miRNA-125b regulates apoptosis of human non-small cell lung cancer via the PI3K/Akt/GSK3β signaling pathway

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Abstract. The present investigation demonstrated that regulation of microRNA (miR)-125b affected the apoptosis of human non-small cell lung cancer (NSCLC) through targeting of the PI3K/Akt and Wnt/β-catenin signaling pathways. The expression of miR-125b was assessed in patients with NSCLC, which demonstrated that miR-125b expression in NSCLC tissue was higher than that in para-carcinoma tissue. Furthermore, survival analysis of patients with NSCLC over 3 years indicated that the overall survival (OS) and disease-free survival (DFS) rates of patients with low miR-125b expression were higher than those of patients with high miR-125b expression. Proliferation and apoptosis assays were subsequently conducted in the human NSCLC cell line A549 using MTT assay and Annexin V-FITC/PI kits, respectively. Caspase-3 activity ELISA and western blot analysis were also used to assess caspase-3 activity and the protein expression of Bax, Akt, phosphorylated (p)-Akt, p-GSK3β, Wnt and β-catenin. It was observed that downregulation of miR-125b inhibited the proliferation and induced the apoptosis of A549 cells. Downregulation of miR-125b also suppressed the protein expression of p-Akt, Wnt and β-catenin, and increased caspase-3 activity and Bax protein expression in A549 cells. In addition, downregulation of miR-125b combined with the PI3K inhibitor LY294002 enhanced cell growth inhibition, suppression of p-GSK3β, Wnt and β-catenin protein expression and promotion of caspase-3 activity in A549 cells. These results revealed that the downregulation of miR-125b regulates apoptosis in human NSCLC through the suppression of the PI3K/Akt/GSK3β and Wnt/β-catenin signaling pathways.

Introduction

Primary bronchogenic carcinoma (also known as lung cancer) refers to malignant tumors that originate in the bronchus, mucous gland, bronchiolar epithelium or pulmonary alveolar epithelium (1). Lung cancer is among the most common malignant tumors in China. Its rate of morbidity has markedly increased over recent years, and is now ranked highest in a number of cities. Furthermore, its yearly incidence now exceeds 500,000 cases (2), with a male:female ratio of ~7-7:1. However, as the biological behaviors of tumors differ, lung cancer is generally divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) for convenient clinical treatment (3). The latter includes all epithelium-derived lung cancers excluding SCLCs. At present, the first-choice therapeutic method for early-stage lung cancer is surgical treatment (4), while the main treatment methods for locally advanced lung cancer include radiotherapy, chemotherapy, surgery and comprehensive treatment involving a combination of these methods (3).

In glycogen synthase kinase (GSK)-3β, phosphorylation of the ninth serine inactivates the kinase, which can prevent the Wnt signal transduction pathway and cause degradation of the protein, inhibiting apoptosis (5). Meanwhile, phospho(p)-GSK-3 prevents GSK-3β from interacting with β-catenin and cyclin D1, enabling the overexpression of β-catenin and cyclin D1, which favors the binding of cyclin D1 to CDK4/6 to form a complex that promotes the G1/S phase transition in cells (6). The cells can then enter into the S phase of the cell cycle, and may ultimately overproliferate and malignantly transform (6).

Research into the PI3K/Akt signaling pathways now spans >10 years. After PI3K phosphorylation, the second messenger phosphatidylinositol (PIP3) is generated on the plasmalemma (7). PIP3 can then bind to the N-terminal of Akt, which contains a PH structural domain for such binding.

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This activates Akt and transfers it to the cytosolic side of the plasmalemma (8), where Akt itself activates or suppresses downstream target proteins through phosphorylation, which ultimately stimulates the proliferation, differentiation, apoptosis and migration of target cells (9). As major kinases of inositol and phosphatidylinositol, PI3K family members are considered to be primary cancer genes, and are composed of the regulatory subunit p85 and catalytic subunit p110. The catalytic subunit is responsible for catalyzing phosphorylation of the third hydroxyl of the inositol ring (10). Akt is a serine/threonine protein kinase with a molecular weight of 57 ku, and is the mammalian congener of the viral protooncogene Akt (11). Following activation of the PI3K/Akt signaling pathway, activated Akt exerts its effects through the activation of multiple downstream effector molecules, including Bad, caspase-9, FKHR1 and NF-κB, enabling it to participate in the suppression of apoptosis and regulation of the cell cycle (12). At present, a relatively well-established effect of Akt is its upregulation of gene expression through increased transcription of c-Myc. c-Myc is a type of cell cycle-associated factor that enables cells to exit the G0 phase and undergo proliferation (11), and may also promote tumor angiogenesis. Similarly, Akt can activate nitric oxide synthase, stimulate the growth and proliferation of endothelial cells and increase vascular permeability (13). Furthermore, after hemangiectasis, Akt can promote neovascularization, aiding with the provision of sufficient nutrition for tumor cells, and can also promote cell invasion and metastasis.

Previous evidence revealed that microRNAs (miRs) serve important roles in the occurrence and development of human tumors (14). Recently, it has been demonstrated that miR-125b and its target gene play a critical role in the invasion and metastasis of multiple tumor types (15). For example, miR-125b can directly regulate c-Jun protein expression and directly suppress the metastasis of melanoma cells on a transcriptional and translational level (16). It has also been reported that miR-125b can act as a tumor-inhibitory factor that targets the PI3K/Akt signaling pathway, and prevents the invasion and metastasis of cervical cancer cells (16). Therefore, the aim of the present study was to evaluate the effects of miR-125b regulation on the apoptosis of human NSCLC cells and the targeting of the PI3K/Akt and Wnt/β-catenin signaling pathways *in vitro*.

Materials and methods

Patients and specimen selection. Approval for the present study was obtained from the local Ethics Committee along with written informed consent from each patient. From September to December of 2012, human NSCLC specimens from patients (n=91) were obtained from the Department of Oncology of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences. The main characteristics of all human NSCLC specimens from patients are shown in Table I. Every 2 months, a follow-up of NSCLC patients was conducted, over a 3-year period. We analyzed overall survival (OS) and disease-free survival (DFS) in the present study.

Quantative real-time PCR analysis. Total RNA was extracted from NSCLC and para-carcinoma tissues using TRIzol® (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA,

Table I. Association between miR-125b expression and clinicopathological features.

Variables	No. of pts.	miR-125b expression		P-value
		Low (%)	High (%)	
Sex				0.437
Male	79	42 (53.16)	37 (46.84)	
Female	12	4 (33.33)	8 (66.67)	
Age (years)				0.014
≤60	57	21 (36.84)	36 (63.16)	
>60	34	18 (52.94)	16 (47.06)	
Pathological differentiation				0.627
High	7	5 (71.43)	2 (28.57)	
Median	61	23 (37.70)	38 (62.30)	
Low	23	13 (56.52)	10 (43.48)	
Clinical stage				0.139
I	7	3 (42.86)	4 (57.14)	
II	17	6 (35.29)	11 (64.71)	
IIIA	67	38 (56.71)	29 (43.28)	
Vascular invasion				0.074
Negative	84	41 (48.81)	43 (51.19)	
Positive	7	2 (28.57)	5 (71.43)	
Pathological type				0.000
Squamous cancer	39	25 (64.10)	14 (35.90)	
Adenocarcinoma	47	15 (31.91)	32 (68.09)	
Large cell cancer	5	2 (40.00)	3 (60.00)	

pts., patients.

USA). cDNA was synthesized from total RNA (100-200 ng) using M-MLV reverse transcriptase (Promega Corporation, Madison, WI, USA) with random primers (Takara Bio, Inc., Otsu, Japan), according to the manufacturer's protocol. Quantitative real-time PCR analysis was performed using the CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and SYBR®-Green PCR Master Mix kit (Applied Biosystems, Thermo Fisher Scientific, Inc.). Quantative real-time PCR analysis was performed with 1 cycle at 95˚C for 10 min, followed by 40 cycles of 95˚C for 30 sec and 58˚C for 60 sec.

Cell culture. The human NSCLC A549 cell line was purchased from the Shanghai Cell Bank (Shanghai, China), and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (both from Gibco; Thermo Fisher Scientific Inc.) with 100 U/ml streptomycin and 100 U/ml penicillin (both from Thermo Fisher Scientific, Inc.) at 37˚C in a humidified atmosphere containing 5% CO_2.

Transfection and lentiviral transduction. Anti-miR-125b mimics and negative plasmids were transfected into A549 cells using Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific, Inc.), following the manufacturer's protocol.
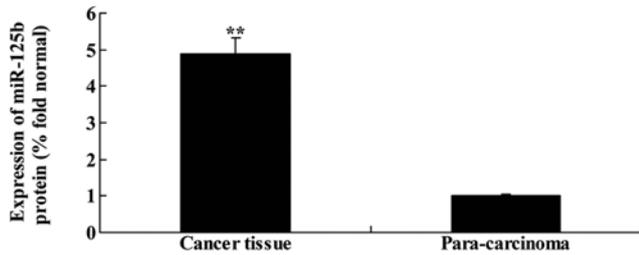
Figure 1. miR-125b expression in human non-small cell lung cancer. Cancer tissue, non-small cell lung cancer tissue; para-carcinoma, para-carcinoma tissue; **p<0.01 compared with para-carcinoma tissue.

After transfection for 24 h, the cells were maintained in new culture medium and used to execute experimental research.

Cell proliferation assay. A549 cells transfected with anti-miR-125b mimics and negative plasmids or treated with LY294002 (1 μM) for 48 h were assayed 96 h later using an MTT assay kit (50 μg/ml; Sigma-Aldrich, St. Louis, MO, USA) for 4 h. Subsequently, the culture medium was removed and 150 μl of dimethyl sulfoxide (DMSO) (Sigma-Aldrich) was added into cells to dissolve. The absorbance was assessed using a Genesys™ 15 ultraviolet spectrophotometer (Thermo Fisher Scientific, Inc.) at 492 nm.

Cell apoptosis assay. A549 cell transfected with anti-miR-125b mimics and negative plasmids or treated with LY294002 (1 μM) for 48 h was assayed 6 h later using Annexin V-FITC/PI apoptosis assay. Annexin V-FITC (BD Biosciences, San Jose, CA, USA) was added and incubated for 20 min at room temperature in the dark. Then, PI (BD Biosciences) was also added and incubated for 5 min at room temperature in the dark. Then, cell apoptosis was examined using a FACScan flow cytometer.

Caspase-3 activity assay. A549 cells transfected with anti-miR-125b mimics and negative plasmids or treated with LY294002 (1 μM) for 48 h were assayed 96 h later using a caspase-3 activity ELISA kit for 1 h at 37˚C. Caspase-3 activity was assessed using a Genesys™ 15 ultraviolet spectrophotometer (Thermo Fisher Scientific, Inc.) at 405 nm.

Western blot analysis. A549 cells transfected with anti-miR-125b mimics and negative plasmids or treated with LY294002 (1 μM) 48 h later. A549 cells were lysed with a hypotonic buffer containing a protease inhibitor cocktail (both from Beyotime Institute of Biotechnology, Shanghai, China). Protein concentration was determined by the BCA method (Beyotime Institute of Biotechnology). Equal protein (50 μg) was separated by 10% SDS-polyacrylamide gel and blotted onto a polyvinylidene difluoride (PVDF) membranes (BD Biosciences). The membranes were incubated with Akt (1:500), phosphorylated (p)-Akt (1:500), p-GSK3β (1:500), Wnt (1:500), β-catenin (1:300), Bax (1:300) and GAPDH (1:500) (all from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 4˚C overnight. Following washing with PBS with Tween-20 (Sigma-Aldrich) twice, membranes were incubated with horseradish peroxidase-conjugated secondary antibody

IgG (Santa Cruz Biotechnology, Inc.). Subsequently the membranes were revealed with an Amersham ECL Western Blotting Detection kit (EMD Millipore, Billerica, MA, USA).

Statistical analysis. Data are expressed as the mean ± standard deviation. Comparison between the means of two groups was performed using Student's t-test and one-way analysis of variance followed by post hoc pairwise comparisons using Tukey's test. p<0.05 was considered to indicate a statistically significant difference.

Results

miR-125b expression in human non-small cell lung cancer. Patients with NSCLC (n=91) were recruited from the Oncology Department of the Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, and the expression of miR-125b was assessed in all patients using quantitative PCR. As shown in Fig. 1, the expression of miR-125b in NSCLC tissue was higher than that in para-carcinoma tissue.

Association between miR-125b expression and clinicopathological features. To determine the regulatory role of miR-125b in human NSCLC, we examined the association between miR-125b expression and the clinicopathological features of patients. As shown in Table I, differences were observed between miR-125b expression and the pathological type of NSCLC, and between miR-125b expression and the age of patients with NSCLC.

Association between miR-125b expression and OS and DFS rates. A follow-up of NSCLC patients was conducted over a 3-year period, in which the OS and DFS rates of patients were recorded. As shown in Fig. 2A and B, the 3-year OS and DFS rates in patients with low miR-125b expression were higher than those in patients with high miR-125b expression.

Downregulation of miR-125b inhibits the proliferation of A549 cells. To assess the effects of miR-125b on the proliferation of A549 cells, we investigated cell growth with an MTT assay. Firstly, downregulation of miR-125b significantly suppressed the expression of miR-125b in A549 cells when compared with a negative control group (Fig. 3A). Notably, we found that miR-125b markedly suppressed A549 cell proliferation at 24 and 48 h when compared with the negative control group (Fig. 3B).

Downregulation of miR-125b induces apoptosis in A549 cells. To examine whether downregulation of miR-125b induced the apoptosis of A549 cells, as indicated by the aforementioned results, an Annexin V-FITC/PI apoptosis assay was used to analyze changes in the rate of apoptosis. The results revealed that downregulation of miR-125b significantly increased the rate of apoptosis and promoted cell death in A549 cells when compared with the negative control group (Fig. 4).

Downregulation of miR-125b suppresses the protein expression of p-Akt, p-GSK3β, Wnt and β-catenin in A549 cells. We used western blot analysis to detect the protein expression of p-Akt, p-GSK3β, Wnt and β-catenin in A549 cells. The results revealed that the downregulation of miR-125b significantly
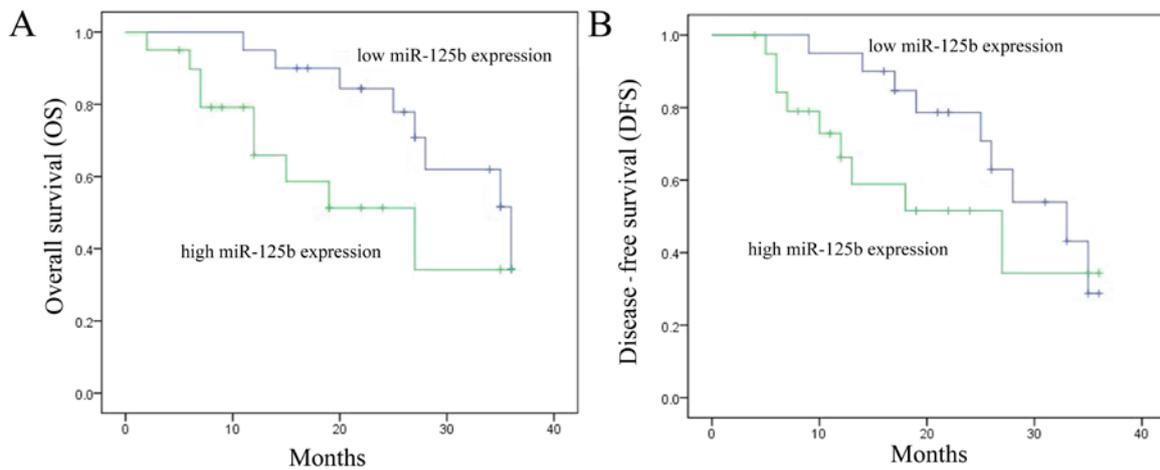
Figure 2. Association between miR-125b expression and OS and DFS rates. (A) OS and DFS (B) rates were recorded from a 3-year follow-up of patients with non-small cell lung cancer. OS, overall survival; DFS, disease-free survival.
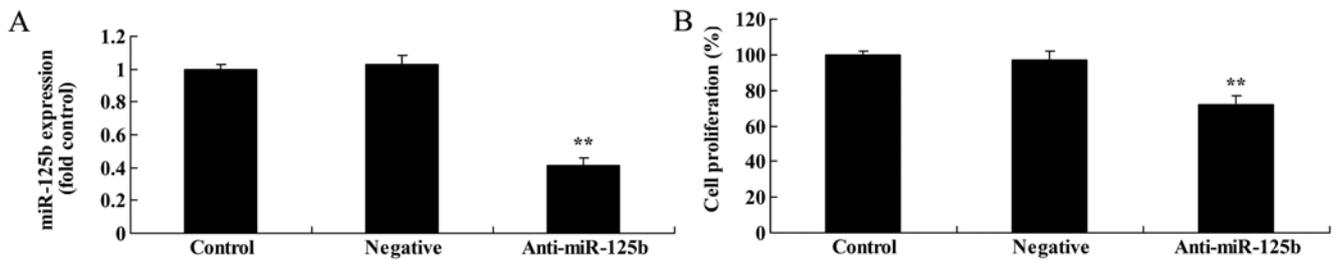
Figure 3. Downregulation of miR-125b inhibits the proliferation of A549 cells. Downregulation of miR-125b inhibited (A) miR-125b expression and (B) proliferation in A549 cells; **p<0.01 compared with the control group.
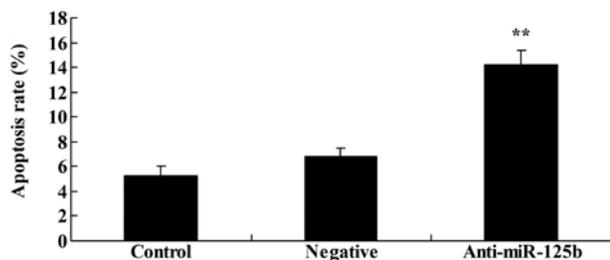
Figure 4. Downregulation of miR-125b induces apoptosis in A549 cells; **p<0.01 compared with the control group.

suppressed the expression of p-Akt (Fig. 5), p-GSK3β (Fig. 6), Wnt and β-catenin (Fig. 7) in A549 cells when compared with the negative control group.

Downregulation of miR-125b increases caspase-3 activity in A549 cells. To validate the effects of miR-125b on the apoptosis of A549 cells, the effects of miR-125b downregulation on the activity of caspase-3 were assessed using a caspase-3 activity ELISA kit. The results revealed that the downregulation of miR-125b significantly promoted caspase-3 activity in A549 cells when compared with the negative control group (Fig. 8).

Downregulation of miR-125b increases Bax protein expression in A549 cells. The effect of miR-125b on the apoptosis of A549 cells was also validated by assessing the protein

expression of Bax following miR-125b downregulation by western blot analysis. As shown in Fig. 9, the downregulation of miR-125b significantly increased Bax expression in A549 cells when compared with the negative control group.

Downregulation of miR-125b combined with a PI3K inhibitor suppresses PI3K and p-Akt protein expression in A549 cells. Based on the aforementioned results, we explored the effect of a PI3K inhibitor on the growth of A549 cells after the downregulation of miR-125b. LY294002 was used as the PI3K inhibitor to suppress the PI3K/Akt pathway in A549 cells following miR-125b downregulation. As shown in Fig. 10, LY294002 markedly inhibited the expression of PI3K and p-Akt in A549 cells following the downregulation of miR-125b at 48 h, relative to a control group with only miR-125b downregulation.

Downregulation of miR-125b combined with a PI3K inhibitor enhances cell growth inhibition. We performed an MTT assay to assess the effects of miR-125b on the proliferation of A549 cells following PI3K inhibition. When compared with miR-125b downregulation alone, the PI3K inhibitor markedly inhibited the proliferation of A549 cells at 48 h following the downregulation of miR-125b (Fig. 11).

Downregulation of miR-125b combined with a PI3K inhibitor suppresses the protein expression of p-GSK3β, Wnt and β-catenin in A549 cells. To determine whether the
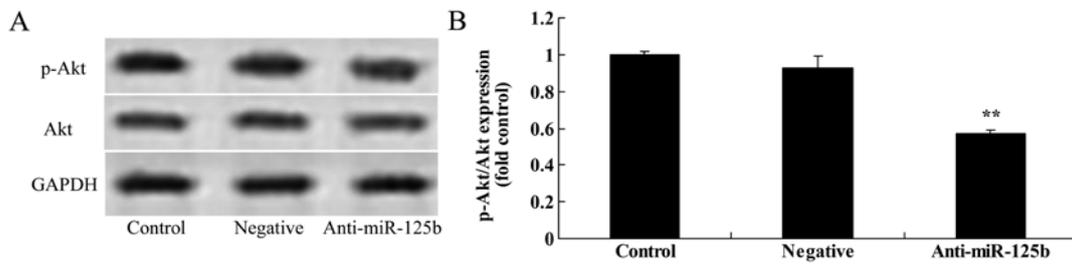
Figure 5. Downregulation of miR-125b suppresses the protein expression of p-Akt in A549 cells. Downregulation of miR-125b suppressed the protein expression of p-Akt, as indicated by (A) western blotting and (B) statistical analysis of p-Akt expression in A549 cells; **p<0.01 compared with the control group.
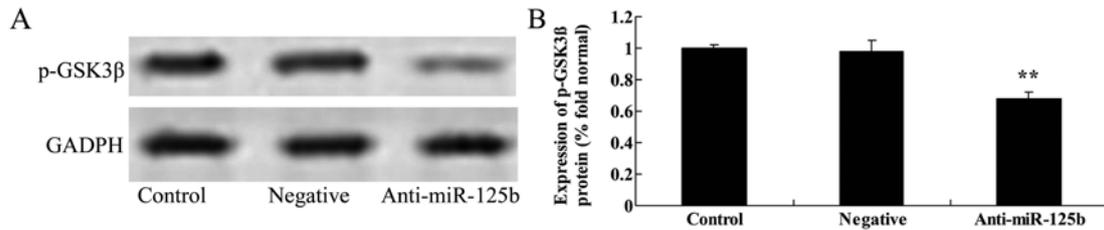
Figure 6. Downregulation of miR-125b increases Bax protein expression in A549 cells. Downregulation of miR-125b suppressed the protein expression of Bax, as indicated by (A) western blotting and (B) statistical analysis of Bax expression in A549 cells; **p<0.01 compared with the control group.
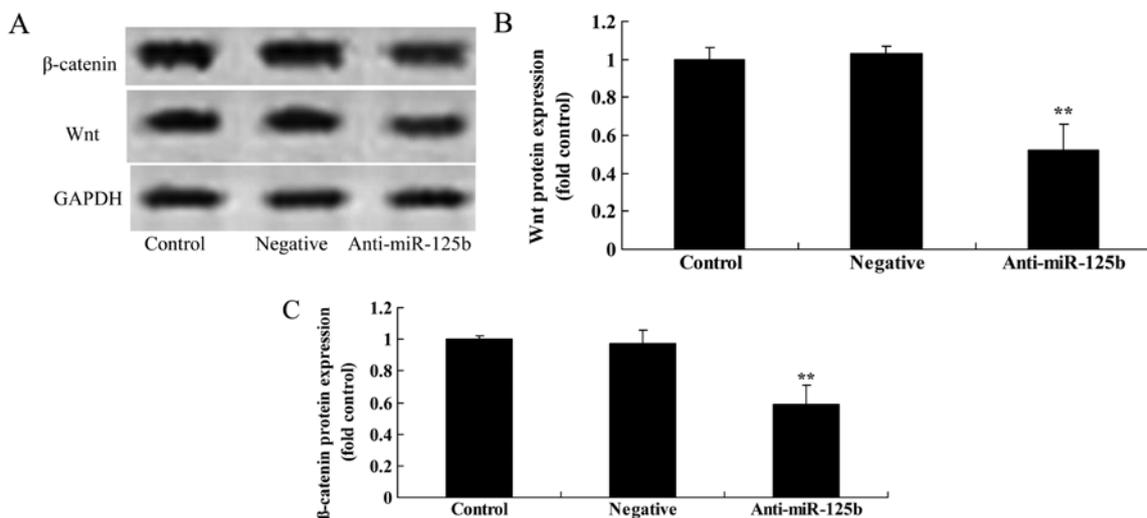
Figure 7. Downregulation of miR-125b suppresses the protein expression of Wnt and β-catenin in A549 cells. Downregulation of miR-125b suppressed the protein expression of Wnt and β-catenin, as indicated by (A) western blotting, (B) and statistical analysis of Wnt and (C) β-catenin expression in A549 cells; **p<0.01 compared with the control group.
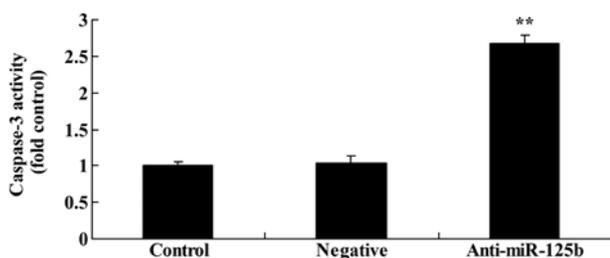
Figure 8. Downregulation of miR-125b increases caspase-3 activity in A549 cells; **p<0.01 compared with the control group.

in A549 cells, western blot analysis was used to assess the expression of the proteins. Suppression of PI3K significantly decreased the expression levels of p-GSK3β (Fig. 12), Wnt and β-catenin (Fig. 13) in A549 cells following the downregulation of miR-125b at 48 h, relative to a control group with only miR-125b downregulation.

Downregulation of miR-125b combined with a PI3K inhibitor promotes caspase-3 activity and Bax expression in A549 cells. To determine whether the downregulation of miR-125b combined with a PI3K inhibitor further promoted caspase-3 activity in A549 cells, caspase-3 activity in A549 cells was assessed using the caspase-3 activity ELISA kit. The protein expression of Bax was also evaluated. The activation of

downregulation of miR-125b combined with a PI3K inhibitor affected the expression of p-GSK3β, Wnt and β-catenin
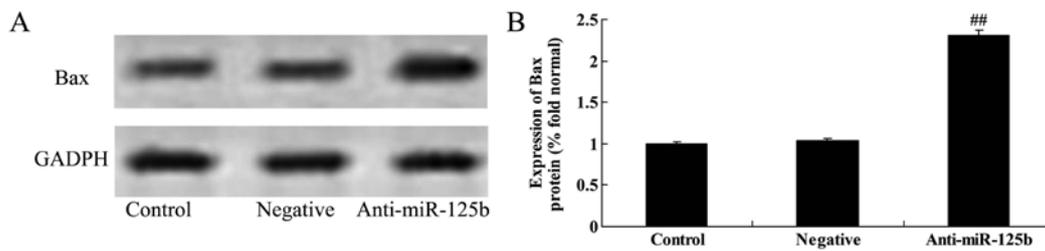
Figure 9. Downregulation of miR-125b increases Bax protein expression in A549 cells. Downregulation of miR-125b increased the protein expression of Bax, as indicated by (A) western blotting and (B) statistical analysis of Bax expression in A549 cells; ##$p<0.01$ compared with the control group.
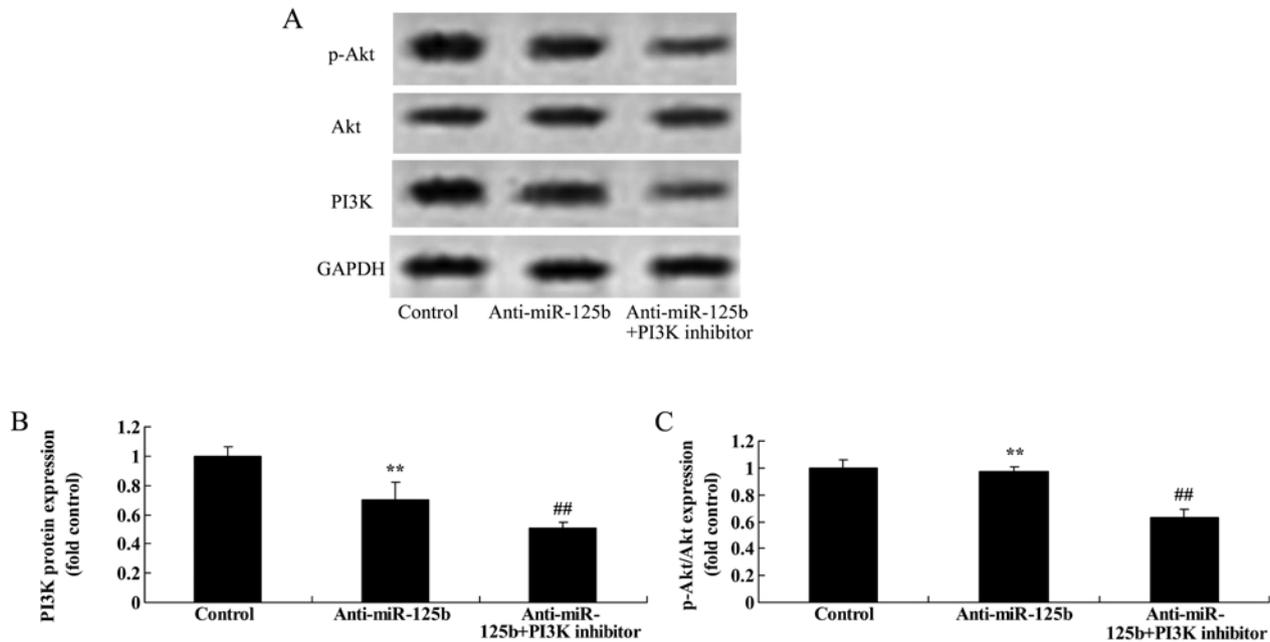
Figure 10. Downregulation of miR-125b combined with a PI3K inhibitor suppresses the protein expression of PI3K and p-Akt in A549 cells. Downregulation of miR-125b combined with a PI3K inhibitor suppressed PI3K and p-Akt protein expression, as indicated by (A) western blotting and statistical analysis of (B) PI3K and (C) p-Akt expression in A549 cells; **$p<0.01$ compared with the control group; ##$p<0.01$ compared with the control group.
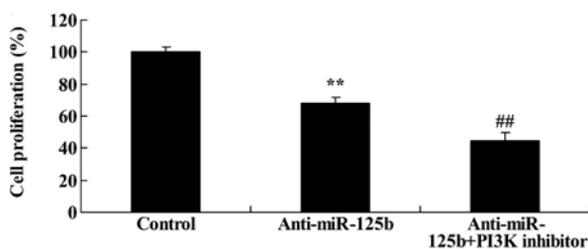
Figure 11. Downregulation of miR-125b combined with a PI3K inhibitor enhances cell growth inhibition; **$p<0.01$ compared with the control group; ##$p<0.01$ compared with the control group.

caspase-3 and expression of Bax in A549 cells following downregulation of miR-125b was significantly increased by the PI3K inhibitor at 48 h, relative to a control group with only miR-125b downregulation (Fig. 14).

Discussion

The treatment of cancer is a medical science challenge that to date has had limited success. Although tumor treatment has made progress in recent years, there is currently no fundamental method of curing cancer. Lung cancer is among the most common types of malignant tumors (17). A survey by the World Health Organization revealed that the morbidity rate of lung cancer in many countries and regions ranked the highest of all malignant tumors (18). This is particularly the case for China, where the morbidity rate of lung cancer is higher than that in America and other Western countries. NSCLC accounts for ~70% of all lung cancer cases (19). Despite the use of surgical treatment for early-stage NSCLC, its 5-year survival rate remains relatively low at ~30%, while late-stage NSCLC has a one-year survival rate of ~10% even after chemotherapy (20). To the best of our knowledge, the present study is the first to demonstrate that the downregulation of miR-125b could suppress the proliferation and promote the apoptosis of A549 cells.

The activated substrates of PI3K serve as second messengers on the plasmalemma and bind with the signaling proteins Akt and PDK1 via interactions with their pH structure domains (21). After Akt is recruited to the cytomembrane, it undergoes Ser124 and Thr450 phosphorylation, which promotes its catalytic activity (13). Akt can also be activated
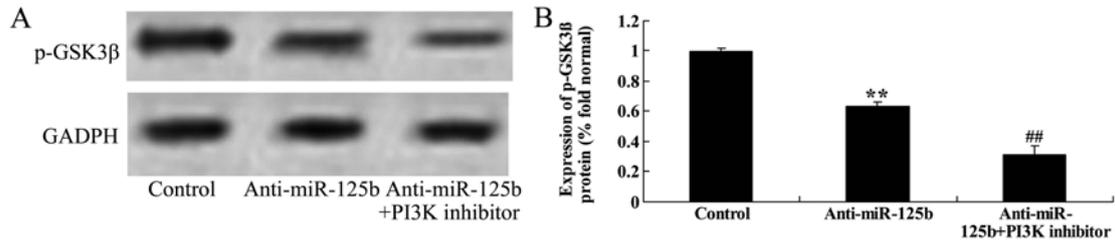
Figure 12. Downregulation of miR-125b combined with a PI3K inhibitor suppresses the protein expression of p-GSK3β in A549 cells. Downregulation of miR-125b combined with a PI3K inhibitor suppressed p-GSK3β protein expression, as indicated by (A) western blotting and (B) statistical analysis of p-GSK3β expression in A549 cells; **p<0.01 compared with the control group; ##p<0.01 compared with the control group.
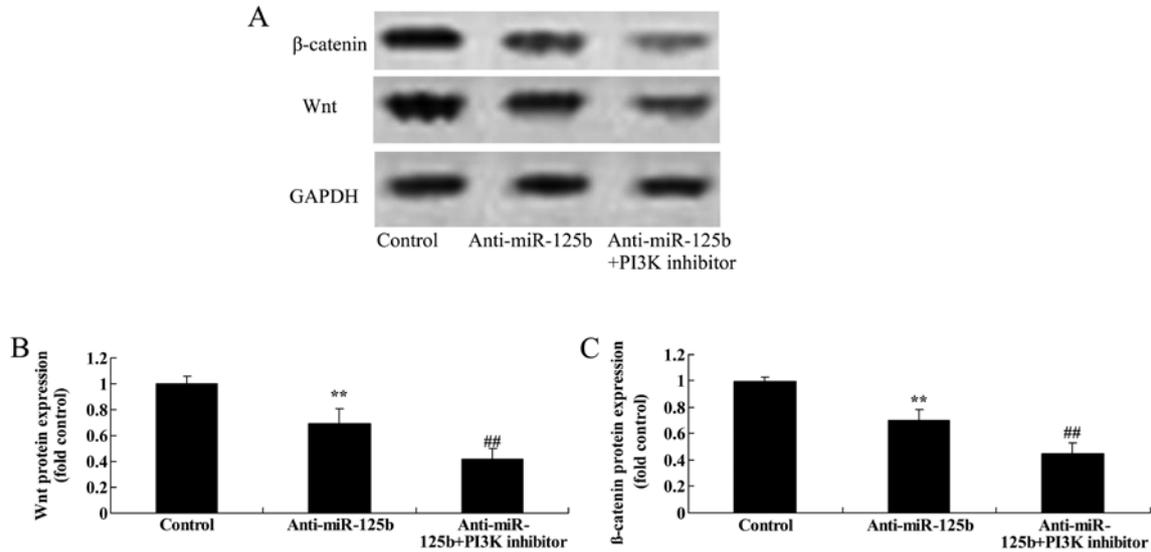
Figure 13. Downregulation of miR-125b combined with a PI3K inhibitor suppresses Wnt and β-catenin protein expression in A549 cells. Downregulation of miR-125b combined with a PI3K inhibitor suppressed Wnt and β-catenin protein expression, as indicated by (A) western blotting and statistical analysis of (B) Wnt and (C) β-catenin expression in A549 cells; **p<0.01 compared with the control group; ##p<0.01 compared with the control group.
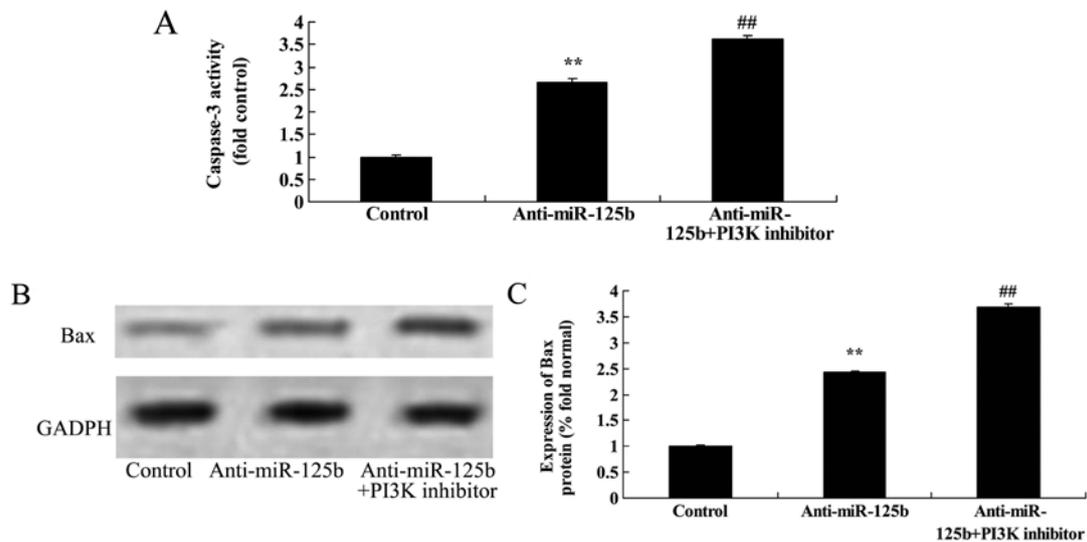
Figure 14. Downregulation of miR-125b combined with a PI3K inhibitor promotes caspase-3 activity and Bax expression in A549 cells. Downregulation of miR-125b combined with a PI3K inhibitor promoted (A) caspase-3 activity and (B and C) Bax expression in A549 cells; **p<0.01 compared with the control group.

through phosphorylation of Ser473 mediated by PDK2 enzymes, such as integrin-linked kinase (11). The PI3K/Akt pathway induces tumor occurrence via multiple mechanisms: it downregulates the protein expression of p53 in the cell

nucleus by promoting the nuclear translocation of Mdm2 tumor proteins (22); it promotes the abnormal proliferation of cancer cells at a transcriptional and translational level through excessive activation (23); and it can suppress the process of apoptosis through multiple mechanisms, including via inhibitory effects on the conformational changes of pre-apoptotic Bax, and via the phosphorylation of other apoptotic structures at the mitochondrial level (24). In the present study, we found that the downregulation of miR-125b significantly suppressed the protein expression of p-Akt in A549 cells. Furthermore, the present results revealed that a PI3K inhibitor suppressed the proliferation of A549 cells following the downregulation of miR-125b. Similarly, Shi *et al* reported that a PI3K inhibitor combined with a miR-125b inhibitor suppressed cell proliferation in glioma stem cell cancer through inactivation of the Wnt/β-catenin signaling pathway (25). These data suggest that alterations in p-Akt expression could be involved in the regulatory effect of miR-125b downregulation on lung cancer progression.

Glycogen synthase kinase 3β (GSK3β) is a serine-threonine protein kinase. GSK3β can phosphorylate a variety of substrates, including metabolic and signaling proteins, cell structural proteins and transcription factors, and plays an important role in cell growth and development as well as tumor occurrence (26). GSK3β is an important kinase in the Wnt signaling pathway, where it acts as a key inhibitory factor that blocks Wnt signaling to inhibit tumor development (27). In the Wnt signaling pathway, GSK3β also serves as a key regulatory kinase that mediates the phosphorylation state of β-catenin in the β-catenin/Axin/APC complex (27). GSK3β is active in the absence of Wnt signaling, and catalyzes phosphate addition to four sites within the amino terminus of β-catenin, leading to its degradation and ultimately the inhibition of Wnt signal transduction (28). Our results revealed that the downregulation of miR-125b significantly decreased the protein expression of p-GSK3β in A549 cells.

β-catenin is not only a component of the cytomembrane epithelial cadherin/β-catenin complex, but is also a critical molecule within the intracellular Wnt signal transduction pathway (29). β-catenin acts as an important link on the cytomembrane, where it aids to transduce downstream signals of the Wnt pathway into the nucleus (30). The β-catenin gene *CTNNB1* is located on chromosome 3p22 and has a length of 23.2 kb, which includes 16 exons. The third exon is regarded as a multi-functional factor. β-catenin is expressed on the cytomembrane of normal epithelial tissues (31). Its characteristics in the cytomembrane are shown in the figure. There are four ligands in the cytomembrane (32). Upon receipt of outside stimulation, signals are conducted to the cytoplasm from the cytomembrane, which involves phosphorylation of intracellular β-catenin. Phosphorylation of β-catenin enables its dissociation from the membrane and translocation to the nucleus, where it promotes gene transcription (33). Ectopic expression of β-catenin on the cytomembrane is generally rare, although a previous study of NSCLC showed that the levels of β-catenin in tumor tissues were significantly higher than those in normal lung tissues (32). The aberrant expression of β-catenin proteins has no obvious correlation with different tumor types or stages. However, the rate of aberrant expression of β-catenin has been reported to differ between

NSCLC tumors with different degrees of differentiation and different lymphatic metastasis statuses (31). A codon can be coded into the phosphorylated locus of GSK3β. The mutation of the locus can cause abnormal accumulation of β-catenin, leading to cell proliferation and potentially tumorigenesis (30). The present study revealed that the downregulation of miR-125b suppressed the protein expression of Wnt and β-catenin in A549 cells. In addition, PI3K inhibition combined with miR-125b downregulation suppressed the expression of Wnt and β-catenin in A549 cells to a greater extent than miR-125b downregulation alone. Zang *et al* reported that the E6 protein of human papilloma virus type 16 promoted cell growth through the downregulation of miR-125b and the activation of the Wnt/β-catenin signaling pathway in esophageal cancer (34). Collectively these experiments indicate that PI3K/Wnt/β-catenin signaling may play a role in the suppression of NSCLC cell growth through the downregulation of miR-125b.

In summary, this study demonstrates for the first time that the downregulation of miR-125b can suppress the proliferation and promote the apoptosis of A549 cells, potentially through the suppression of PI3K/Wnt/β-catenin expression. These findings may provide new insights into the mechanisms of miR-125 regarding its effects on PI3K/AKT and GSK3β/Wnt/β-catenin signaling and the development of NSCLC, and may aid in the development of potential diagnostic or therapeutic strategies for NSCLC.

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