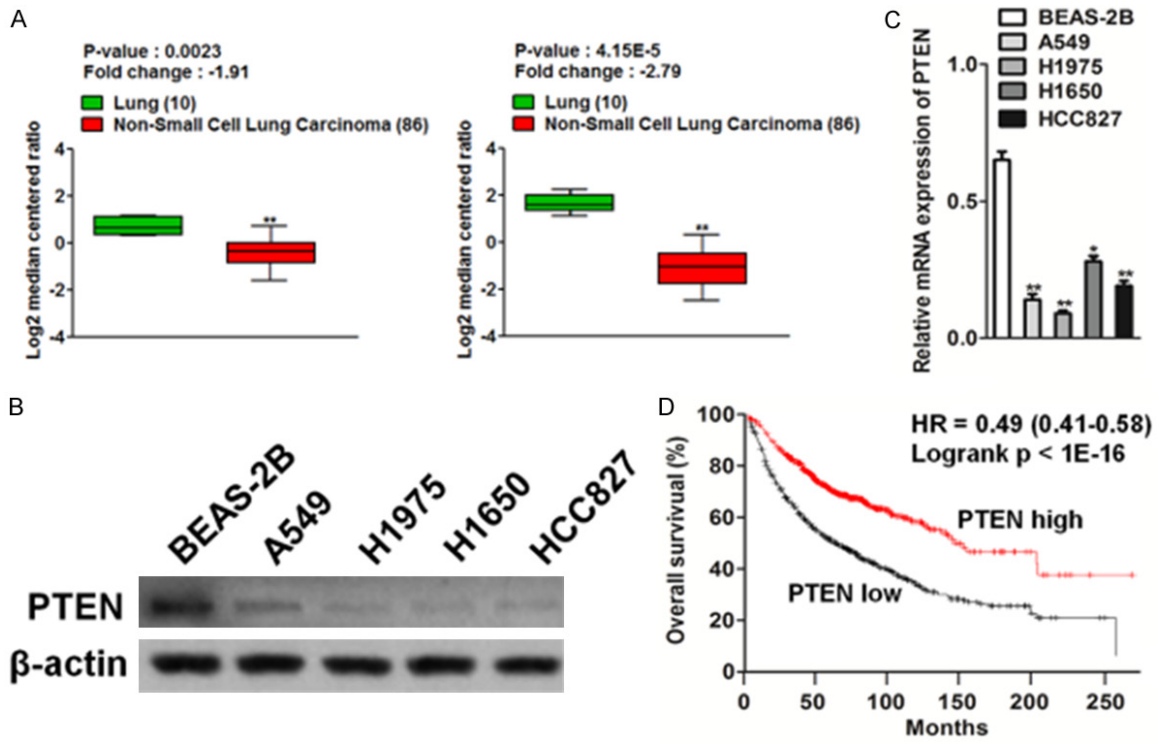

Figure 1. PTEN is significantly down-regulated in human NSCLC cancer tissues and cell lines. A. Box plots show decreased levels of PTEN in NSCLC (right) compared with normal lung tissues (left) in two microarray data sets. **P<0.01, compared with normal lung tissues. B. Western blotting analysis of the levels of PTEN in various NSCLC cancer cell lines. β-actin was used as loading controls. Expression of PTEN was quantified and normalized to that in BEAS-2B cells, and shown in the graph to the right. C. qRT-PCR analysis of PTEN mRNA levels in untransformed BEAS-2B cells and various NSCLC cancers cell lines. PCR values were normalized to the levels of β-actin. Data were presented as the mean ± SD from three independent measurements. D. The Kaplan-Meier curve for non-small lung cancer cell (NSLCC) patients with high or low PTEN mRNA level was get from Kaplan-Meier Plotter (http://kmplot.com). HR = 0.49, P<1E-16.

an Agilent microarray scanner (Agilent Technologies) at 535 nm for Cy3 and 625 nm for Cy5, controlled by Agilent Scan Control 7.0 software. Data were extracted with Agilent Feature Extraction 9.1 software. Differentially expressed targets were identified using processed signal intensities and *p*-value log ratios as described elsewhere [14].

Xenograft tumor assay

Female BALB/c nu/nu mice (4-6 weeks-old) were obtained from Shanghai Slack laboratory animal co., LTD and maintained in specific pathogen-free conditions. All experimental protocols were approved by the Committee for the Care and Use of Laboratory Animals of Qilu Hospital of Shandong University and all experiments were performed in accordance with the approved guidelines and regulations. For the subcutaneous tumor model, cells transfected with PTENshRNA or plasmid were suspended in PBS at a concentration of 1×10^5 cells/μl. A volume of 0.1 ml of cells was subcutaneously injected into the right flank of anesthetized mice (n = 6 for each group), and the mice were observed for 3 weeks. The xenograft tumor volume was measured every 3 days and calculated as $(W^2 \times L)/2$, where W and L refer to the shorter and longer dimensions of the tumor, respectively. Tumor specimens were fixed in 10% formalin and embedded in paraffin, and 5 μm sections were cut. For immunohistochemical assays, endogenous peroxidases were blocked in 3% hydrogen peroxide. Immunohistochemistry for PTEN was carried out on tumor tissue sections using the Biogenex IHC detection system according to the manufacturer's guidelines with anti-PTEN antibody. Images were taken and analyzed using the ZEN 2011 imaging software on a Zeiss inverted micro-

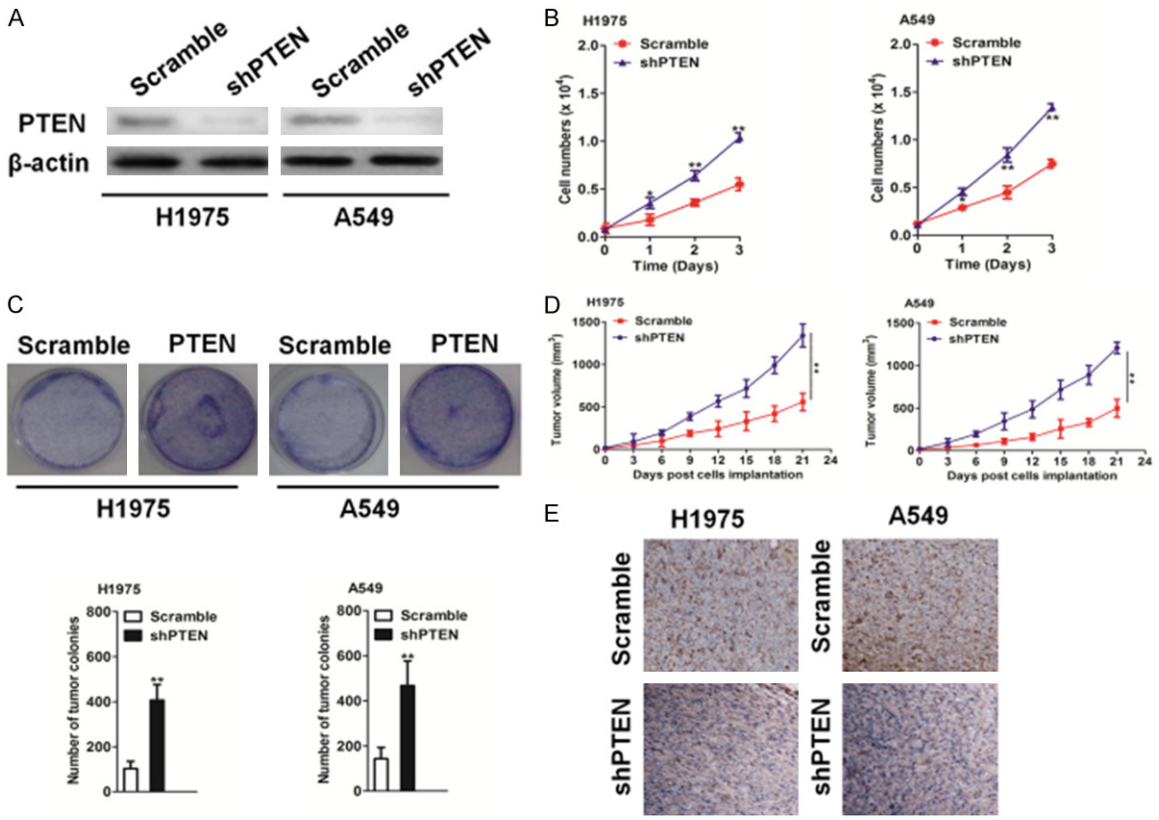

Figure 2. PTEN knock-down promotes tumorigenicity in NSCLC cells. A. Western blot was performed to test the efficiency and specificity of PTEN knockdown plasmid. PTENshRNA transfection was shown completely loss of PTEN relative to parental H1975 and A549 cells. B. Cell growth rate from parental and PTEN knockdown H1975 cells (left) and A549 cells (right) at indicated time point. The data are presented as mean ± SD. C. Soft-agar colony formation assay of parental and PTEN knockdown H1975 and A549 cells. 10^4 of indicated cells were plated in complete medium containing 0.1% agarose and cultured for 3 weeks. The colonies were stained with 1% crystal violet and counted. D. Tumor growth curve for H1975 and A549 xenogaft tumor models. E. Representative PTEN immunohistochemistry staining for expression of PTEN of NSCLC tumor graft grown in nude mice (Size bar, 50 μm).

scope (CarlZeiss) under 200-fold magnification.

Results

Low PTEN expression predicts poor prognosis in NSCLC cancer patients

To investigate whether PTEN and its associated factors are involved in human NSCLC cancer progression, we first examined their expression patterns using the publicly accessible Oncomine microarray database. In two independent clinical data sets containing PTEN information [15, 16], PTEN expression was markedly reduced in NSCLC cancer tissues, when compared with matched normal tissues (**Figure 1A**). The association between cancer progression and reduced PTEN expression was also confirmed in a panel of NSCLC cancer cell lines. As shown in **Figure 1B**, PTEN was expressed at relatively high levels in the untransformed human bronchial epithelial cells (BEAS-2B), but was markedly reduced in all four metastatic NSCLC cancer lines examined (A549, H1975, H1650, and HCC827). This reduction was partially due to a decrease in PTEN mRNA levels as shown by qRT-PCR (**Figure 1C**). To study the function of PTEN in NSCLC, Kaplan-Meier curves for NSCLC patients with high or low PTEN mRNA levels were generated using Kaplan-Meier Plotter (http://kmplot.com). As shown in **Figure 1D**, low PTEN expression was associated with poor prognosis.

PTEN loss enhances growth in NSCLC cancer and lung cancer cells

To investigate the effect of PTEN in NSCLC cancer cells, PTEN was knocked down by transfection of shRNA plasmid in NSCLC cancer cell lines H1975 and A549. Complete efficiency

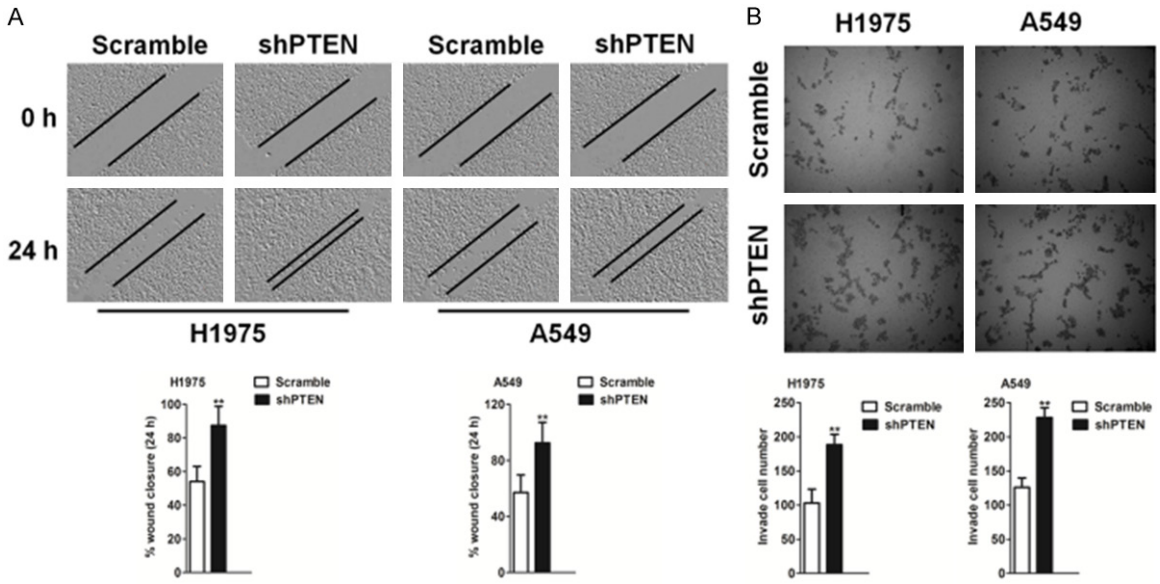

Figure 3. Silencing PTEN induces migration and invasion in NSCLC cells. A. In vitro wound healing assay with human H1975 and A549 cells after knockdown PTEN expression. Image was acquired at 0, 24 h time points after scratching (upper panel). Quantification of wound closure was calculated (lower panel). B. Representative staining of invasive potentials of human H1975 and A549 cells from in vitro Transwell assay (upper panel). Quantification of invasive cells per field was analyzed (lower panel). Statistical analyses were performed by the Student's t test. The following symbols were used to indicate significant differences: $^{**}P<0.01$.

and specificity of PTEN knockdown was shown relative to parental H1975 and A549 cells by western blot analysis (**Figure 2A**). Intriguingly, we observed that both H1975 and A459 cells with knockdown of PTEN grew significantly more rapidly than the parental cells (**Figure 2B**), indicating a negative role for PTEN in regulating cell growth. Consistently, frequencies of colony formation from PTEN-knockdown H1975 and A459 cells were increased dramatically in soft agar colony formation assays (**Figure 2C**). We next examined the impact of PTEN knockdown on tumor growth in vivo. PTEN-deficient tumor cells from both H1975 and A549 exhibited significantly stronger progression, as evidenced by larger tumor volume over the treatment period (**Figure 2D**). H1975 and A549 tumor grafts grown in nude mice also showed low PTEN expression (**Figure 2E**). Taken together, these results suggest that PTEN negatively regulates NSCLC growth and progression.

PTEN loss enhances cancer metastasis

Given that the invasive property of tumor cells contributes to poor prognosis, we next assessed whether PTEN affects cell migration and invasion. Wound healing assays indicated that knockdown of PTEN in both H1975 and A549 cells resulted in significantly increased cell migration compared with the parental cells (**Figure 3A**). A quantitative analysis demonstrated that the wound closure rate was increased from 57% to 92% in A549 cells and from 54% to 87% in H1975 cells when PTEN was knocked down (**Figure 3A**). Consistently, we observed in a Transwell experiment that more cells invaded into the bottom wells when PTEN was knocked down (**Figure 3B**). These results suggest that PTEN regulates the migration and invasion abilities of tumor cells in vitro.

Overexpressed PTEN inhibits tumor growth

To functionally dissect the precise role of overexpressed PTEN in tumor growth, we stably expressed an empty vector and a vector expressing PTEN plasmid in the H1975 and A549 cell lines. Functional assessment of the exogenous PTEN was achieved using western blot analysis (**Figure 4A**). Ectopic expression of PTEN decreased cellular proliferation (**Figure 4B**), and colony formation assays revealed that enhanced PTEN expression inhibited the formation of tumor colonies (**Figure 4C**). In addition, we examined the effects of PTEN on NSCLC cell growth in vivo, using preclinical nude mouse models of H1975 and A549 cell

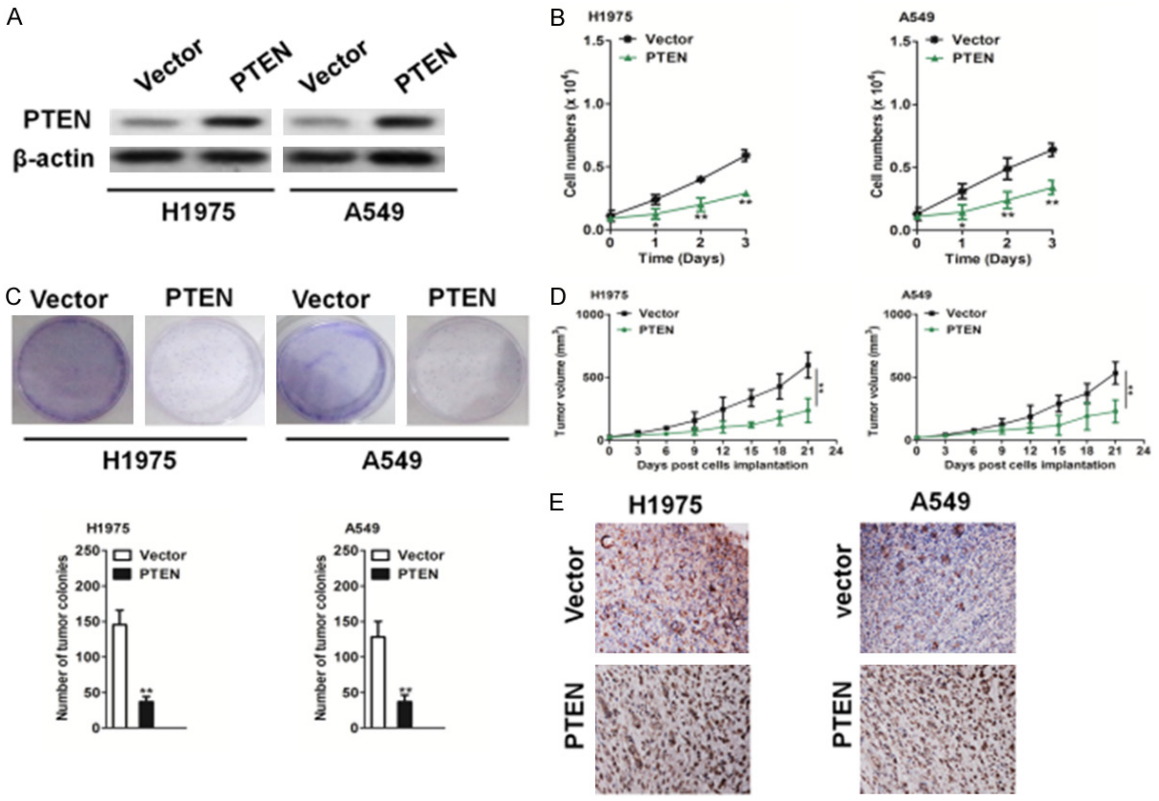

Figure 4. Enhanced expression of PTEN decreases NSCLC cells migration and invasion. A. The expression of PTEN in T98G NSCLC cells transfected with the vector expressing PTEN plasmid was evaluated by western blotting assay. B. Up-regulation of PTEN caused a significant growth promotion of H1975 NSCLC cells as revealed by proliferation assay. Values shown were the mean absorbance ± SD for five wells from one experiment, and were representations of three independent experiments. C. Colonies were shown in purple post staining with crystal violet. D. Tumor growth kinetics (mean ± SD) of vector control H1975 or PTEN over-expressing cells in nude mice (n = 6 each). Data in this figure were presented as the mean ± SD, and $^{**}P<0.01$ was determined by the Student's t test. E. Immunohistochemistry identifies the expression of PTEN from mice inoculation with PTEN over-expressing cells was significantly distinct than cells transfected with vector.

lines. PTEN overexpressing or control cells were implanted subcutaneously into the posterior flank of nude mice (n = 6). Remarkably, PTEN overexpressing cells impaired solid tumor growth within the inoculation site (**Figure 4D**). H1975 and A549 tumor grafts grown in nude mice inoculated with cells overexpressing PTEN exhibited higher PTEN expression (**Figure 4E**). Lastly, wound healing and Transwell invasion assays showed that PTEN overexpression significantly decreased cellular mobility (**Figure 5A**) and cell invasion (**Figure 5B**). Taken together, our findings indicate that PTEN overexpression ablates NSCLC cell metastasis in vitro and proliferation in vivo.

PTEN regulates migration and invasion through integrin αVβ6

In order to unravel the cellular pathways involved in PTEN-mediated migration and invasion, we performed gene expression analysis in control and PTEN-depleted H1975 cells. We selected a panel of genes involved in the regulation of migration and invasion (Z score ≥2 or ≤-2, *p*-value <0.05). The most downregulated gene was integrin αVβ6, which activates the notch receptor signaling pathway (**Figure 6A**). Integrin αVβ6 regulates multiple cancer-associated processes including proliferation, survival, EMT, metastasis, and angiogenesis. Interestingly, lung cancers generally have higher levels of integrin αVβ6 expression, which is associated with reduced disease-free survival. We confirmed that ectopic expression of PTEN downregulated both integrin αVβ6 protein subunits. Notably, shRNA-based PTEN depletion remarkably increased the expression of integrin αVβ6 in H1975 cells, and PTEN overexpression remarkably inhibited the expression of integrin αVβ6 (**Figure 6B**). To explore the impact of

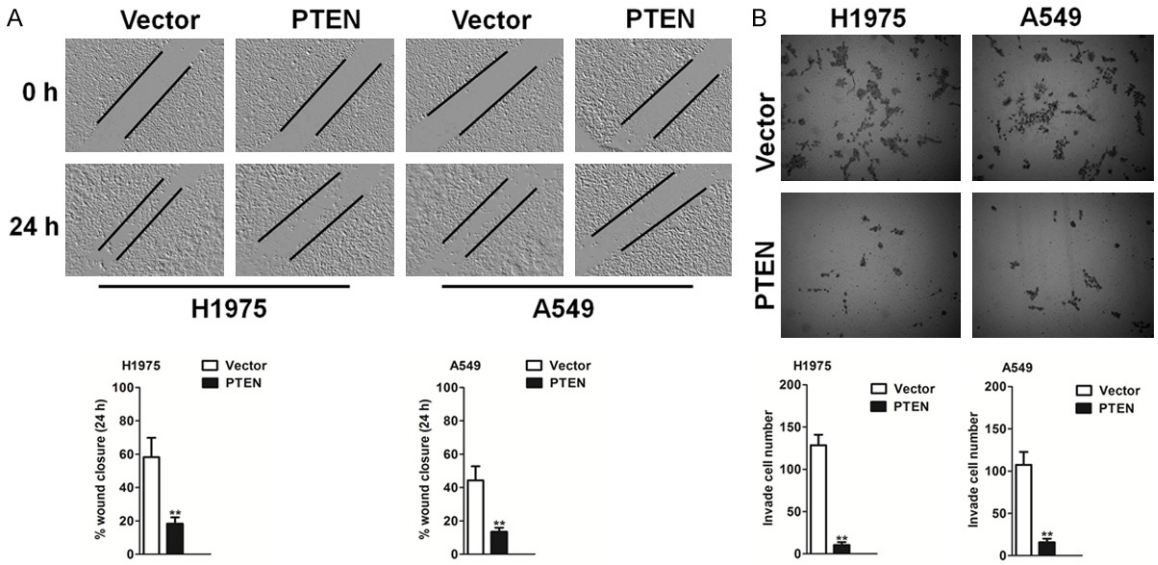

Figure 5. PTEN over-expressing suppresses migration and invasion in H1975 cells. A. Wound healing assay. Confluent cell monolayers were wounded, and wound closure was monitored at 0 hour and 24 hour. Quantification of wound closure was calculated. B. Invasion assay. H1975 control or cells transfected with PTEN plasmid were subjected to a Transwell invasion assay. The invasived cells were stained with 1% crystal violet and counted. Data were collected from five fields in three independent experiments. Quantification of invasive cells per field was analyzed. For indicated comparisons, $**P<0.01$.

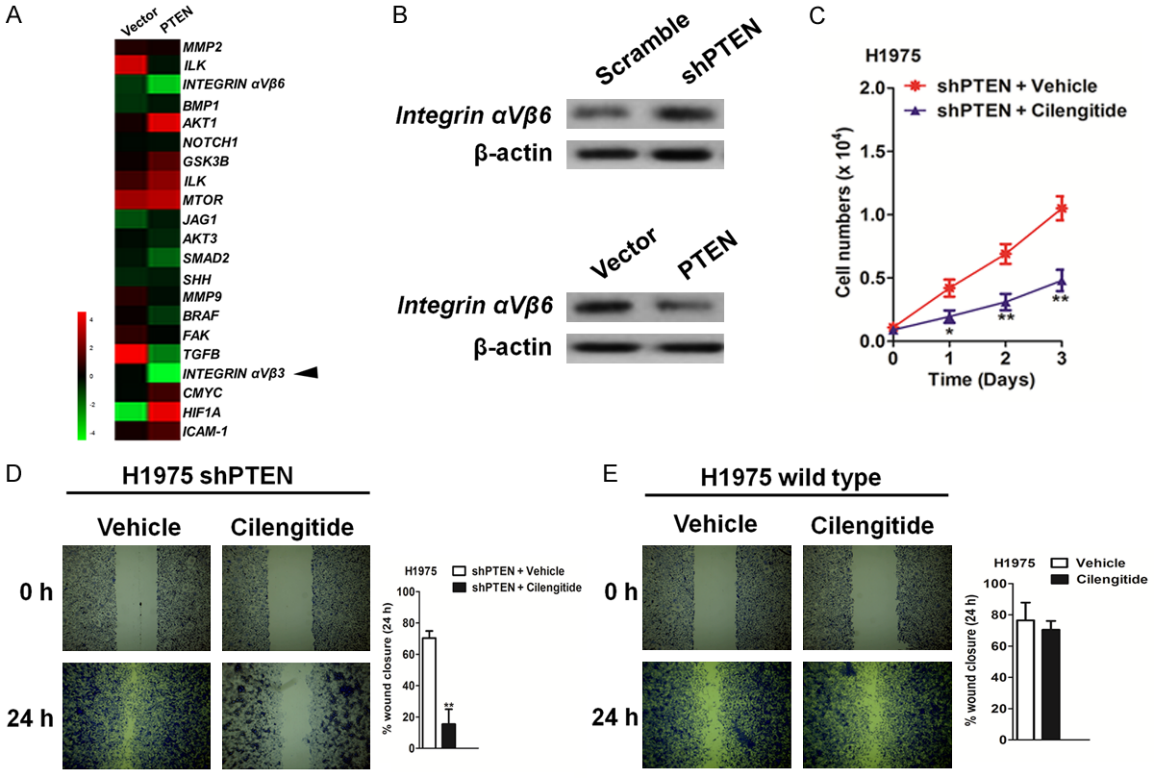

Figure 6. Integrin αVβ6 acts downstream of PTEN to regulate H1975 cells migration and invasion. A. Microarray-based gene expression. B. The protein level of integrin αVβ6 in PTEN-knockdown NSCLC H1975 cells after transfection with the indicated shRNAs or PTEN over-expression H1975 as determined by immunoblotting analysis. C. Cell growth rate from cilengitide (2 μM) and vehicle treated PTEN knock-down H1975 cells. D. Analysis of migration potential from cilengitide and vehicle treated PTENshRNA H1975 cells by a wound healing assay (left) and the quantification of wound closure (right). Bars show means ± SD of three independent experiments. Statistical analyses

were performed by using the Student's t test. E. Analysis of migration potential from cilengitide and vehicle treated PTEN wild-type H1975 cells by a wound healing assay (left) and the quantification of wound closure (right). Bars show means ± SD of three independent experiments. Statistical analyses were performed by the Student's t test. For indicated comparisons, $**P<0.01$.

blocking integrin αVβ6 on PTEN-knockdown cells, we next treated PTEN-knockdown cells with cilengitide, a known inhibitor of integrin αVβ6. As expected, the growth rate (**Figure 6C**) of cilengitide-treated cells was significantly decreased compared with vehicle-treated cells. Wound healing assays also showed that cilengitide-treated PTEN-knockdown H1975 cells displayed significantly less invasion in vitro (**Figure 6D**). In addition, to assess the effect of integrin αVβ6 inhibition in PTEN wild-type cells, we treated H1975 cell lines without PTEN knockdown with cilengitide, and found that treatment in PTEN-normal cells did not significantly reduce wound healing (**Figure 6E**), indicating that suppression of integrin αVβ6 was significantly affected in PTEN-deficient tumors.

Discussion

The issues of tumor recurrence, drug resistance and enhanced metastasis remain a challenge in the treatment and clinical management of NSCLC [17]. Despite a series of investigations aimed at gaining a better understanding of NSCLC genetic alterations, the ability to translate these findings into effective treatments has been limited by the difficulty of identifying functionally relevant drivers of the disease [18]. The processes of cancer metastasis are complex. In the first step of metastasis, cancer cells dissociate from a primary tumor by losing cell-cell contact and transforming into a mesenchymal phenotype, resulting in increased motility and invasive abilities. Hence, identifying molecular drivers of NSCLC will be crucial to a better understanding of its biology and, ultimately, to the development of therapies to prevent metastasis [19]. Here, we identified that PTEN can modulate integrin signaling, which modulates further downstream events of tumor cell migration, invasion, and dissemination in vivo. Ectopic expression of PTEN in NSCLC cells decreased their capacity for migration and invasion. In contrast, in metastatic NSCLC cells that express low levels of endogenous PTEN, transfection of specific shRNA against PTEN increased migration and invasion abilities. We also used NSCLC cell lines to determine that PTEN supports cell proliferation and tumor growth in vivo. On depletion of PTEN using shRNA, NSCLC cells showed accelerated primary tumor growth in vivo, and diminished tumor colony size and induced cell proliferation in vitro. Conversely, cells with PTEN overexpression had decreased growth rates both in vitro and in vivo.

PTEN, a kinase receptor expressed in endothelial cells, is also expressed by human epithelial cancer cells, such as those derived from pancreatic, colorectal, and breast tumors, and melanomas [20]. Activation of PTEN promotes the epithelial-mesenchymal transition and an aggressive phenotype in specific cancer cells. Recent studies support the crucial role that PTEN plays in mediating the epithelial-mesenchymal transition and consequent aggressive disease traits. In addition to its role in angiogenesis, PTEN might mediate a variety of hitherto unappreciated biological functions, such as liver regeneration, inflammatory processes, and cancer metastasis. Recent studies have also observed increased expression of PTEN in grade 4 diffusely infiltrating astrocytoma. However, whether PTEN has a role in the metastatic potential of NSCLC has not yet been determined. Consequently, the role of PTEN in mediating lung cancer tumor development has been investigated in the current study, which has shown that the expression of PTEN significantly correlates with overall survival in NSCLC patients.

Microarray analysis identified an unbiased list of downstream regulatory genes potentially responsible for phenotypic changes in NSCLC cells resulting from PTEN overexpression [21]. Near the top of this list was integrin αVβ6, a signaling molecule that promotes primary cell growth, and stimulates invasion and metastasis. Several studies have demonstrated that the integrin αVβ6 signaling pathway contributes to cancer cell plasticity through the regulation of cellular adhesion to the extracellular matrix, increasing cell motility and invasion, and contributes to poor patient outcome. In this study, we have shown that ectopic expression of PTEN decreases the expression of integrin αVβ6, whereas elimination of PTEN mark-

edly increases integrin αVβ6 protein levels in NSCLC cells. The functional role of integrin αVβ6 in PTEN-mediated metastasis is also revealed by the finding that inhibition of integrin αVβ6 decreases the PTEN-mediated cell migration and invasion [22]. Inhibition of integrin αVβ6 expression in H1975 and A549 cells ectopically expressing PTEN appears to play a major role in mediating tumor growth and progression.

NSCLC is one of the most aggressive form of solid tumor due to its highly invasive nature that impedes the surgical removal of all tumor cells, making relapse inevitable [23]. However, the mechanisms used by NSCLC cells to metastasize into the surrounding tissue are still unclear. Our findings provide strong evidence that PTEN potentially affects NSCLC metastasis by using a loss-of-function model. In summary, our in vitro and in vivo studies have demonstrated that PTEN-knockdown H1975 and A549 cells have profound pathogenic effects on NSCLC progression. This study reveals the critical contribution of overexpression of PTEN to suppression of cancer progression through inhibition of integrin αVβ6 signaling, and identifies PTEN as a potential therapeutic target for controlling tumor aggression.

Acknowledgements

Yan Xia Yu wrote the manuscript. Hong Liu modified the article.

Disclosure of conflict of interest

None.

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