The PTEN tumor suppressor inhibits human airway smooth muscle cell migration

HAIBING LAN[1*], HAOHAI ZHONG[2*], YANG GAO[1], DUNQIANG REN[3], LILI CHEN[2],
DACHENG ZHANG[1], WENYAN LAI[4], JIAN XU[5] and YALING LUO[1]

[1]Department of Respiratory Diseases, Nanfang Hospital, Southern Medical University, Guangzhou 510515;
[2]Department of Respiratory Diseases, Huizhou Municipal Center Hospital, Huizhou 516001; [3]State Key Laboratory of
Respiratory Disease, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of
Guangzhou Medical College, Guangzhou 510120; [4]Laboratory of Cardiovascular Diseases,
[5]Department of Kidney Transplantation, Nanfang Hospital, Southern Medical University,
Guangzhou 510515, Guangdong Province, P.R. China

Abstract. Airway remodeling in asthma is characterized by increased airway smooth muscle (ASM) mass, accompanied by cell migration. It is well known that the proliferation and migration of ASM cells (ASMCs) play a key role in airway remodeling, but the precise mechanism modulating these cellular events remains unclear. One of the genes most likely to be involved in this process is the phosphatase and tensin homolog (PTEN) gene, whose deletion from chromosome 10 can inhibit the proliferation and migration of many cell types. In this study, we investigated the effects of PTEN on human ASMCs. The cells were infected with recombinant adenovirus containing wild-type PTEN cDNA (Ad-PTEN), and the results were compared with those from the uninfected cells and those infected with the GFP-labeled adenovirus vector. Cell proliferation was measured using the MTT method. Cell migration was determined by wound-healing and transwell assays. The expressions of PTEN, phospho-Akt, Akt, phospho-$ERK_{1/2}$, $ERK_{1/2}$, phospho-focal adhesion kinase (FAK) and FAK, were examined by Western blot analysis. The results show that PTEN is expressed endogenously in ASMCs, and that Ad-PTEN inhibits the proliferation and migration of these cells. In addition, the Ad-PTEN treatment decreased the phosphorylation of Akt and FAK but not that of $ERK_{1/2}$. In conclusion, this study demonstrates that PTEN overexpression inhibits the proliferation and migration of human ASMCs by down-regulating the activity of the Akt and FAK signaling pathways.

Introduction

Airway remodeling, an important feature of asthma, is partly characterized by the increase in the number of airway smooth muscle (ASM) cells (ASMCs) in the airway wall (1-3). It has been suggested that the cell migration contributes to the pathogenesis of ASMC hyperplasia and airway remodeling (4). The migration of these cells to sites close to the disrupted reticular basement membrane, is a process similar to the formation of neointima in atherosclerosis and post-angioplasty restenosis.

The phosphatase and tensin homolog (PTEN) protein is encoded by a tumor-suppressor gene located on human chromosome 10 (5). Deletions and mutations of the PTEN gene have been observed in many types of human cancers, including breast, brain and prostate tumors (6,7). The gene product functions as a dual specificity phosphatase and it modulates protein and lipid phosphatase activity (8). PTEN regulates a number of normal cell processes, such as growth, adhesion, migration and apoptosis (9).

Some studies have shown that the enforced expression of PTEN leads to the decreased activity of Akt/PKB, which is a downstream target of PI3K signaling (10-12). Akt/PKB, a serine-threonine protein kinase, is a critical regulator in many different cellular processes, such as proliferation, apoptosis, cell cycle progression, angiogenesis and migration. PTEN has also been proposed to be a negative modulator of the mitogen-activated kinase (MAPK) pathways, where it blocks Shc phosphorylation (with the exception of the Akt pathway). The inhibition of ERK1/2 MAPK could lead to the inhibition of cell proliferation and motility (8,13,14). The focal adhesion (FA) kinase (FAK) is another important substrate of PTEN. This kinase is a non-receptor protein tyrosine kinase, activated mainly in FAs. It is important in cell-extracellular matrix

Correspondence to: Dr YaLing Luo, Department of Respiratory Diseases, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, P.R. China
E-mail: luoyaling513@163.com

*Contributed equally

(ECM) interactions, which affect cell migration, proliferation and survival (15). It has been reported that PTEN directly interacts with FAK, reducing its tyrosine phosphorylation, and thus resulting in the inhibition of cell migration, spreading and FAs (16,17). In particular, the phosphorylation of tyrosine-397 (Tyr397), a major auto-phosphorylation site of FAK, is necessary for FAK-induced cell migration.

Many studies have confirmed that the PTEN tumor suppressor inhibits the migration of a variety of cell types, including human fibroblast and tumor cells (16,17). Given the possibility that PTEN can suppress the migration of vascular smooth muscle cells (VSMCs) (18,19), its decreased level in OVA-induced asthma suggests a potential role for PTEN in airway remodeling (20). We therefore hypothesized that PTEN would regulate the migration and proliferation in cultured human ASMCs. In this study, we demonstrate the expression of endogenous PTEN in these cells, as well as PTEN overexpression after infection with a recombinant adenovirus, which inhibits the activation of p-Akt and p-FAK. In addition, the overexpression of PTEN suppresses the migration and proliferation of human ASMCs.

Materials and methods

Cell culture. Human ASMCs were isolated from the lobar or main bronchus obtained from lung resection donors, using procedures approved by the Division of Thoracic Surgery and the Human Ethics Committee of Nanfang Hospital, Southern Medical University. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 U/ml streptomycin (all from Gibco, USA). Cells from passages 3-8 were used for the experiments.

Immunocytochemical characterization. The cells were subcultured and grown to near confluence. After washing twice with ice-cold phosphate buffered saline (PBS), the cells were suspended in ice-cold 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. They were washed and subsequently incubated with anti-α smooth muscle actin antibody (Bios, Beijing, China). After incubation, the cells were washed twice with PBS and further incubated with goat anti-rabbit IgG (Bios). The positive specimens were visualized using a microscope and then photographed.

Adenovirus construction and infection. Recombinant adeno-viruses encoding human wild-type PTEN cDNA (Ad-PTEN) and a control virus containing no cDNA insert [empty virus, GFP-labeled adenovirus vector (Ad-GFP)], were constructed using the pAdxsi system, and the viruses were amplified as described previously (21). ASMCs were grown in 25 cm² culture flasks in DMEM containing 10% FBS. When the cells were grown to 70% confluence, the medium was changed to DMEM containing no FBS, and viruses were added to the medium at a multiplicity of infection (MOI) of 100 and incubated at 37˚C, 5% CO_2 for 4 h. Then the culture medium with 10% FBS was added. After 18 h, the medium was changed to fresh DMEM containing 10% FBS, followed by different treatments, as indicated. As a control, an identical group of cells was kept uninfected (mock), but was incubated for 18 h in the same manner. The infection rate of the cells was ~96% at a MOI of 100.

MTT proliferation assay. Cells were cultured in triplicate in 96-well plates, first for 24 h in DMEM containing 10% FBS, followed by 24 h of arrested growth in DMEM containing 0.1% FBS. Briefly, the cells were either mock-infected or infected with Ad-PTEN or Ad-GFP for 24 h, then the medium was replaced with fresh DMEM containing 10% FBS, and the cells were incubated for an additional 18 h. After discarding the medium and adding MTT (Sigma-Aldrich) solution (5 mg/ml MTT in PBS, 20 μl/well), the cells were incubated for 4 h at 37˚C, 5% CO_2. The MTT stain was aspirated, and 150 μl of DMSO were added to each well. The plates were then agitated for 5 min. This was followed by the detection procedure in the Universal Microplate Reader, at 490 nm.

Wound-healing assay. ASMCs were seeded onto 6-well plates and allowed to grow to 90% confluence in DMEM containing 10% FBS, followed by serum deprivation for 24 h in DMEM medium. They were then infected as described above for 18 h. The cell monolayer was wounded using a sterile 200 μl pipette tip to create a cell-free zone. The wounded ASMCs were washed three times with PBS to remove cell debris and then treated with DMEM containing no FBS. The cells were observed 24 h later using an Olympus IX-70 inverted microscope with a Spot RT Color Camera (Diagnostic Instruments). Wound-healing assay was performed at least three times.

Migration assay. Migration assays were performed using the transwell chamber apparatus (Corning, NY, USA). Cell culture membrane inserts (8 μm pore size), in 24-well format (Corning), were coated with 50 μg/ml rat tail collagen I overnight at 4˚C and then air-dried for 1 h. The confluent cells were serum-deprived using DMEM containing 0.5% bovine serum albumin (BSA) for 24 h before the migration assays. The cells were either mock-infected or infected with Ad-PTEN or Ad-GFP for 44 h, and were then collected using trypsin (0.025%; Invitrogen Canada Inc.). They were counted, centrifuged and re-suspended at a density of 1x10⁶ cells/ml. Finally, 100 μl of cell suspension were added to the upper compartment of the transwell chamber, and DMEM with 0.1% BSA was added to the lower chamber. Cells in the transwell chamber were incubated at 37˚C, 5% CO_2 for 4 h. Non-migrated cells were scraped off and the membranes were fixed with methanol/acid solution. The cells that migrated to the underside of the membrane were stained with 0.1% crystal violet solution (Sigma-Aldrich). The membranes were mounted on slides and the cells were manually counted (the number of nuclei in a standard central field). The number of cells that migrated to the lower surface of the membrane was counted at x200 magnification. Five random high-power fields were counted for each sample. The data presented in this study are from at least three independent experiments.

Immunohistochemistry for the cell actin cytoskeleton. For fluorescent labeling of the actin cytoskeleton, cells were grown in 35-mm dishes on glass coverslips coated with
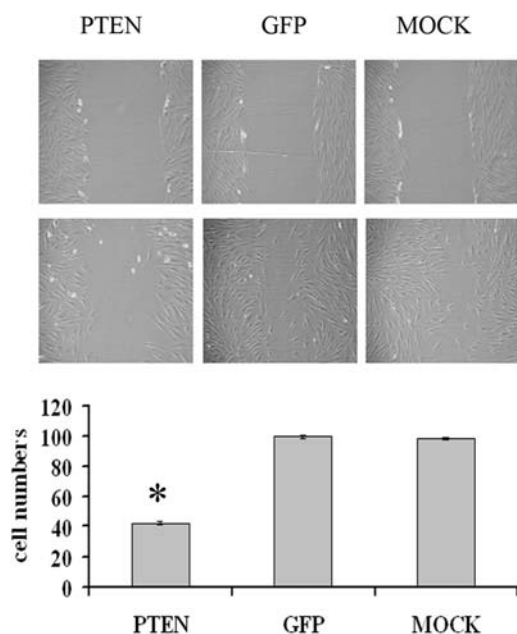
Figure 1. Effect of PTEN on migration of ASMCs in *in vitro* wound-healing assay. Photographs were taken 48 h after wounding (x100 magnification). The number of cells migrating to the wound area was quantified. *P<0.05 vs. Ad-GFP or mock. Data represent the means ± SD of three independent experiments.
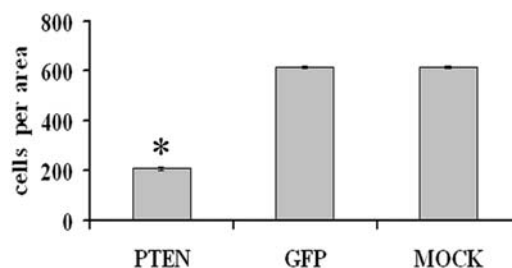

Figure 2. Effects of PTEN overexpression on migration of ASMCs using the transwell chamber apparatus. The number of cells migrating to the membrane was quantified in five random high-power fields. *P<0.05 vs. Ad-GFP or mock. Data represent the means ± SD of three independent experiments.

0.10% gelatin. When grown to 70% confluence, they were infected for 48 h as previously described. Following this treatment, the cells were washed once with 1 ml cold PBS for 3 min, before fixing for 20 min in 1 ml formalin (4% formaldehyde in PBS). They were then gently washed three times with 1 ml PBS for 5 min, permeabilized for 10 min with 0.1% Triton buffer (1 μl Triton X-100 in 1000 μl PBS) and then washed again three times in PBS. Rhodamine-labeled phalloidin (Molecular Probes) (1:50 in 1% BSA in PBS) was added onto the center of the cover slip and the slips were incubated for 1 h at room temperature. Finally, the cells were washed three times with 1 ml PBS at room temperature for 3 min. The cover slips were mounted in 50% glycerol in PBS. The images were obtained using a Leica laser scanning confocal microscope (Leica, Germany) at x20 magnification.

Western blot analysis. Forty-eight hours after infection, total protein was extracted by the addition of 200 μl lysis buffer. The samples were separated by SDS 10% polyacrylamide gel electrophoresis (PAGE) and electroblotted onto PVDF membranes. The membranes were incubated overnight at 4˚C with the following primary antibodies: PTEN, phospho-Akt (Ser473), total Akt, phospho-ERK, total ERK, phospho-FAK (Try397) and total FAK (all from Cell Signaling Technology, MA, USA), diluted in primary antibody dilution buffer. The levels of proteins and phosphoproteins were estimated using horseradish peroxidase linked secondary antibodies, and the ECL system (Thermo, USA). GAPDH (Santa Cruz, CA, USA) was used as the internal control in all the Western blotting procedures.

Statistical analysis. Data were expressed as the means ± SD of at least three separate experiments. A one-way ANOVA and Student-Newman-Keuls tests were used for statistical analysis, and a value of p<0.05 was considered statistically significant. All analyses were performed using SPSS Version 13.0 (Statistical Software for Social Sciences, Chicago, IL).

Results

Characterization of ASMCs. The confluent ASMC growth exhibited the typical 'hill and valley' appearance under an inverted light microscope, and single ASMCs had a fusiform shape. The immunocytochemical staining of human ASMCs with anti-α-actin antibody revealed the positive expression of α-actin.

PTEN overexpression inhibits ASMC migration and actin cytoskeletal rearrangement. *In vitro* wound-healing assay indicated that, compared to the similar wound area at 0 h, the cell-free areas in the Ad-PTEN group 48 h after wounding, were significantly larger than those in the Ad-GFP and uninfected groups. The difference was not caused by the adenovirus alone, as it had no inhibitory effect on cell migration. No significant differences were observed between the mock and Ad-GFP groups (Fig. 1). These results indicate that the migration of ASMCs was down-regulated by the overexpression of PTEN.

In order to further confirm the effect of PTEN on ASMC migration, we also performed *in vitro* transwell chamber migration analysis. In the migration assay, growth-arrested ASMCs were plated in transwell plates. As shown in Fig. 2, the number of cells migrating per area in the mock and Ad-GFP groups was higher than in the Ad-PTEN group, and no significant difference was found between the two control groups. These results also demonstrate that PTEN overexpression inhibits ASM cell migration, while endogenous PTEN does not have this effect.

We then examined the F-actin cytoskeletal rearrangement in ASMCs. The cells treated with DMEM alone contained only a few relatively short and flimsy actin stress fibers, and no protrusions were found at the leading edge of the cells at 48 h. The infection with Ad-GFP did not alter the actin fibers. However, PTEN overexpression inhibited most of the actin rearrangement (Fig. 3). These data suggest that PTEN overexpression inhibits the migration of ASMCs, partially by inhibiting the rearrangement of actin filaments.
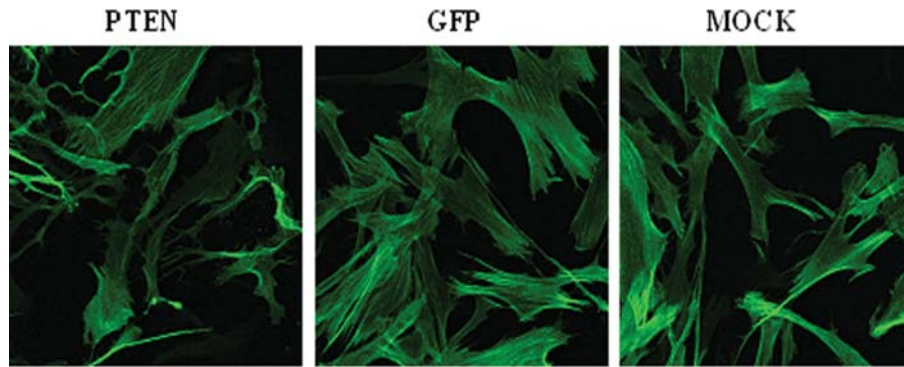
Figure 3. Effects of PTEN on ASMC actin cytoskeleton. Visualization of stress (actin) fibers: The expression of actin fibers (rhodamine phalloidin-positive green fibers) was observed (x400 magnification).
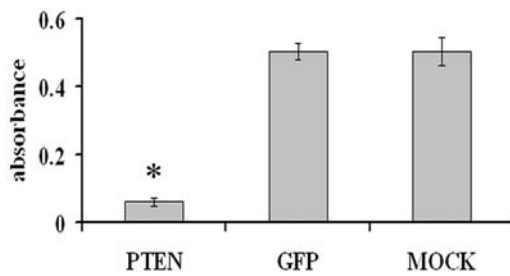

Figure 4. Effects of PTEN on the proliferation of ASMCs. The proliferation was assessed by absorbance. *P<0.05 vs. Ad-GFP or mock. Data represent the means ± SD of three independent experiments.
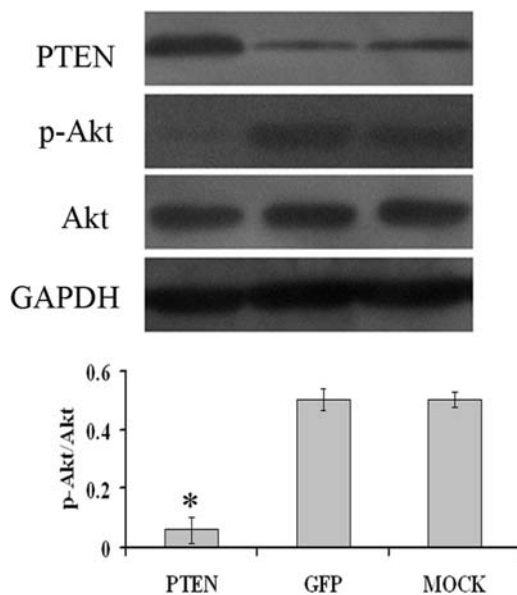

Figure 5. The expression of PTEN, phospho-Akt, total Akt and GAPDH in ASMCs. The cells were lysed, and the proteins were separated by SDS-PAGE and probed with antibodies against PTEN, phospho-Akt, total Akt and GAPDH. *P<0.05 vs. Ad-GFP or mock.

PTEN overexpression inhibits ASMC proliferation. We also investigated the effect of PTEN overexpression on ASMC proliferation. Growth rates were evaluated by MTT assay (Fig. 4). In the Ad-PTEN group, the cell proliferation rate
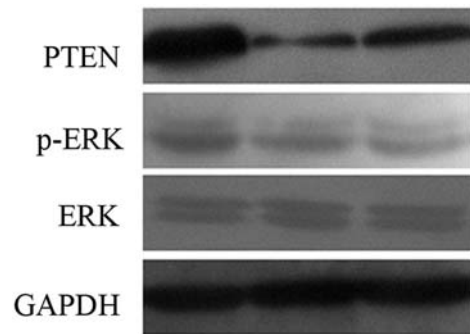

Figure 6. The expression of PTEN, phospho-ERK, total ERK and GAPDH in ASMCs. The cells were lysed, and the proteins were separated by SDS-PAGE and probed with antibodies against PTEN, phospho-ERK, total ERK and GAPDH. #P>0.05 vs. Ad-GFP or mock.

decreased visibly, but no significant difference was observed between the Ad-GFP and mock-infected group. These findings demonstrate that PTEN overexpression blocks ASMC proliferation.

PTEN overexpression inhibits activation of Akt, but not $ERK_{1/2}$. We evaluated the phosphorylation status of Akt and $ERK_{1/2}$ (Figs. 5 and 6). We found that phospho-Akt was significantly attenuated in the cells infected with Ad-PTEN, while phospho-$ERK_{1/2}$ showed no significant change. This suggests that PTEN overexpression affects ASMC migration and proliferation by inhibiting phospho-Akt activation. However, it had no effect on the phosphorylation level of $ERK_{1/2}$.

PTEN overexpression blocks activation of FAK. In order to determine whether FAK is involved in the ASMC migration,
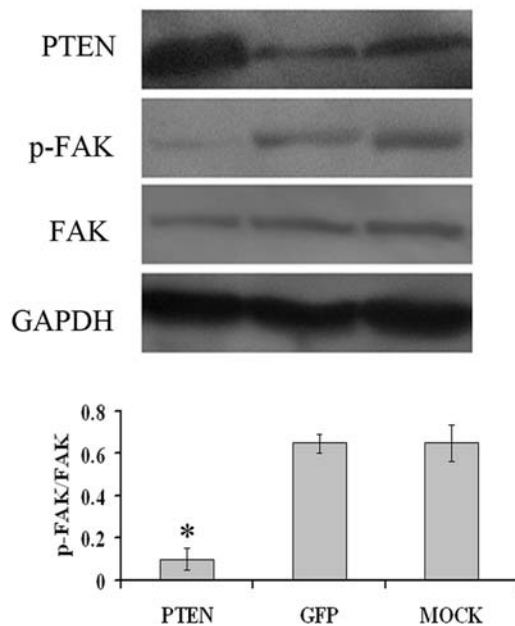
Figure 7. The expression of PTEN, phospho-FAK, total FAK and GAPDH in ASMCs. The cells were lysed, and the proteins were separated by SDS-PAGE and probed with antibodies against PTEN, phospho-FAK, total FAK and GAPDH. *P<0.05 vs. Ad-GFP or mock.

we performed immunoblot experiments using a phosphorylation-specific antibody against FAK. PTEN overexpression drastically down-regulated the FAK phosphorylation at Py397. The level of phosphorylation of FAK in the Ad-PTEN group was reduced in comparison to the control groups. The mock and Ad-GFP treatments did not affect the FAK phosphorylation significantly. These data show that the phosphorylation level of FAK is well correlated with cell migration. Therefore, we suggest that PTEN overexpression can block ASMC migration via the FAK cascade (Fig. 7).

Discussion

The PTEN tumor suppressor plays an important role in regulating cell proliferation, apoptosis and migration. However, little is known about the role of PTEN in the migration of human ASMCs.

In this study, we confirm the hypothesis that the overexpression of PTEN, an inositol phosphatase specific for PI (3,4), P_2 and PIP3 (22), inhibits cultured human ASMC migration and proliferation. PTEN was expressed endogenously in these cells without affecting their migration, but PTEN overexpression inhibited the Akt and FAK pathways in ASMCs, without blocking the phosphorylation of $ERK_{1/2}$. Taken together, these findings suggest that PTEN overexpression could prevent airway remodeling in asthma.

The results of both the wound-healing and *in vitro* transwell assays confirm that PTEN overexpression inhibits the migration of ASMCs. The changes in the rearrangement of the F-actin cytoskeleton of these cells also show a similar PTEN effect.

There are possibly several mechanisms involved in the inhibition of ASMC migration by PTEN. Firstly, PI3K/Akt

signaling has been linked to cellular migration in a variety of cell types (23). In our study, we observed a significant reduction in Akt phosphorylation after Ad-PTEN infection, which is consistent with the results from certain other studies. This suggests that Akt is involved in the PTEN-induced inhibition of cell migration and proliferation as an effector downstream of PTEN (24-26). Secondly, the reduction in FAK phosphorylation indicates that it could also be involved in this process. PTEN has been shown to suppress cell migration and proliferation by dephosphorylating FAK at the Tyr397 autophosphorylation site (13). Intimal hyperplasia has been reported to be correlated with the overexpression of FAK in smooth muscle cells (27), whereas the overexpression of FRNK, an endogenous inhibitor of FAK, inhibits the migration and proliferation of smooth muscle cells (28). All these studies postulate that FAK plays an important role in cell migration, and that it could also be a potential therapeutic target for airway remodeling treatment. We confirm that PTEN overexpression inhibits the migration of ASMCs by lowering the phosphorylation levels in the Akt and FAK pathways. However, the $ERK_{1/2}$ phosphorylation was not affected. $ERK_{1/2}$ has been reported to be both a participant and non-participant in chemotaxis (13,29), and its function varies in different cell types. The activation of the $ERK_{1/2}$ signaling pathway is important in the migration of mouse fibroblasts and human aortic smooth muscle cells (30,31). However, in this study, the overexpression of PTEN did not inhibit the activation of $ERK_{1/2}$. Our data are consistent with certain previous studies on endothelial cell growth, in which PTEN overexpression did not alter the $ERK_{1/2}$ activation (32).

ASM mitogenesis depends chiefly on the stimulation of the $ERK_{1/2}$ and PI3K/Akt pathways (33). Bronchoalveolar lavage fluid from the asthmatic airways increases $ERK_{1/2}$ activation and the number of cultured human ASMCs (34). This is consistent with the opinion that the $ERK_{1/2}$ signaling pathway is an important regulator of cell cycle entry and progression in ASM (35). Many studies have investigated the interactions between PI3K and $ERK_{1/2}$. Both have been shown to be activated as part of a parallel or independent pathway (36,37). In our study, PTEN overexpression, which blocks the PI3K/Akt pathway, inhibits the activation of Akt but not $ERK_{1/2}$. Other studies have noted that the constitutive activation of PI3K in bovine tracheal myocytes is sufficient for transcription from the cyclin D1 promoter but does not induce $ERK_{1/2}$ activation (38). Similarly, we could assume that PI3K signaling occurs independently of $ERK_{1/2}$ in ASMCs.

The proliferation of ASMCs is one of the reasons for the pathological changes in the airway of asthmatic patients. Our data clearly show that exogenous PTEN inhibits the proliferation of human ASMCs. Another study has also demonstrated that PTEN overexpression inhibits cell proliferation, migration and survival in rabbit VSMCs (18). These data suggest that PTEN plays an important role in the regulation of cell proliferation in certain smooth muscle cells. However, the mechanism of action of PTEN in the proliferation of ASMCs remains poorly understood. It is likely that the inhibition of VSMC proliferation by PTEN was mediated

in part by an increase in apoptosis (18). However, other findings have indicated that exogenous PTEN suppresses cell growth and cell cycle progression without inducing cell death (39,40). These findings could explain the cell type and species differences in PTEN-regulated apoptosis. The discrepancies could be attributable to the differences in PTEN signaling in VSMC and other cell types. Further studies are required in order to clarify the role of PTEN in ASMC proliferation during airway remodeling.

In summary, our data demonstrate that PTEN over-expression inhibits the ASMC proliferation and migration. These results contribute to a better understanding of the mechanisms by which PTEN suppresses bronchial inflammation and airway remodeling.

Acknowledgements

References

1. Dunnill MS, Massarella GR and Anderson JA: A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis, and emphysema. Thorax 24: 176-179, 1969.
2. Carroll N, Elliot J, Morton A and James A: The structure of large and small airways in nonfatal and fatal asthma. Am Rev Respir Dis 147: 405-410, 1993.
3. Ebina M, Takahashi T, Chiba T and Motomiya M: Cellular hypertrophy and hyperplasia of airway smooth muscle underlying bronchial asthma. Am Rev Respir Dis 148: 720-726, 1993.
4. Bentley JK and Hershenson MB: Airway smooth muscle growth in asthma: proliferation, hypertrophy, and migration. Proc Am Thorac Soc 5: 89-96, 2008.
5. Li DM and Sun H: TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res 57: 2124-2129, 1997.
6. Steck PA, Pershouse MA, Jasser SA, et al: Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15: 356-362, 1997.
7. Li J, Yen C, Liaw D, et al: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275: 1943-1947, 1997.
8. Chu EC and Tarnawski AS: PTEN regulatory functions in tumor suppression and cell biology. Med Sci Monit 10: RA235-RA241, 2004.
9. Yamada KM and Araki M: Tumor suppressor PTEN: modulator of cell signaling, growth, migration and apoptosis. J Cell Sci 114: 2375-2382, 2001.
10. Davies MA, Lu Y, Sano T, et al: Adenoviral transgene expression of MMAC/PTEN in human glioma cells inhibits Akt activation and induces anoikis. Cancer Res 58: 5285-5290, 1998.
11. Davies MA, Koul D, Dhesi H, et al: Regulation of Akt/PKB activity, cellular growth, and apoptosis in prostate carcinoma cells by MMAC/PTEN. Cancer Res 59: 2551-2556, 1999.
12. Stambolic V, Suzuki A, de la Pompa JL, et al: Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 95: 29-39, 1998.
13. Gu J, Tamura M, PanKov R, Danen EH, Takino T, Matsumoto K and Yamada KM: Shc and FAK differentially regulate cell motility and directionality modulated by PTEN. J Cell Biol 146: 389-403, 1999.
14. Gu J, Tamura M and Yamada KM: Tumor suppressor PTEN inhibits interin- and growth factor-mediated mitogen-actived protein (MAP) kinase signaling pathways. J Cell Biol 143: 1375-1383, 1998.
15. Yang YC, Ho TC, Chen SL, Lai HY, Wu JY and Tsao YP: Inhibition of cell motility by troglitazone in human ovarian carcinoma cell line. BMC Cancer 7: 216, 2007.
16. Tamura M, Gu J, Danen EH, Takino T, Miyamoto S and Yamada KM: PTEN interactions with focal adhesion kinase and suppression of the extracellular matrix-dependent phosphatidylinositol 3-kinase/Akt cell survival pathway. J Biol Chem 274: 20693-20703, 1999.
17. Tamura M, Gu J, Matsumoto K, Aota S, Parsons R and Yamada KM: Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science 280: 1614-1617, 1998.
18. Huang J and Kontos CD: Inhibition of vascular smooth muscle cell proliferation, migration, and survival by the tumor suppressor protein PTEN. Arterioscler Thromb Vasc Biol 22: 745-751, 2002.
19. Hata JA, Petrofski JA, Schroder JN, et al: Modulation of phosphatidylinositol 3-kinase signaling reduces intimal hyperplasia in aortocoronary saphenous vein grafts. J Thorac Cardiovasc Surg 129: 1405-1413, 2005.
20. Kwak YG, Song CH, Yi HK, Hwang PH, Kim JS, Lee KS and Lee YC: Involvement of PTEN in airway hyperresponsiveness and inflammation in bronchial asthma. J Clin Invest 111: 1083-1092, 2003.
21. Zhong HH, Luo YL, Lai WY, Xu J, Chen LL and Ye H: Construction of a recombinant adenovirus vector containing PTEN and its expression in airway smooth muscle cells. Nan Fang Yi Ke Da Xue Xue Bao 29: 859-863, 2009.
22. Maehama T and Dixon JE: The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4, 5-trisphosphate. J Biol Chem 273: 13375-13378, 1998.
23. Day RM, Lee YH, Park AM and Suzuki YJ: Retinoic acid inhibits airway smooth muscle cell migration. Am J Respir Cell Mol Biol 34: 695-703, 2006.
24. Stewart AL, Mhashilkar AM, Yang XH, et al: PI3 kinase blockade by Ad-PTEN inhibits invasion and induces apoptosis in RGP and metastatic melanoma cells. Mol Med 8: 451-461, 2002.
25. Hlobilková A, Knillová J, Bártek J, Lukás J and Kolár Z: The mechanism of action of the tumour suppressor gene PTEN: Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 147: 19-25, 2003.
26. Dasari VR, Kaur K, Velpula KK, et al: Upregulation of PTEN in glioma cells by cord blood mesenchymal stem cells inhibits migration via downregulation of the PI3K/Aktpathway. PLoS One 5: e10350, 2010.
27. Owens LV, Xu L, Marston WA, et al: Over-expression of the focal adhesion kinase (p125FAK) in the vascular smooth muscle cells of intimal hyperplasia. J Vasc Surg 34: 344-349, 2001.
28. Taylor JM, Mack CP, Nolan K, Regan CP, Owens GK and Parsons JT: Selective expression of an endogenous inhibitor of FAK regulates proliferation and migration of vascular smooth muscle cells. Mol Cell Biol 21: 1565-1572, 2001.
29. Rönnstrand L and Heldin CH: Mechanisms of platelet-derived growth factor-induced chemotaxis. Int J Cancer 91: 757-762, 2001.
30. Glading A, Uberall F, Keyse SM, Lauffenburger DA and Wells A: Membrane proximal ERK signaling is required for M-calpain activation downstream of epidermal growth factor receptor signaling. J Biol Chem 276: 23341-23348, 2001.
31. Mu H, Wang X, Lin P, Yao Q and Chen C: Nitrotyrosine promotes human aortic smooth muscle cell migration through oxidative stress and ERK1/2 activation. Biochim Biophys Acta 1783: 1576-1584, 2008.
32. Huang J and Kontos CD: PTEN modulates vascular endothelial growth factor-mediated signaling and angiogenic effects. J Biol Chem 277: 10760-10766, 2002.
33. Zhou L and Hershenson MB: Mitogenic signaling pathways in airway smooth muscle. Respir Physiol Neurobiol 137: 295-308, 2003.
34. Naureckas ET, Ndukwu IM, Halayko AJ, Maxwell C, Hershenson MB and Solway J: Bronchoalveolar lavage fluid from asthmatic subjects is mitogenic for human airway smooth muscle. Am J Respir Crit Care Med 160: 2062-2066, 1999.

35. Lew DB, Dempsey BK, Zhao Y, Muthalif M, Fatima S and Malik KU: Beta-hexosaminidase-induced activation of p44/42 mitogen-activated protein kinase is dependent on p21Ras and protein kinase C and mediates bovine airway smooth-muscle proliferation. Am J Respir Cell Mol Biol 21: 111-118, 1999.
36. Xue L, Murray JH and Tolkovsky AM: The Ras/phosphatidylinositol 3-kinase, and Ras/ERK pathways function as independent survival modules each of which inhibits a distinct apoptotic signaling pathway in sympathetic neurons. J Biol Chem 275: 8817-8824, 2000.
37. Nguyen KT, Wang WJ, Chan JL and Wang LH: Differential requirements of the MAP kinase and PI3 kinase signaling pathways in Src- versus insulin and IGF-1 receptors-induced growth and transformation of rat intestinal epithelial cells. Oncogene 19: 5385-5397, 2000.
38. Page K, Li J, Wang Y, Kartha S, Pestell RG and Hershenson MB: Regulation of cyclin D1 expression and DNA synthesis by phosphatidylinositol 3-kinase in airway smooth muscle cells. Am J Respir Cell Mol Biol 23: 436-443, 2000.
39. Li J, Simpson L, Takahashi M, Miliaresis C, Myers MP, Tonks N and Parsons R: The PTEN/MMAC1 tumor suppressor induces cell death that is rescued by the AKT/protein kinase B oncogene. Cancer Res 58: 5667-5672, 1998.
40. Furnari FB, Huang HJ and Cavenee WK: The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells. Cancer Res 58: 5002-5008, 1998.