High expression of PINK1 promotes proliferation and chemoresistance of NSCLC

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Abstract. PTEN-induced putative kinase 1 (PINK1) was identified initially as a gene upregulated in cancer cells which regulates cellular processes of significance in cancer cell biology, including cell survival, stress resistance and the cell cycle. However, the expression and function of PINK1 in non-small cell lung cancer (NSCLC) has not been determined yet. We demonstrated high PINK1 expression in NSCLC tumor tissues and cell lines as assessed by western blot and immunohistochemistry (IHC) assays. In addition, IHC analysis revealed that PINK1 expression was associated with a more invasive tumor phenotype and poor prognosis. Furthermore, *in vitro* studies using upregulation and knockdown of PINK1 confirmed that PINK1 promoted cell proliferation of NSCLC, which might be through as the NF-κB pathway. Moreover, we also demonstrated that downregulation of PINK1 enhanced cisplatin (CDDP)-induced NSCLC cell apoptosis. Together, our findings indicate that PINK1 plays a significant role in NSCLC progression and chemoresistance, and highlights its potential role as a target in future anticancer therapies.

Introduction

Lung cancer is one of the leading causes of cancer-related death in the world (1). Non-small cell lung cancer (NSCLC) is a major type of lung cancer accounting for nearly 85% of all lung cancer cases. Despite great efforts in diagnostic procedures and therapeutic options, it is often diagnosed at an advanced stage and the overall 5-year survival rate is only approximately 15% (2). For treatment of lung cancer, platinum-based combination chemotherapy is still the standard first-line treatment for NSCLC, particularly for tumors without EGFR mutation or ALK translocation (3). However, chemoresistance is a major obstacle in the treatment of lung cancer. Therefore, the discovery of new biomarkers is critical not only for early detection of the disease but also for the prediction of chemotherapeutic efficacy.

A Parkinson's disease-associated gene, PINK1 [phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-induced kinase 1] was initially identified in HeLa cells as a gene upregulated by overexpression of the main tumor suppressor, PTEN (4). Loss of function in PINK1 could cause autosomal recessive forms of Parkinson's disease (5). The PTEN-induced putative kinase 1 (PINK1) gene encodes a 581-amino acid protein with a mitochondrial targeting sequence, a highly conserved serine/threonine protein kinase domain and a regulatory C-terminal sequence (6). Research during the past decade revealed that PINK1 plays an important role in pro-survival, anti-apoptosis and cytoprotection (7-9), mechanistically via proteasomal and autophagic pathways, PI3-kinase/Akt, NF-κB pathway and calcium-dependent signaling (10-13). Substantial studies have indicated a potential role for PINK1 in cancer cell biology, including cell survival, mitochondrial homeostasis, stress resistance and the cell cycle (14-16). As a tumor oncogene, PINK1 is critical for activation of a well-known oncogenic pathway, insulin-like growth factor-1-dependent Akt signaling (17).

Moreover, deletion of PINK1 increases sensitivity of cancer cells to paclitaxel (18). Although PINK1 dysfunction has been related with the progression of numerous cancers such as breast cancer, colorectal cancer and endometrial carcinoma, the precise role of PINK1 in the development of NSCLC remains unknown. However, numerous studies demonstrate that NF-κB signaling is intimately linked with cellular adhesion, migration, invasion, and chemoresistance of NSCLC (19,20).

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Furthermore, it has been reported that PINK1 could specifically bind to TRAF6, which results in auto-ubiquitination of TRAF6 and activation of NF-κB pathway (21). Therefore, we assume that PINK1 might play a role in NSCLC development through NF-κB signaling.

In the present study, we determined the expression of PINK1 in NSCLC tissues and NSCLC cell lines. In addition, we explored the correlation between PINK1 expression and various clinicopathological features as well as its prognostic value for NSCLC patients. Furthermore, we explored the role of PINK1 expression in regulating cell cycle progression, cell proliferation, and drug resistance in NSCLC cells. This study might provide a better understanding of the mechanism underlying NSCLC development.

Materials and methods

Patients and tissue samples. Paired specimens of NSCLC were obtained from 114 patients, who underwent surgery without preoperative systemic chemotherapy at the Surgery Department of the Affiliated Hospital of Nantong University between 2008 and 2016. Immediately after surgical removal, NSCLC specimens were fixed in formalin and embedded in paraffin, and 5-μm sections were prepared for immunohistochemistry. All human tissues were collected in accordance with protocols approved by the ethics committee of the Affiliated Hospital of Nantong University. The main clinical and pathological variables of patients are summarized in Table I.

Western blot analysis. The tissues and cell samples were immediately homogenized in a lysis buffer containing 50 mM Tris-Cl, pH 8.0, 0.1% NP-40, 150 mM NaCl, 1 mM EDTA, 60 mM β-glycerophosphate, 0.1 mM NaF, 0.1 mM sodium orthovanadate, and complete protease inhibitor cocktail (Roche Diagnostics) and then centrifuged at 12,000 x g, 4˚C for 15 min to collect the supernatant. Protein concentrations were determined using a BCA protein assay kit (Bio-Rad, Hercules, CA, USA). Subsequently, the supernatants were added with equal volume of 2X sodium dodecyl sulfate (SDS) sample buffer and boiled for 15 min. The protein samples were subjected to 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) separation and then transferred to polyvinylidene difluoride filter (PVDF) membranes (Millipore, Bedford, MA, USA). Next, the membranes were blocked with 5% no-fat milk in TBST (150 mM NaCl, 20 mM Tris and 0.05% Tween-20) for 2 h and then incubated with primary antibodies overnight at 4˚C.

The primary antibodies used for western blotting were as follows: rabbit polyclonal anti-PINK1 antibody (Abgent, San Diego, CA, USA), mouse monoclonal anti-p27 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-cyclin D1 antibody (Santa Cruz Biotechnology), mouse monoclonal anti-p65 antibody (Santa Cruz Biotechnology), rabbit polyclonal anti-GAPDH antibody (Santa Cruz Biotechnology), rabbit polyclonal anti-cleaved-caspase-3 antibody (Santa Cruz Biotechnology), and rabbit polyclonal anti-cleaved poly (ADP-ribose) polymerase (PARP) antibody (Immunoway, Newark, DE, USA). Secondary antibody incubation was performed using horseradish peroxidase linked IgG (Pierce Biotechnology, Rockford, IL, USA) at a dilution of 1:5000. The detection of chemiluminescent signals was performed by ECL method (Zhongshan Biotechnology Co., Ltd., Beijing, China).

Immunohistochemistry and immunohistochemistrical evaluation. Immunohistochemistry was performed in accordance with previous reports. In brief, paired tissue sections were dewaxed, washed, and blocked. Afterward, tissue sections were incubated overnight at 4˚C with primary antibodies: anti-PINK1 (1:100) or mouse monoclonal anti-Ki-67 (1:100; Santa Cruz Biotechnology), followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. After rinsing in water, the sections were counterstained with hematoxylin, dehydrated, and cover slipped. The slides were then mounted for observation under a fluorescence microscope.

All immunostained sections were independently examined by three pathologists in a blinded manner without knowledge of the clinical and pathological variables of the patients. At least five high-power fields in each specimen were randomly selected, and the cytoplasm or nuclear staining was examined under a high magnification. More than 500 cells were examined to determine the mean percentage of signal-positive cells. For determining PINK1 expression, the intensity of immunostaining was assessed as 0 (negatively or poorly staining), 1 (moderately staining), and 2 (strongly staining), and according to PINK1 expression ratio (50%, 75%), we divided patients into three groups: low expression group (<50%) scored 1, moderate expression group (50-75%) scored 2, and high expression group (>75%) scored 3. Then, we multiplied the two scores and divided patients into two groups according to the average scores (4.2): high-expression group (>4.2) and low expression group (≤4.2). The expression of proliferation marker Ki-67 was scored in a semi-quantitative fashion: high expression (≥50%) and low expression (<50%) (22).

Cell culture and transfection. The human NSCLC cell lines (A549, H1299 and Spca-1) and normal human bronchial epithelial cell line BEAS-2B were obtained from the Institute of Cell Biology, Academic Sinica, and all cells were cultured in the 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum in 5% CO_2 at 37˚C.

The PINK1-siRNA and control-siRNA were purchased from Genechem (Shanghai, China). The PINK1-specific siRNA target sequence was as follows: PINK1-siRNA#1 was 5'-TCC TCGTTATGAAGAACTA-3', PINK1-siRNA#2 was 5'-AAG CCATCTTGAACACAAT-3', PINK1-siRNA#3 was 5'-GCTG GAGGAGTATCTGATA-3', and PINK1-siRNA#4 was 5'-AGC GTAGCATGTCTGATTT-3'. A549 and H1299 cells were grown in dishes until they reached 80% confluence. The medium was replaced 6 h later with fresh medium for transfection. A549 and H1299 cells were transfected with PINK1-siRNA or control-siRNA according to the manufacturer's instructions. Cells were collected for western blotting, CCK-8, and flow cytometry assays after transfection for 36 h. A549 and H1299 cells were seeded the day before transfection using 1640 with 10% fetal bovine serum (FBS) without antibiotics. A549 and H1299 cells were transfected with the PINK1-siRNA or the control siRNA using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's protocol, and the media were replaced with 1640 supplemented with 10% FBS at

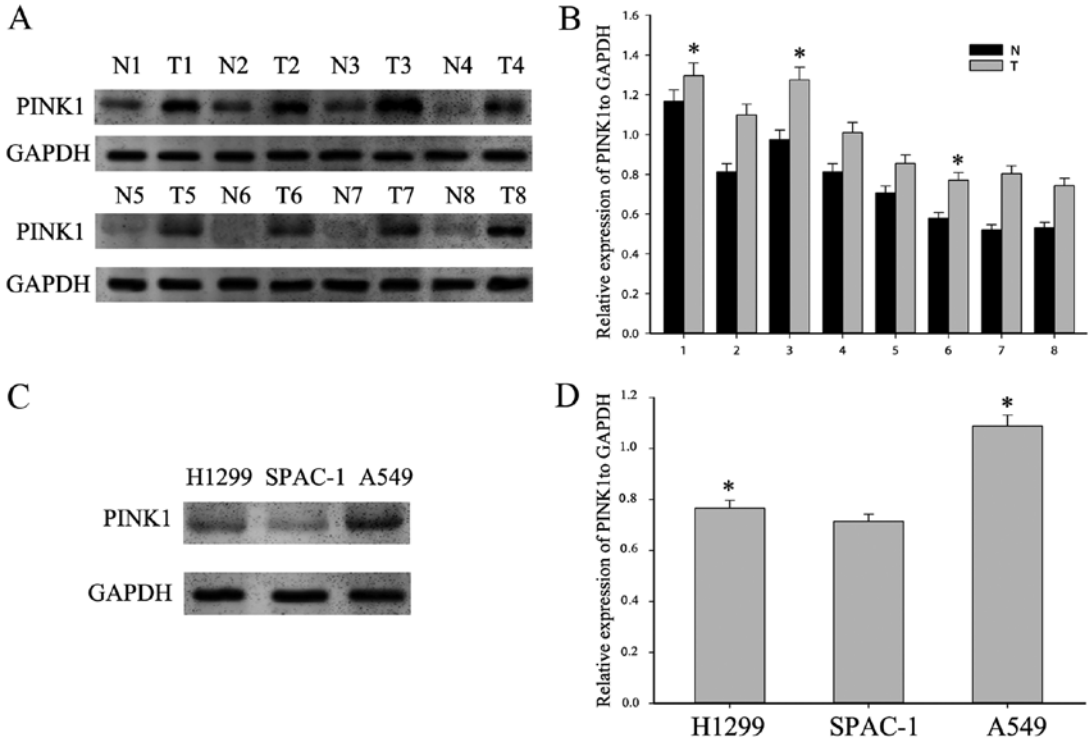

Figure 1. Expression profile of PINK1 in human NSCLC tissues and cell lines. (A) Expression of PINK1 in eight representative paired samples of NSCLC tissues (T) and adjacent non-tumorous (N) tissues. (B) The bar chart indicates the ratio of PINK1 protein to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by densitometry in NSCLC tissues. (C) PINK1 is expressed in three NSCLC cells. (D) The bar chart of the ratio of PINK1 protein to GAPDH by densitometry in the indicated cell lines. The data are mean ± SEM. *P<0.05.

6 h after transfection. The transfected cells were subjected to subsequent experiments at 48 h after transfection.

Cell cycle analysis and cell proliferation assay. Cells were harvested, washed twice with ice-cold 1 ml phosphate buffered saline (PBS), and fixed in 70% ethanol for 24 h at 4˚C. Then, the cells were washed three times with ice cold 1 ml PBS and incubated with 1 mg/ml RNase A for 30 min at 37˚C. Subsequently, cells were stained with 50 μg/ml propidium iodide (PI; Becton Dickinson, San Jose, CA, USA) in 0.5% Tween-20 in PBS and subjected to analysis of cell cycle distribution using a BD FACScan flow cytometer (Becton Dickinson) coupled with Cell Quest acquisition and analysis programs.

Cell proliferation was evaluated using the Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) in accordance with the manufacturer's protocol. Briefly, A549 and H1299 cells were seeded at a cell density of $2x10^4$ cells per well into a 96-well plate and grown overnight. For the measurement of CCK-8 absorbance, the cells were incubated with 10 μl CCK-8 reagent coupled with 100 μl 1640 medium for another 2 h at 37˚C in an incubator. The absorbance was recorded at a test wavelength of 490 nm and a reference wavelength of 650 nm using a microplate reader (Bio-Rad). These experiments were repeated for at least three times with similar results.

Colony formation assay. Cells were seeded in a 6-well plate at $5x10^3$ cells per well and cultured for 10 days. Colonies were fixed for 5 min using 10% formaldehyde and then stained with 1.0% crystal violet for 30 sec, and cell numbers were counted.

Apoptotic analysis. The apoptosis assays were performed at 72 h after the cells were transfected with PINK1-siRNA or negative control. The A549 and H1299 cells transfected with PINK1-siRNA or control-siRNA were washed three times in ice-cold PBS, resuspended in 100 μl of 1X 19 binding buffer and incubated with Annexin V-FITC (Bestbio, China) for 15 min at 4˚C in the dark, according to the manufacturer's instructions. After staining, the cells were incubated with propidium iodide for 5 min at 4˚C in the dark and then analyzed using a flow cytometer (Beckman, Palo Alto, CA, USA).

Statistical analysis. The SPSS 17.0 software package was used for all statistical analysis. The association between PINK1 and Ki-67 expression and clinicopathological features was analyzed using the χ^2 test. For analysis of the survival data of patients, the Kaplan-Meier curves and the log-rank test were performed. Multivariate analysis was constructed using the Cox proportional hazards model. The hazard ratio and its 95% confidence interval were recorded for each variable. P<0.05 was considered statistically significant. All values were expressed as mean ± SEM (23).

Results

PINK1 is upregulated in human NSCLC tissues. To detect a possible involvement of PINK1 in NSCLC, western blot analysis was performed to examine the expression pattern of PINK1 in eight paired NSCLC and adjacent non-tumorous tissues. As shown in Fig. 1A and B, PINK1 expression was remarkably higher in tumorous tissues than in adjacent non-tumorous

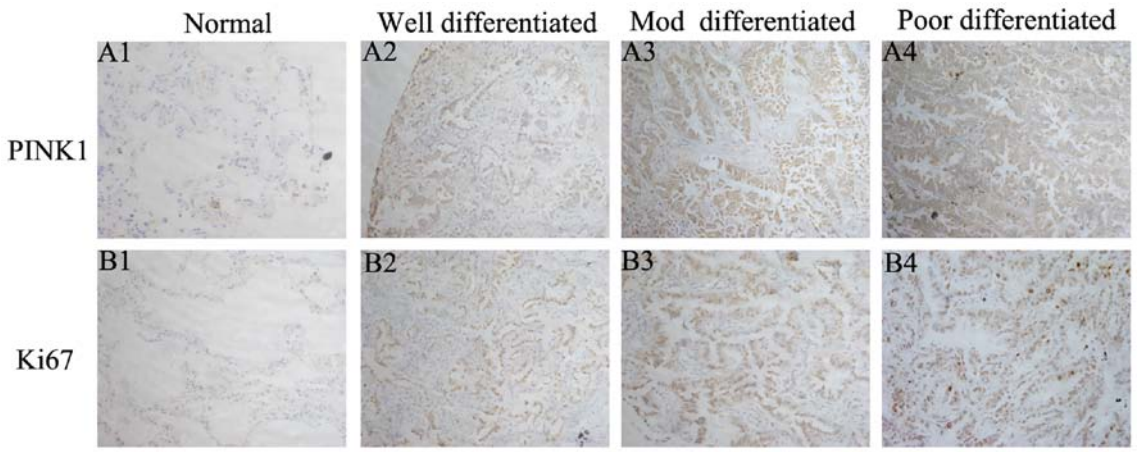

Figure 2. Immunohistochemical analysis of PINK1 and Ki-67 expression in paraffin-embedded NSCLC tissues. PINK1 and Ki-67 were highly expressed in NSCLC. According to the intensity of PINK1 and Ki67 expression, we divided the samples into normal (A1, B1), well differentiated (A2, B2), moderately differentiated (A3, B3), and poorly differentiated (A4, B4), respectively (x200 magnification).

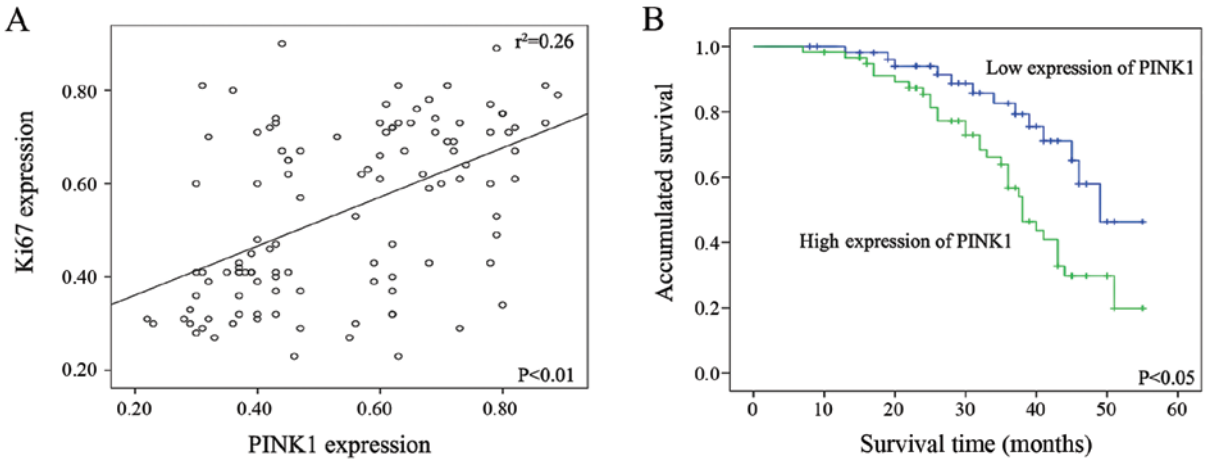

Figure 3. (A) Relationship between PINK1 and Ki67 expression in NSCLC. Scatter plot of PINK1 versus Ki67 with regression line showing a significant correlation using the Pearson's correlation coefficient (P<0.01). (B) Kaplan-Meier survival curves for low PINK1 expression versus high PINK1 expression in 114 patients of NSCLC showed a highly significant separation (P<0.05, log-rank test).

ones. Next, we examined the basic expression of PINK1 in three human NSCLC cell lines, A549, H1299, and SPCA-1. We found that PINK1 was highly expressed in NSCLC cell lines (Fig. 1C). Moreover, we analyzed the expression of PINK1 in 114 NSCLC tissues using immunohistochemical assay (Fig. 2). NSCLC tumor tissues displayed cytoplasmic diffuse staining of PINK1, together with a prominent membrane staining. While for Ki-67 which is a marker for cell proliferation, its immunoreactivity was found predominantly in the nucleus. As expected, PINK1 was highly expressed in poorly differentiated specimens compared with well-differentiated ones, which was consistent with Ki-67. There was low or even no expression for both markers in adjacent non-tumor tissues. Thus, PINK1 was upregulated in NSCLC specimens and might be associated with tumor cell proliferation.

Correlation of PINK1 expression with clinicopathological variables in NSCLC patients. To further confirm the correlation of PINK1 and Ki-67 expression by twos, Spearman's correlation test was next performed by percentage of positive malignant cells. A significant positive correlation was found between the expression status of PINK1 and that of Ki-67. Spearman's correlation coefficient (γ^2) for PINK1-Ki-67 equals to 0.037 (P<0.01) (Fig. 3A).

In addition, we further evaluated the association of PINK1 expression with clinicopathological variables including Ki67 by Pearson's χ^2 test. The level of PINK1 and Ki-67 expression was divided into high group and low group according to the cutoff value stated in the afore-mentioned methods. The data are summarized in Table I. According to Table I, PINK1 expression was correlated with tumor size (P=0.008), lymph node metastasis (P=0.001), histological differentiation (P=0.045), clinical stage (P=0.035) and Ki-67 expression (P=0.000), while there was no correlation with other variables such as age (P=0.349), gender (P=0.708), and smoking status (P=0.206).

High expression of PINK1 predicted poor prognosis of NSCLC patients. Next, we used Kaplan-Meier analysis to determine the effect of PINK1 expression level on patient survival. The result revealed that NSCLC patients with high PINK1 expression was significantly associated with poor

Table I. Expression of PINK1 in 114 human lung adenocarcinoma tissues.

Clinicopathological features	Total	PINK1 expression		P-value
		Low	High	
Age (years)				0.349
<60	50	22	28	
≥60	64	33	31	
Gender				0.708
Female	59	27	32	
Male	55	28	27	
Tumor size (cm)				0.008[a]
<3	49	31	18	
≥3	65	24	41	
Smoking status				0.206
Yes	85	38	47	
No	29	17	12	
Lymph node metastasis				0.001[a]
0	60	38	22	
>0	54	17	37	
Clinical stage				0.035[a]
I	67	39	28	
II	30	11	19	
III	17	5	12	
Histological differentiation				0.045[a]
Well	26	7	19	
Mod	63	35	28	
Poor	25	13	12	
Ki-67 expression				0.000[a]
Low	51	31	20	
High	63	24	39	

Statistical analyses were performed by Pearson's χ^2 test. [a]P<0.05 was considered significant.

Table II. Contribution of various potential prognostic factors to survival by Cox regression analysis in 114 NSCLC specimens.

Characteristics	Hazard ratio	95.0% confidence interval	P-value
Age	0.982	0.948-1.017	0.317
Gender	0.846	0.538-1.861	0.998
Clinical stage	1.227	0.746-2.019	0.420
Tumor size	1.759	0.731-4.234	0.207
Histological differentiation	0.833	0.479-1.450	0.518
Lymph node metastasis	1.307	0.632-2.704	0.470
Ki67 expression	0.422	0.189-0.943	0.035[a]
PINK1 expression	0.436	0.195-0.976	0.043[a]

Statistical analyses were performed by the Cox regression analysis. [a]P<0.05 was considered significant.

overall survival rate, compared with those with low PINK1 expression (Fig. 3B). In addition, Cox proportional survival analysis showed that both PINK1 (P=0.043), and Ki67 expression (P=0.035) were independent prognostic factors in patients with NSCLC (Table II).

PINK1 overexpression promotes proliferation of NSCLC cells. In a recent study, a novel function for PINK1 was discovered as a positive regulator of cell cycle progression that can promote cancer-associated phenotypes (24). Given the fact that PINK1 expression was tightly associated with the expression of Ki-67 (Fig. 3), which is a cell proliferation marker, we presume that PINK1 expression would be related with proliferation and play a role in the regulation of cell cycle progression in NSCLC cells. Thus, we decided to overexpress PINK1 in A549 and H1299 to investigate its effect on cell proliferation. CCK8 assay showed that overexpression of PINK1 increased the proliferation rate in both cell lines (Fig. 4A). Colony formation assay also confirmed that overexpression of PINK1 upregulated the colony numbers of indicated cells (Fig. 4B).

To further explore the role of PINK1 in promoting cell proliferation, we investigated the cell cycle progression of A549 and H1299 following PINK1 overexpression. Flow cytometry analysis revealed that overexpression of PINK1 increased the S-phase cell population from 24.08 to 37.25% in A549 and from 22.59 to 29.37% in H1299 with a concomitant decrease in G1 phase (Fig. 4C). As a CDK inhibitor p27 and CDK regulator cyclin D1 are extremely important in regulating G1/S transition, we analyzed the expression levels of p27 and cyclin D1 using western blotting. Our results showed that p27 was downregulated, while cyclin D1 was upregulated in cells with PINK1 overexpression compared with negative control (Fig. 4D). Since it has been reported that PINK1 could bind to TRAF6 and TAK1, and facilitate the autodimerization and autoubiquitination of TRAF6, which leads to the activation of the NF-κB pathway, we presume that NF-κB pathway might be a downstream signaling of PINK1 in NSCLC. We analyzed the expression of p65, which is one of the members in the NF-κB family since its activation, and found that the level of p65 was correlated positively with the expression of PINK1 (Fig. 4D). Therefore, these data indicated that PINK1 expression might have an effect on the proliferation of NSCLC cells and alter cell cycle progression through the NF-κB pathway.

Knockdown of PINK1 inhibits NSCLC proliferation. To further verify the role of PINK1 in NSCLC cell proliferation and explore the possible downstream signaling, we knocked down PINK1 expression in A549 cells using a lentivirus-mediated RNA interference approach. A549 and H1299 cells were transiently transfected with PINK1-siRNA#1, PINK1-siRNA#2, PINK1-siRNA#3, PINK1-siRNA#4 and control siRNA for 36 h. To determine the efficiency of transfection, western

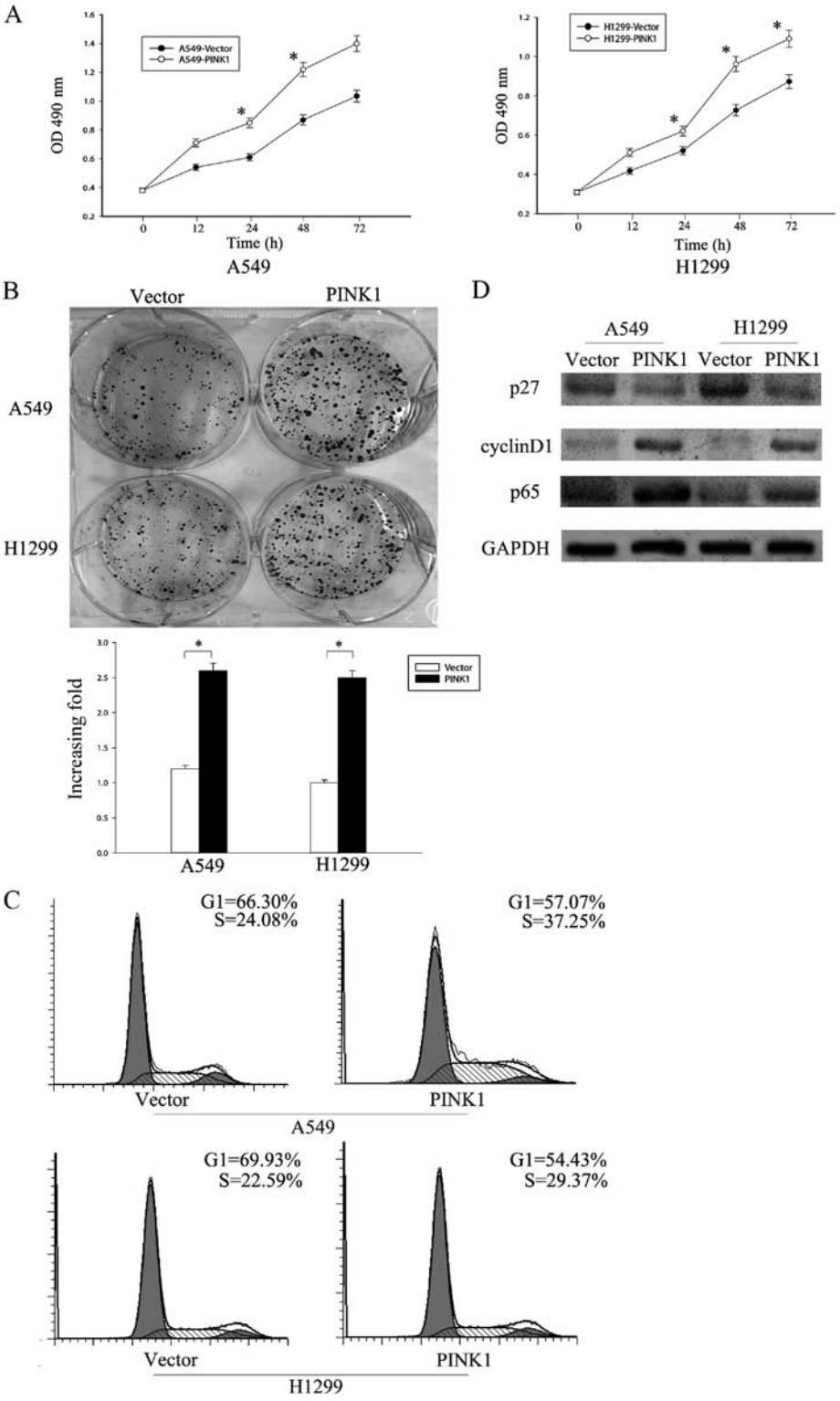

Figure 4. Overexpression of PINK1 accelerates cell proliferation and the cell cycle. (A) Cell proliferation analysis using the CCK-8 assay indicated that overexpression of PINK1 significantly upregulated cell proliferation. The data are mean ± SEM (*P<0.05, compared with the control group). (B) Representative micrographs (upper) and quantification (lower) of cell colonies. (C) Flow cytometry analysis. The cell cycle of indicated lung cancer cells transfected the expression vector of PINK1 or empty vector. (D) Western blot analyzed the expression levels of p27, p65, and cyclin D1 when PINK1 was overexpressed. GAPDH was used as the loading control. *P<0.05. The data are mean ± SEM.

blotting was used. As shown in Fig. 5A, PINK1 protein levels markedly reduced in both A549 and H1299 cells infected with PINK1-siRNA, especially in PINK1-siRNA#2, compared with cells treated with control-siRNA cells. Thus, we used PINK1-siRNA#2 to carry out the following experiments. Since PINK1 was reported to activate the NF-κB signaling, western blot assay was performed to detect the expression of p65 which is an important member in NF-κB signaling family and cell cycle-related protein including p27 and cyclin D1. As shown in Fig. 5B, knockdown of PINK resulted in decrease of

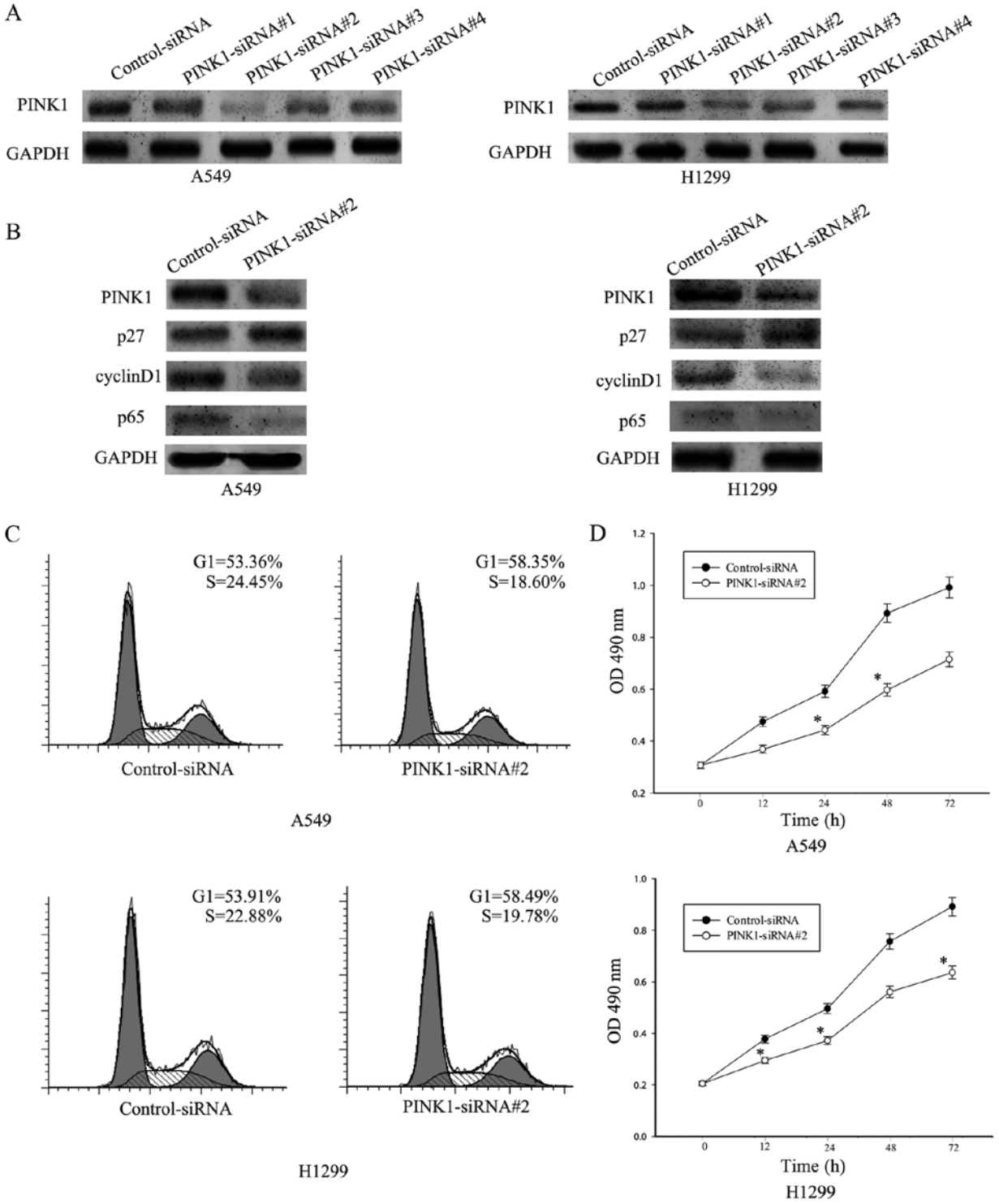

Figure 5. Knockdown of PINK1 suppressed A549 and H1299 cells proliferation. (A) PINK1 expression was detected by western blotting after transfection for 48 h with PINK1-siRNA in A549 and H1299 cells, while PINK1-siRNA#2 achieved the best effected downregulation. (B) Western blot analyzed the expression levels of p27, p65, and cyclin D1 when PINK1 was knocked down. GAPDH was used as the loading control. (C) Flow cytometric analysis showed that knockdown of PINK1 led to an increase of cell number on the G0/G1 phase and a concomitant reduction in the number of cells in the S phase, compared with control-siRNA-transfected cells. (D) Cell proliferation analysis using the CCK-8 assay indicated that PINK1-siRNA#2-transfected NSCLC cells exhibited significantly reduced cell proliferation. The data are mean ± SEM (*P<0.05, compared with the control group).

p65 and cyclin D1, with concomitant increase of p27 which is a CDK inhibitor. Thus these results indicated that PINK1 expression might promote cell cycle progression through NF-κB signaling.

Furthermore, flow cytometry analysis of cell cycle showed that A549 and H1299 cells transfected with PINK1-siRNA#2 had an increase of cell number in the G0/G1 phase from 53.36% to 58.35% and the number in the S phase decreased from 24.45% to 18.60% in A549, and from 53.91% to 58.49% in the G0/G1 phase in H1299 and from 22.88% to 19.78% in the S phase in H1299 (Fig. 5C). CCK-8 assay was used to test the effect of PINK1 on NSCLC cell growth rate. Knockdown

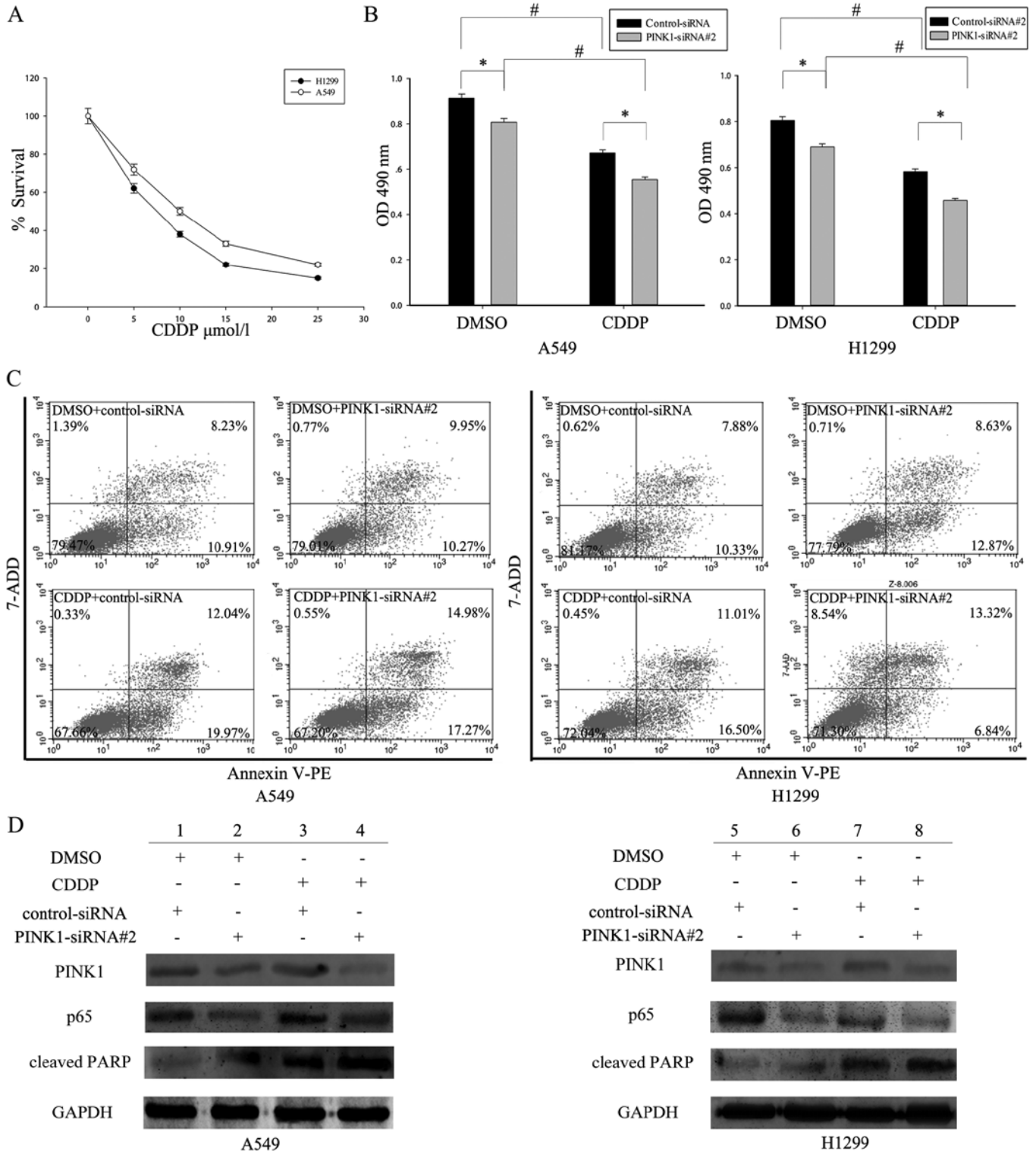

Figure 6. PINK1-mediated activation of the NF-κB pathway enhanced cell survival and drug resistance in NSCLC. (A) A549 and H1299 cells were treated with different concentrations of cisplatin (CDDP) (0, 5, 10, 15, 20, and 25 μM/l) for 48 h, and cell viability was measured using the CCK-8 assay. (B) After PINK1-SiRNA#2 transfection for 48 h, A549 and H1299 cells were treated with CDDP (25 μM/l) stimulation or not. After 24 h, the cell proliferation was determined by CCK-8 assay. *P<0.05,statistically different with the control group; $^{\#}$P<0.05, statistically different with the doxorubicin-treated group, the data are mean ± SEM. (C) Interference of PINK1 promoted CDDP-triggered cell apoptosis in A549 and H1299 cells as analyzed by Annexin V-FITC/7-ADD double staining. (D) Western blot analysis of the expression of apoptosis-related proteins in control siRNA or PINK1-siRNA#2 transfected A549 and H1299 cells treated with or without CDDP.

of PINK1 can attenuate the cell proliferation compared with cells treated with control-siRNA (Fig. 5C). Taken these results together, knockdown of PINK1 could inhibit proliferation of NSCLC cells.

Depletion of PINK1 sensitizes NSCLC cells to cisplatin (CDDP). A cytoprotective and chemoresistant function for PINK1 has been highlighted by some studies, supporting PINK1 as a target in cancer therapeutics (25). Therefore, we

investigated whether PINK1 could confer chemoresistance to *cis*-diamminedichloroplatinum (CDDP), a first-line drug for treating NSCLC patients. Firstly, CCK8 assay was performed to determine the sensitivity of NSCLC cells to CDDP. The cell proliferation rate was decreased in a dose-dependent manner, and drug sensitivity reached the highest level at the concentration of 25 μmol/l (Fig. 6A). In addition, to further confirm the contribution of PINK1 to CDDP sensitivity in NSCLC cells, CCK8 assay and flow cytometry assay were performed to demonstrate the cell viability level and apoptosis rate following PINK1 knocking down and CDDP addition. It showed that CDDP addition led to obvious decrease of cell viability and increase of cell apoptosis (Fig. 6B and C, comparing the lower panels to the upper panels).

In addition, depletion of PINK1 augmented the cytotoxic effect of CDDP in NSCLC cells (Fig. 6B and C comparing the right panels to the left panels). Moreover, to test whether PINK1-induced CDDP resistance depends on NF-κB activity, A549 and H1299 cells were treated with CDDP for 24 h following PINK1 addition. Western blot assay was then used to determine the expression of p65, as well as cleaved caspase-3 and PARP1, which are apoptosis markers. As shown in Fig. 6D, PINK1 knockdown resulted in decreased expression of p65 (compare lane 2 to 1, lane 4 to 3 in A549 cells; compare lane 6 to 5, lane 8 to 7 in H1299 cells). Consequently, PINK1-depleted NSCLC cells had much higher level of apoptosis markers compared with the control silenced cells, especially following CDDP treatment (compare lane 3 to 1, lane 4 to 2 in A549 cells; compare lane 7 to 5, lane 8 to 6 in H1299 cells). Taken together, PINK1 might enhance cell survival and drug resistance by NF-κB signaling.

Discussion

Despite great progress in diagnostic and therapeutic strategies, non-small cell lung cancer is still one of the most common causes of cancer death due to late diagnosis, high metastasis and chemoresistance. As molecular characterization of lung cancer has significantly affected treatment strategies, it is necessary to discover novel therapeutic targets that can complement present chemotherapy. Although increasing attention has been drawn toward PINK1 in a number of processes including cancer (13,14,26), whether PINK1 was involved in NSCLC carcinogenesis has yet not been clarified. In this study, we investigated the potential role of PINK1 in NSCLC development. We found that PINK1 was significantly overexpressed in NSCLC tissues and NSCLC cell lines, and correlated with clinical pathologic variables of NSCLC. Univariate and multivariate analysis indicated that PINK1 was an independent prognostic indicator for the survival of NSCLC patients. Moreover, we showed that PINK1 knockdown resulted in decreased NSCLC cell proliferation rate, colony formation ability, and increased cell cycle arrest. In addition, our results also indicated that PINK1 could induce lung cancer cell survival and chemoresistance through the nuclear factor-κB (NF-κB) pathway. Taken together, these results revealed that PINK1 might be a novel therapeutic target for NSCLC.

PINK1 [phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-induced kinase 1], a serine/threonine kinase, is widely expressed, and localizes in mitochondria and cytosol (27). PINK1 plays a role in mitochondrial homeostasis and dynamics, including bioenergetics, mitophagy, fission and fusion. The role of PINK1 in cancer biology is controversial. However, increasing attention to this kinase in regulating cell survival systems indicated that PINK1 has a potential role in tumorigenesis. Early studies indicated that PINK1 protein is highly expressed in breast, colorectal and endometrial cancer tissues. Moreover, knockdown of PINK1 inhibits proliferation, colony formation and migration (16), and increases the sensitivity of cancer cells to numerous stressors (28,29). Besides, PINK1 was tightly associated with the major oncogenic PI3-kinase/Akt axis. Recent studies showed that PINK1 can activate Akt via the mTORC2/mitochondrial control axis to enhance invasiveness in cancer cells (14), and accelerated cancer stem cell renewal through Notch signaling (30). Furthermore, loss of PINK1 may sensitize breast cancer cells to paclitaxel (18), while overexpression of PINK1 can override the sensitization and lead to chemoresistance (31,32). This highlighted PINK1 could be a novel target for chemoresistance in cancer.

NF-κB pathway plays an important role in cell proliferation, differentiation and chemoresistance in many solid cancers. The NF-κB family of transcription factors has five members, Rel, p65, RelB, p50 and p52. Once activated, the NF-κB was activated and transferred into the nucleus to maintain homeostasis. It was reported that PINK1 can bind to TRAF6 and TAK1, and finally activate NF-κB pathway, which indicated PINK1 may induce lung cancer cell survival and drug resistance by activating the NF-κB pathway. In this study, we found that the PINK1 knockdown could inhibit activation of NF-κB signaling, which consequently might promote proliferation, cell cycle progression and chemoresistance of NSCLC cells.

In summary, this study showed that PINK1 may contribute to proliferation and chemoresistance of NSCLC through NF-κB pathway. As a result, PINK1 may be a novel target for NSCLC. However, further studies are needed to clarify the precise role of PINK1 in NSCLC pathogenesis.

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