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ORIGINAL ARTICLE

# The PVT1/miR-216b/Beclin-1 regulates cisplatin sensitivity of NSCLC cells via modulating autophagy and apoptosis

**Liangfeng Chen[1] · Xiaobing Han[1] · Zhongzhou Hu[1] · Liangxin Chen[1]**

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## Abstract

**Purpose** The efficacy of cisplatin-based chemotherapy remains an open question for chemo-resistance in non-small cell lung cancer (NSCLC). This study aimed to explore the role and mechanism of long noncoding RNA plasmacytoma variant translocation 1 (PVT1) in cisplatin sensitivity of NSCLC.

**Methods** Paired tumor and adjacent tissues were collected from forty patients with NSCLC. The clinical value of PVT1 was investigated according to clinicopathological parameters of patients. Cisplatin-sensitive or -resistant cells (A549 or A549/DDP) were used for *in vitro* experiments. Cell viability, apoptosis, autophagy and animal experiments were conducted to investigate cisplatin sensitivity. The expressions of PVT1, microRNA-216b (miR-216b) and apoptosis- or autophagy-related proteins were measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) or western blot assay, respectively. Luciferase reporter assay and RNA immunoprecipitation (RIP) assay were conducted to probe the interaction between miR-216b and PVT1 or Beclin-1.

**Results** PVT1 was highly expressed and associated with poor prognosis of NSCLC patients ($^{*}P<0.05$). PVT1 knockdown enhanced cisplatin-induced viability inhibition and apoptosis induction in A549/DDP cells, but addition of PVT1 caused an opposite effect in A549 cells ($^{*}P<0.05$, $^{\#}P<0.05$). Moreover, accumulation of PVT1 facilitated autophagy of NSCLC cells and tumor growth in vivo ($^{*}P<0.05$, $^{\#}P<0.05$). In addition, miR-216b interacted with PVT1 or Beclin-1. Beclin-1 reversed miR-216b-mediated effect on autophagy and apoptosis of NSCLC cells ($^{*}P<0.05$,$^{\#}P<0.05$). Besides, Beclin-1 protein expression was regulated by PVT1 and miR-216b ($^{*}P<0.05$, $^{\#}P<0.05$).

**Conclusions** PVT1 may function as a competing endogenous RNA for miR-216b to inhibit cisplatin sensitivity of NSCLC through regulating apoptosis and autophagy via miR-216b/Beclin-1 pathway, providing a novel target for improving chemotherapy efficacy of NSCLC.

**Keywords** PVT1 · NSCLC · Cisplatin sensitivity · Autophagy · miR-216b · Beclin-1

## Introduction

Lung cancer is the predominant form of tumor malignancy with approximately 18% 5-year survival rate because of high metastasis [1]. Non-small lung cancer (NSCLC) accounts for more than 80% of lung cancer [2]. Chemotherapy is one of the main strategies for NSCLC treatment. Cisplatin-based chemotherapy is widely used for NSCLC treatment, but later the efficiency is unsatisfactory for the development of resistance [3]. To date, the mechanism that underlies cisplatin resistance in NSCLC is not fully known, thus, enhancing cisplatin sensitivity shows a great promise in NSCLC treatment.

Emerging finding has highlighted the interaction of long noncoding RNAs (lncRNAs) with development of cisplatin resistance [4]. LncRNAs are reported to play vital roles in cancer progression, metastasis, prognosis and drug resistance in NSCLC [5]. LncRNA human plasmacytoma variant translocation1 (PVT1), a promising lncRNA, is associated with cisplatin resistance via modulating apoptotic pathways in ovarian cancer cells [6]. Besides, enrichment of PVT1 promotes development of cisplatin resistance in gastric and colorectal cancer [7, 8]. Moreover, PVT1 has been suggested to be implicated in the poor prognosis of patients with NSCLC and its depletion promotes radio-sensitivity through

✉ Liangfeng Chen
chenliangfeng2019@163.com

[1] Department of Oncology, Xinyang Central Hospital, No. 1 Siyi Road, Shihe District, Xinyang 464000, China

regulating miR-195 expression [9]. However, little is known about PVT1 addressing cisplatin sensitivity of NSCLC.

Functional lncRNA is known to serve as a competing endogenous RNA (ceRNA) to regulate microRNAs (miRNAs) and target mRNA [10]. MiRNAs are one class of small noncoding RNAs with a length of 18–25 nucleotides and play essential roles in cell proliferation, apoptosis, epithelial–mesenchymal transition as well as chemo-resistance in NSCLC [11]. The available evidence indicates that miR-216b regulates cell proliferation and invasion via modulating sex determining region Y-box 9 in NSCLC [12]. Moreover, miR-216b has been suggested to enhance cisplatin sensitivity of ovarian cancer, revealed by reducing cell viability and promoting apoptosis [13]. However, the effect of miR-216b on cisplatin sensitivity and its mechanism are largely unknown.

Cisplatin sensitivity is associated with drug uptake or efflux, DNA repair, apoptosis and autophagy [4]. Beclin-1, a biomarker of autophagy, has an important impact on cisplatin sensitivity of anaplastic thyroid carcinoma [14]. Moreover, Beclin-1 has been reported to be related with malignancy and poor clinical outcome in NSCLC [15]. In this study, we recruited NSCLC patients with cisplatin-based chemotherapy and investigated the clinical significance of PVT1 in patients and its effect on cisplatin sensitivity in A549 and resistant A549/DDP cells. Moreover, we analyzed whether it was associated with apoptosis and autophagy and explored the interaction among PVT1, miR-216b and Beclin-1.

**Table 1** Correlation between lncRNA PVT1 expression and clinical features ($n=40$)

| Clinical features | Number | PVT1 expression | | *P* value |
|---|---|---|---|---|
| | | Low *n* (%) | High *n* (%) | |
| Age (years) | | | | |
| < 65 | 24 | 14 (58.33%) | 10 (41.67%) | 0.8961 |
| ≥ 65 | 16 | 9 (56.25%) | 7 (43.75%) | |
| Gender | | | | |
| Female | 11 | 6 (54.54%) | 5 (45.46%) | 0.8159 |
| Male | 29 | 17 (58.62%) | 12 (41.38%) | |
| Tumor size | | | | |
| > 5 cm | 14 | 4 (35.71%) | 10 (64.29%) | 0.0408* |
| ≤ 5 cm | 26 | 19 (69.23%) | 7 (30.73%) | |
| Lymph node metastasis | | | | |
| Negative | 23 | 17 (73.91%) | 6 (26.09%) | 0.0146* |
| Positive | 17 | 6 (35.29%) | 11 (64.71%) | |
| TNM stage | | | | |
| Ia + Ib | 17 | 13 (76.47%) | 4 (23.53%) | 0.01* |
| IIa + IIb | 14 | 8 (57.14%) | 6 (42.86%) | |
| IIIa | 9 | 2 (22.22%) | 7 (77.78%) | |
| Smoking history | | | | 0.6287 |
| Smokers | 29 | 16 (55.17%) | 13 (44.83%) | |
| Never smokers | 11 | 7 (63.64%) | 4 (36.36%) | |

## Materials and methods

### Patients and tissues

The study was accepted by the Institutional Research Ethics Committee of Xinyang Central Hospital and written informed consent was obtained from all patients with NSCLC. In this study, NSCLC samples and adjacent normal tissues collected from 40 patients who suffered from the cisplatin-based chemotherapy after surgical treatment. The patients were divided into cisplatin-sensitive or -resistant group according to the response evaluation criteria. All samples were snap frozen in liquid nitrogen immediately and stored at − 80 °C until required. The clinicopathological parameters of patients are shown in Table 1. The survival rates of all patients were analyzed after followed up for 5 years.

### Cell culture and treatment

293T, the normal bronchial epithelial cell line 16HBE and human NSCLC cell lines (A549 and cisplatin resistance A549/DDP) were obtained from American Tissue Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured at 37 °C in 5% $CO_2$ using RMPI 1640 culture medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (Gibco), 1% penicillin and streptomycin (Invitrogen, Carlsbad, CA, USA) during the study. For drug-resistant phenotype, A549/DDP cells were maintained in medium with 1 μg/mL of cisplatin (Sigma, St. Louis, MO, USA).

Short hairpin PVT1 (shPVT1), scrambled control, PVT1-overexpression lentiviral vector (PVT1), miR-216b mimic (miR-216b), miR-216b inhibitor (anti-miR-216b), corresponding negative controls (NC or anti-NC), Beclin-1-overexpression plasmid (Beclin-1), and siRNA against Beclin-1 (siBeclin-1) were obtained from Genepharma (Shanghai, China). Transient transfection with the oligonucleotides or plasmids into A549 and A549/DDP cells was conducted using Lipofectamine 2000 (Invitrogen). Cells were collected for further researches at 48 h after the post-transfection.

For treatment of cisplatin, A549 or A549/DDP cells were cultured with medium supplemented with indicated concentrations (2–16 μg/mL) of cisplatin for further cisplatin sensitivity assay.

### Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA from tissues or cells was extracted using Trizol reagent (Invitrogen) following the manufacturer's instructions. Subsequently, 500 ng of total RNA was used for cDNA synthesis with TaqMan Reverse Transcription Kit or TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). qRT-PCR was carried out using SYBR green (Toyobo, Tokyo, Japan) detection following the amplification instructions. All primers were obtained from Invitrogen: PVT1 (Forward, 5′-CAGCACTCT GGACGGAC-3′; Reverse, 5′-CAACAGGAGAA GCAAAC A-3′), GAPDH (Forward, 5′-TGAACTGAAAGCTCTCCA CC-3′; Reverse, 5′-CTGATGTACCAGTTGGGGAA-3′), miR-216b (Forward, 5′-GCCGCGCTAA AGTGCTTAT AGTG-3′; Reverse, 5′-CACCAGGGTCCGAGGT-3′), U6 (Forward, 5′-GCTTCGGCAGCACATATACTAAAAT-3′; Reverse, 5′-CGCTTCACGAATT TGCGTGTCAT-3′). The data were evaluated with $2^{-\Delta\Delta Ct}$ method and GAPDH or U6 small RNA was regarded as housekeeping gene for normalization of PVT1 or miR-216b, respectively.

### MTT assay

Cell viability was assessed using MTT assay. Transfected A549 and A549/DDP cells were seeded into 96-well plates with $1 \times 10^4$ cells per well and incubated with different concentrations (2–16 μg/mL) of cisplatin for 24 h. Then, cell medium was changed with fresh medium containing 0.5 mg/mL of MTT (Sigma) and incubated for 4 h at 37 °C. Following the removal of the supernatant, dimethyl sulfoxide (DMSO) was added to dissolve formazan. The absorbance at 490 nm was measured with a microplate reader (Bio-Rad, Hercules, CA, USA).

### Flow cytometry

Annexin V-FITC/PI staining (Sigma) was used to investigate cell apoptosis by flow cytometry. After the incubation of 8 μg/mL of cisplatin for 24 h, transfected A549 or A549/DDP cells were resuspended in binding buffer. Then, Annexin V-FITC and PI were introduced into cell suspension for 20 min without light. The positive cells were detected using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

### Western blot

Total proteins were prepared in cell lysis buffer (Thermo Fisher, Wilmington, DE, USA) containing 1% protease inhibitor and quantified by bicinchoninic acid protein assay kit (Sigma) according to the instructions. Denatured samples were loaded onto SDS-PAGE gel and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Following the block in 5% non-fat milk for 2 h at 37 °C, membranes were incubated overnight at 4 °C with primary antibodies against LC3-I, LC3-II, Beclin-1, p62, BCL2, Bax, or β-actin (Abcam, Cambridge, UK). β-actin was used as housekeeping protein in this study. After washing, the membranes were incubated with HRP-conjugated secondary antibodies (Abcam) for 2 h at 37 °C. The enhanced chemiluminescence chromogenic substrate (GE Healthcare, Amersham, UK) was used for visualization of protein bands and densitometry analysis was performed with Image Lab software (Bio-Rad).

### Animal experiment

Every animal effort was performed in accordance with the Guidelines for Care and Use of Laboratory Animal approved by the Ethics Committee of Xinyang Central Hospital during the study. SPF BALB/c nude mice (male, 4-week-old) were obtained from Vital River Laboratory Animal Technology (Beijing, China) with free access to water and food. After hosing in specific pathogen-free microisolator cages with a 12 h light/dark cycle for 1 week, the acclimatized mice were treated with A549 cells stably transfected with PVT1-overexpression lentiviral vector (PVT1) or negative vector via subcutaneous injection. Tumor volume was calculated by $(\text{length} \times \text{width}^2)/2$. Once the size reached 60 $mm^3$, mice were insulted with cisplatin (5 mg/kg) or normal saline by intraperitoneal injection twice per week for 3 weeks. Tumor size was measured every week until six weeks after cell implantation. Reaching end points, mice were killed and tumor specimens were collected for weighting and detecting apoptotic-related protein expressions.

### Subcellular fraction assay

The separation of cytoplasmic and nuclear fractions was conducted using Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher) following the manufacturer's protocols. Total RNA was extracted from each fraction for qRT-PCR assay as above description. The relative expression of PVT1 in cytoplasmic and nuclear fractions was analyzed using GAPDH or U6 as cytoplasmic or nuclear control, respectively.

### Luciferase reporter assay

Online software miRcode was used to probe the putative binding sites of miR-216b with PVT1. The 3′ untranslated regions (3′-UTR) sequences of PVT1 containing wild or mutant seed sequences of miR-216b were amplified and cloned into pGL3 luciferase reporter vector (Promega,

Madison, WI, USA) to generate the wild-type (Wt) luciferase reporter plasmid (PVT1-Wt) or mutant (Mut) plasmid (PVT1-Mut), respectively. Wt or Mut luciferase reporter plasmid was co-transfected with miR-216b mimic or NC in 293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocols. Then, lysed cells were exploited for luciferase activities analysis using Dual-Luciferase Assay Kit (GeneCopoeia, Rockville, MD, USA) after 48 h. The 3′-UTR sequences of Beclin-1 containing Wt or Mut miR-216b binding sites predicted by TargetScan and MiRanda were cloned into pGL3 luciferase reporter vector. Then, the interaction between miR-216b and Beclin-1 was probed in A549 and A549/DDP cells as mentioned above.

### RNA immunoprecipitation (RIP)

Argonaute 2 (Ago2) RIP was performed to probe the link between PVT1 and miR-216b using Magna RIP Kit (Millipore) following the protocol. In brief, treated A549/DDP cells were lysed and added to magnetic beads (Thermo Fisher) bounded with anti Ago2 (CST) or IgG antibody. Following the washes with PBS, the RNA in beads complexes was isolated and detected by qRT-PCR with a positive control (input).

### Statistical analysis

Data were presented as the mean ± standard deviation (S.D.) from three independent experiments. The survival curve of patients was generated by Kaplan–Meier methods and analyzed via Log-rank test. The association between clinical features and PVT1 level was analyzed by Fisher's test. The differences were evaluated by Student's *t* test or one-way ANOVA using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). *P* values less than 0.05 were considered to be statistically significant.

## Results

### PVT1 is significantly upregulated and associated with the poor prognosis in NSCLC

To investigate the potential role of PVT1 in NSCLC, we first detected the expression of PVT1 in NSCLC patient tissues. The data of qRT-PCR assay revealed that the abundance of PVT1 was significantly elevated in cancer tissues as compared with that in normal samples and cisplatin-resistant group showed higher expression of PVT1 than sensitive group (Fig. 1a). To explore the clinical value of PVT1, the patients were divided into low PVT1 expression group ($n = 23$) and high PVT1 expression group ($n = 17$) according to the mean level of PVT1. Table 1 summarized that high expression of PVT1 was associated with tumor size, lymph node metastasis, and TNM stage ($P < 0.05$) but not with age, gender and smoking history ($P > 0.05$). Moreover, survival curve analysis suggested that high level of PVT1 displayed lower survival rate in NSCLC patients (Fig. 1b). In addition, the expression of PVT1 was also examined in NSCLC cells and normal bronchial epithelial cell line 16HBE. Results showed that the abundance of PVT1 was significantly enhanced in A549 cells as compared with that in 16HBE and cisplatin-resistant A549/DDP cells exhibited higher PVT1 level (Fig. 1c).

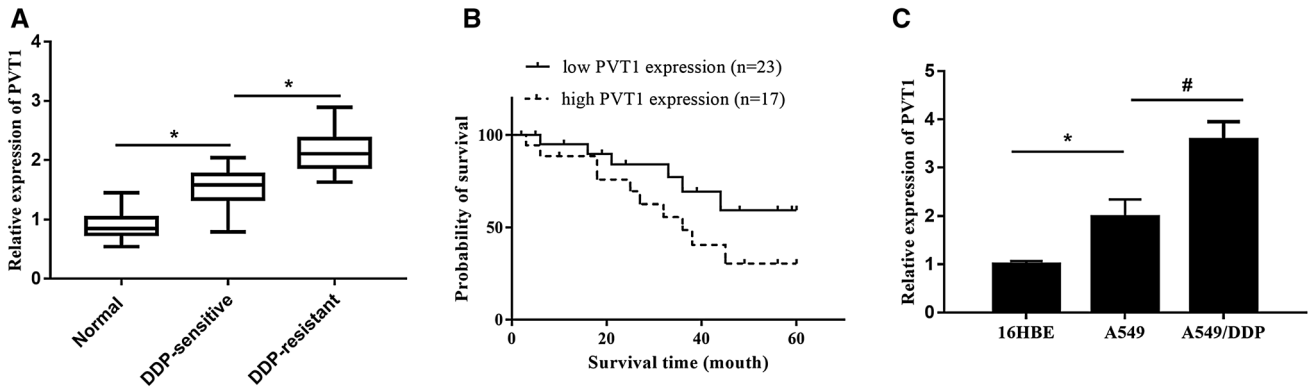



**Fig. 1** PVT1 expression is enhanced in NSCLC-sensitive or -resistant tissues and cells. **a** The expression of PVT1 was detected in NSCLC samples and para-tumor normal tissues by qRT-PCR. **b** All patients who underwent cisplatin-based chemotherapy were followed up for 5 years, and survival probability was evaluated using Kaplan–Meier survival assay. **c** The abundance of PVT1 in A549 and A549/DDP cells was evaluated by qRT-PCR. $^{*}P < 0.05$, $^{\#}P < 0.05$

### PVT1 inhibits cisplatin sensitivity by regulating apoptosis and autophagy in NSCLC cells

To explore the interaction between PVT1 and cisplatin sensitivity, A549/DDP cells were transfected with shPVT1 or scrambled and A549 cells were transfected with PVT1 overexpression vector or empty vector for 24 h. The transfection efficacy was validated by qRT-PCR assay (Fig. 2a, d). Subsequently, cells were exposed with indicated concentrations of cisplatin for 24 h. MTT assay showed that cisplatin insult led to obvious reduction of cell viability, which was aggravated by knockdown of PVT1 in A549/DDP cells, but was attenuated by overexpression of PVT1 in A549 cells (Fig. 2b, e). Moreover, the data of flow cytometry demonstrated that depletion of PVT1 worsened cisplatin-induced cell apoptosis but addition of PVT1 caused an opposite effect (Fig. 2c, f). Meanwhile, the effect of PVT1 on autophagy was also investigated in the two cells. Western blot results exhibited that PVT1 abrogation decreased the ratio of LC3-II/LC3-I and Beclin-1 protein abundance but enhanced p62 protein level in A549/DDP cells compare with scrambled control group (Fig. 2g). However, enrichment of PVT1 played opposing roles in A549 cells (Fig. 2h).

### PVT1 suppresses cisplatin sensitivity in vivo

To further investigate the effect of PVT1 on cisplatin resistance in vivo, BABL/c nude mice were injected subcutaneously with A549 cells stably transfected with PVT1 overexpression lentiviral vector treatment or vector control and treated with cisplatin at indicated time point. Tumor volume and weight were obviously decreased after cisplatin treatment compared with those in control group. However, addition of PVT1 weakened the suppressive effect of cisplatin on tumor growth (Fig. 3a, b). Subsequently, tumor tissues were homogenized for further molecular analysis. Western blot assay showed that anti-apoptotic protein BCL2 expression was significantly decreased and pro-apoptotic protein Bax level was increased in cisplatin-treated group compared with those in control group, which was reversed by the introduction of PVT1 (Fig. 3c).

### MiR-216b is bound to PVT1

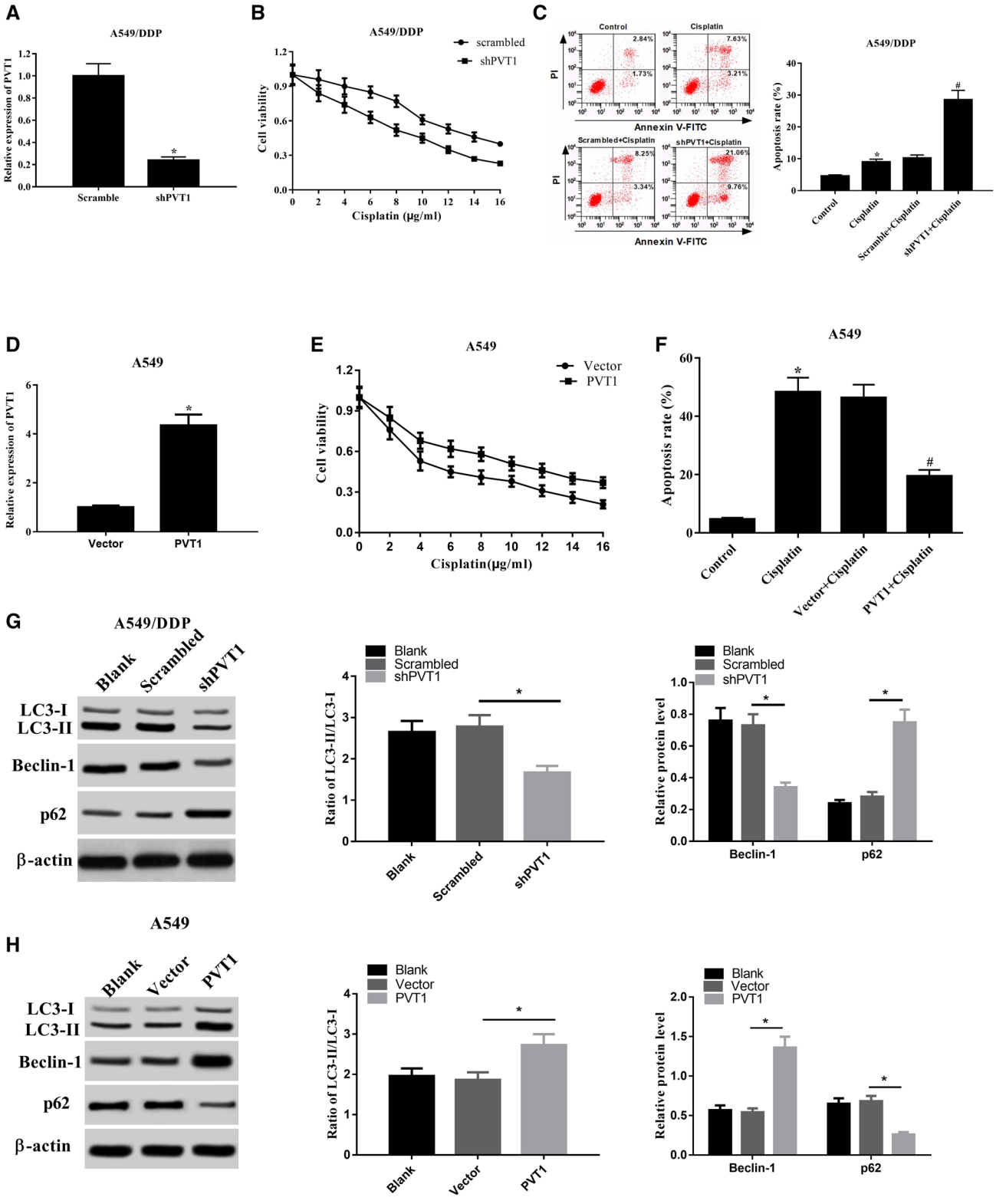
To explore the potential mechanism, subcellular fraction assay was conducted to measure the percentage of PVT1 in the cytoplasmic and nuclear fractions of A549 and A549/DDP cells. qRT-PCR assay uncovered that PVT1 could be located in cytoplasm of two cells, indicating prerequisite for function as a ceRNA in NSCLC (Fig. 4a, b). Intriguingly, bioinformatics analysis provided potential binding sites of miR-216b within the 3′-UTR sequences of PVT1 using miRcode, uncovering that miR-216b expression might be mediated via PVT1 (Fig. 4c). Hence, luciferase activity and Ago2 RIP assay were performed to validate the prediction. Luciferase reporter assay displayed that transfection of miR-216b in 293T cells markedly suppressed the luciferase activity of PVT1-Wt reporter compared with that in NC group, whereas no significant change was observed in PVT1-Mut group (Fig. 4d). Moreover, RIP assay showed that the abundances of PVT1 and miR-216b were higher in anti-Ago2 group than those in anti-IgG group (Fig. 4e). To investigate the effect of PVT1 on miR-216b, cells were transfected with shPVT1 or PVT1 overexpression lentiviral vector. Results showed elevated miR-216b level in A549/DDP cells with shPVT1 transfection and impaired expression in A549 cells with PVT1 overexpression compared with those in corresponding control, respectively (Fig. 4f, g).

### miR-216b enhances cisplatin sensitivity by targeting Beclin-1 in NSCLC cells

Functional miRNA was realized through regulating target gene expression. Hence, one promising gene targeted by miR-216b was needed in this study. TargetScan and MiRanda online predicted the putative binding sites of miR-216b and Beclin-1 (Fig. 5a). The expression of miR-216b was detected and result showed reduced level of miR-216b in NSCLC cells, especially in A549/DDP cells, compared with that in normal cell line (Fig. 5b). Furthermore, luciferase reporter assay described that miR-216b addition induced a strong loss of luciferase activity in A549/DDP cells and its knockdown led to a great increase of luciferase activity in A549 cells with the presence of Beclin-1-Wt reporter plasmid compared with their counterparts, but both of them caused no effect in cells in Beclin-1-Mut group (Fig. 5c). To explore the effect of miR-216b on cisplatin sensitivity, cells were transfected with miR-216b mimic or anti-miR-216b and their negative control. The results of western blot and flow cytometry presented that overexpression of miR-216b inhibited Beclin-1 expression and ratio of LC3-II/LC3-I, but increased p62 level and apoptotic rate in A549/DDP cells, while the opposite was occurred in miR-216-deficiency A549 cells (Fig. 5d–g). To explored whether miR-216b-mediated influence was modulated by Beclin-1, A549/DDP cells were co-transfected with miR-216b mimic and Beclin-1 overexpression vector and A549 cells were co-transfected with anti-miR-216b and siBeclin-1. Results showed that Beclin-1 restoration or silence reversed the effect of miR-216b overexpression or knockdown in the two cells (Fig. 5d–g).

### Beclin is regulated by PVT1 and miR-216b

To further investigate the mechanism of PVT1 as a ceRNA, the effect of PVT1 and miR-216b on Beclin-1 expression


A
A549/DDP
Relative expression of PVT1
Scramble
shPVT1
B
A549/DDP
scrambled
shPVT1
Cell viability
Cisplatin (μg/ml)
C
Control
Cisplatin
Scrambled+Cisplatin
shPVT1+Cisplatin
PI
Annexin V-FITC
2.84%
1.73%
7.63%
3.21%
8.25%
3.34%
21.06%
9.76%
A549/DDP
Apoptosis rate (%)
D
A549
Relative expression of PVT1
Vector
PVT1
E
A549
Vector
PVT1
Cell viability
Cisplatin(μg/ml)
F
A549
Apoptosis rate (%)
Control
Cisplatin
Vector+Cisplatin
PVT1+Cisplatin
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Ratio of LC3-II/LC3-I
Relative protein level

◂**Fig. 2** Knockdown of PVT1 inhibits cisplatin resistance of A549/DDP cells by modulating apoptosis and autophagy. **a** The change of PVT1 expression was detected in A549/DDP cells after transfection of shPVT1 or scrambled by qRT-PCR. **b** The effect of PVT1 knockdown on cell viability was investigated in A549/DDP cells with shPVT1 transfection and cisplatin treatment by MTT assay. **c** Cell apoptosis was detected in A549/DDP cells with shPVT1 transfection and cisplatin treatment by flow cytometry. **d** Altered expression of PVT1 was detected in A549 cells with PVT1-overexpression lentiviral vector or vector transfection by qRT-PCR. **e** Addition of PVT1 regulated A549 cells viability in response to varying concentrations of cisplatin by MTT assay. **f** The effect of PVT1 and cisplatin on cell apoptosis was evaluated in A549 cells by flow cytometry. **g** Inhibitory effect of PVT1 depletion on autophagy-related proteins was evaluated by western blot in A549/DDP cells. **h** The abundances of autophagy-related proteins were detected in A549 cells by western blot. $^{*}P<0.05$, $^{\#}P<0.05$

was evaluated in A549-sensitive and -resistant cells. Western blot data revealed that PVT1 interference markedly resulted in down-regulation of Beclin-1 protein level in the A549/DDP cells, which was alleviated by miR-216b depletion (Fig. 6a). PVT1 overexpression induced up-regulation of Beclin-1 protein level in A549 cells, while the stimulatory effect was abolished by miR-216b addition (Fig. 6b), suggesting that PVT1 might be bound to miR-216b via serving as a competing endogenous RNA (ceRNA) of Beclin-1.

## Discussion

The chemo-resistance has been a great challenge for chemotherapy in human cancers. Desired strategies of enhancing drug sensitivity are required to improve the outcome of NSCLC patients. Cisplatin-based chemotherapy is widely used for NSCLC treatment and lncRNAs play essential roles in cancer progression and drug resistance in NSCLC [5]. In the current study, PVT1 abundance was up-regulated in NSCLC tissues and cells, which is also in agreement with former work [16]. Moreover, we found that cisplatin-resistant tissues or cells displayed higher PVT1 level than sensitive group, suggesting that PVT1 might play an essential role in cisplatin sensitivity of NSCLC. However, the exact role of PVT1 and its underlying mechanism are unclear. This study first investigated the suppressive role of PVT1 in cisplatin sensitivity in NSCLC and first validated the interaction between PVT1 and miR-216b in NSCLC cells.

To explore the clinical value of PVT1, the patients were classified as low or high expression of PVT1 group and data showed that high expression of PVT1 was positively correlated with patients' features, such as tumor size, lymph node metastasis, TNM stage, cisplatin sensitivity and poor survival. These findings suggested that PVT1 could serve as an

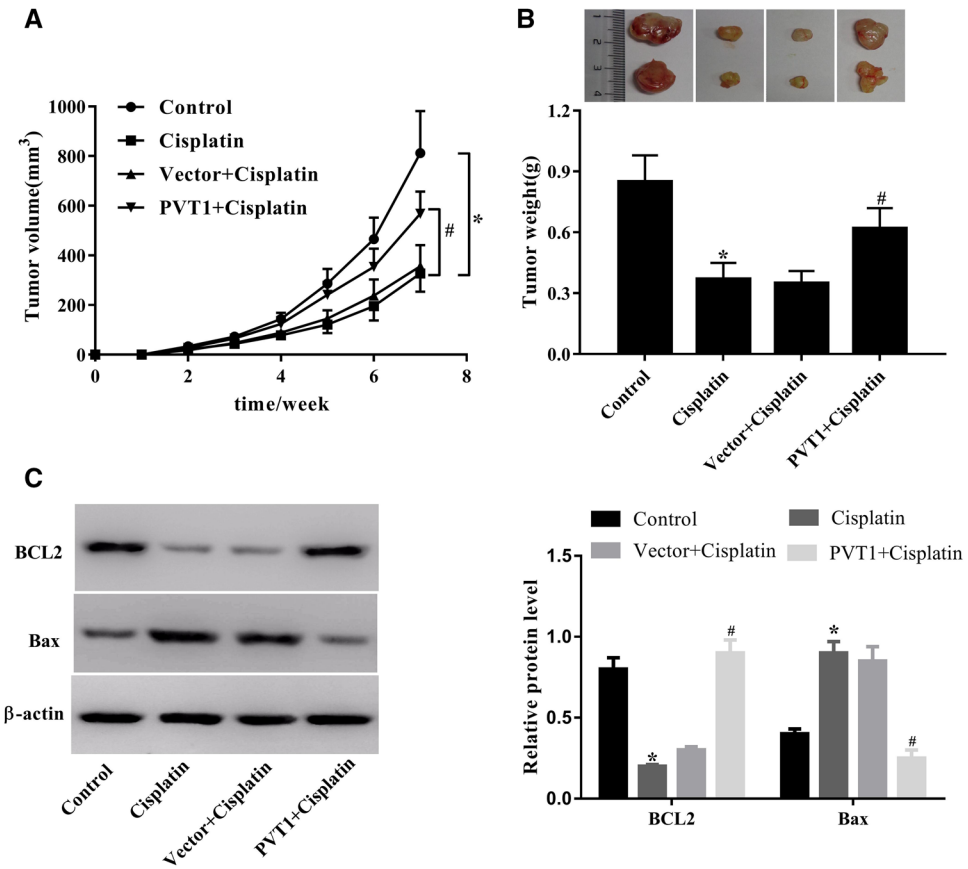


**Fig. 3** Addition of PVT1 enhances cisplatin resistance in vivo. **a** Tumor volume was measured every week after cell injection. **b** Tumor weight was detected at end point. **c** The levels of apoptosis-related proteins BCL2 and Bax were measured by western blot. $^{*}P<0.05$, $^{\#}P<0.05$

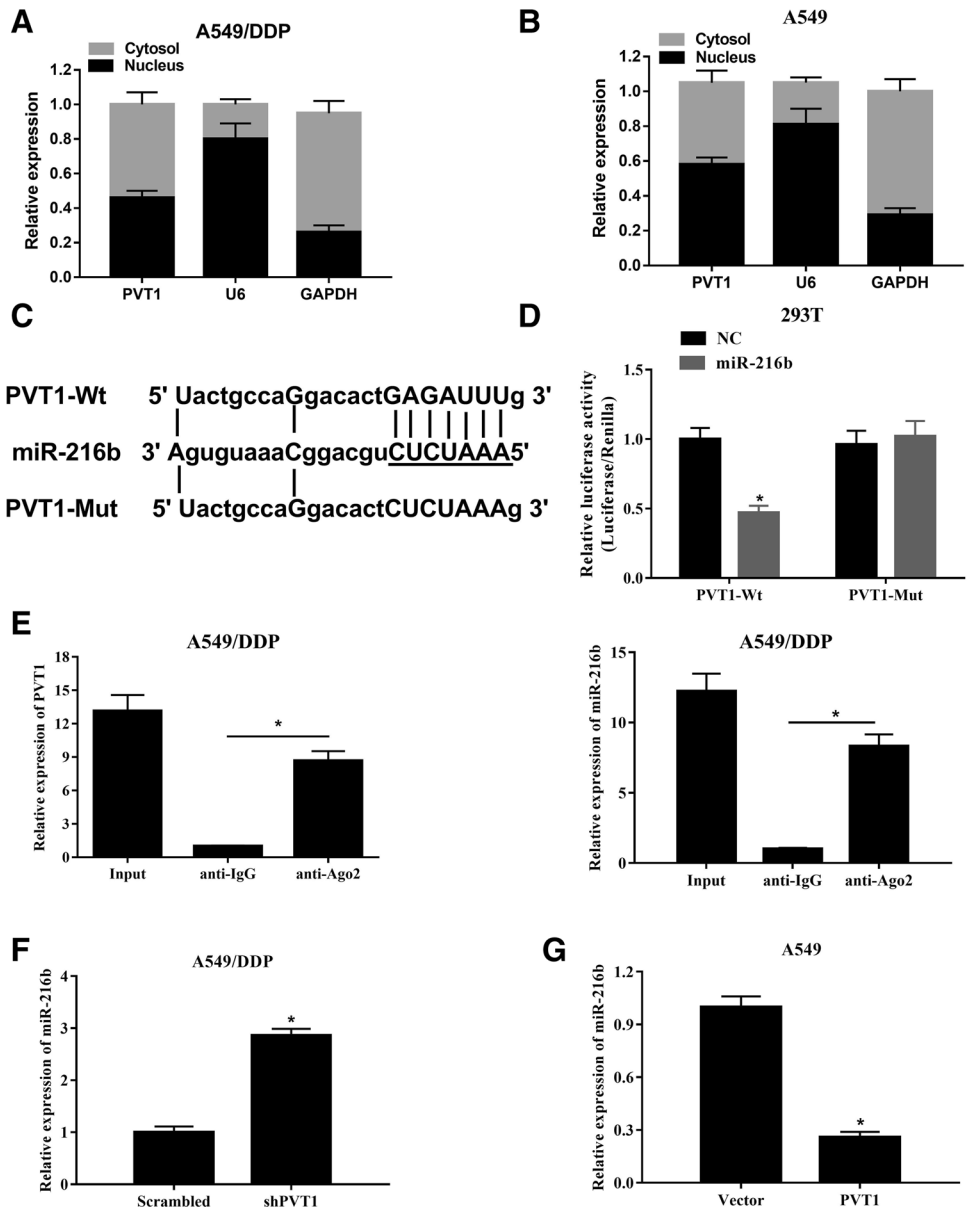


**Fig. 4** PVT1 is bound to miR-216b. **a, b** The percentage of PVT1 in the cytoplasmic and nuclear fractions was measured in A549/DDP and A549 cells by subcellular fraction assay. **c** Putative targeting sequences of PVT1 and miR-216b were predicted by miRcode. **d** Analysis of luciferase activity was performed in 293T cells co-transfected by miR-216b mimic or NC and PVT1-Wt or PVT1-Mut luciferase reporter plasmid. **e** PVT1 and miR-216b levels in the immunoprecipitates were examined by RIP analysis and qRT-PCR. **f, g** The expression of miR-216b was detected in A549/DDP cells transfected with shPVT1 or A549 cells transfected with PVT1 by qRT-PCR. $^{*}P < 0.05$

important predictor for prognosis of NSCLC patients. PVT1 has been reported to serve as a promoter of chemo-resistance in many cancers [6–8, 17]. Here, we also found this efficacy of PVT1, revealed by which knockdown of PVT1 contributed to cisplatin-induced viability inhibition, but its overexpression delayed that. Apoptosis and autophagy are known as two key processes controlling cell survival or death. Cell apoptosis and autophagy influence cell homeostasis and clinical therapeutics, but autophagy could exhibit a positive or negative role in regulating apoptosis in NSCLC [18, 19]. Zhang et al. reported that PVT1 contributed to the development of cisplatin resistance by anti-apoptosis in gastric cancer [7]. In this paper, silence of PVT1 promoted cisplatin-induced apoptosis in A549/DDP cells, while its addition attenuated apoptosis of A549 cells, indicating that the anti-apoptotic of PVT1 might facilitate cisplatin resistance in NSCLC. Moreover, autophagy was reported to delay apoptosis and could improve chemo-resistance [20, 21]. Here, we demonstrated that PVT1 knockdown suppressed autophagy in A549/DDP cells, while PVT1 overexpression promoted autophagy in A549 cells. These findings indicated that PVT1 might increase cisplatin resistance by promoting autophagy which maintained cell homeostasis to make cell survival under adverse conditions.

One of the main mechanisms of lncRNAs is known as a ceRNA by sponging to miRNAs to modulate the derepression of these miRNA targets via post-transcriptional regulation in human cancers [22]. The location of PVT1 in cytoplasm supported the potential of PVT1 as a ceRNA in NSCLC cells. However, there is no evidence in support of

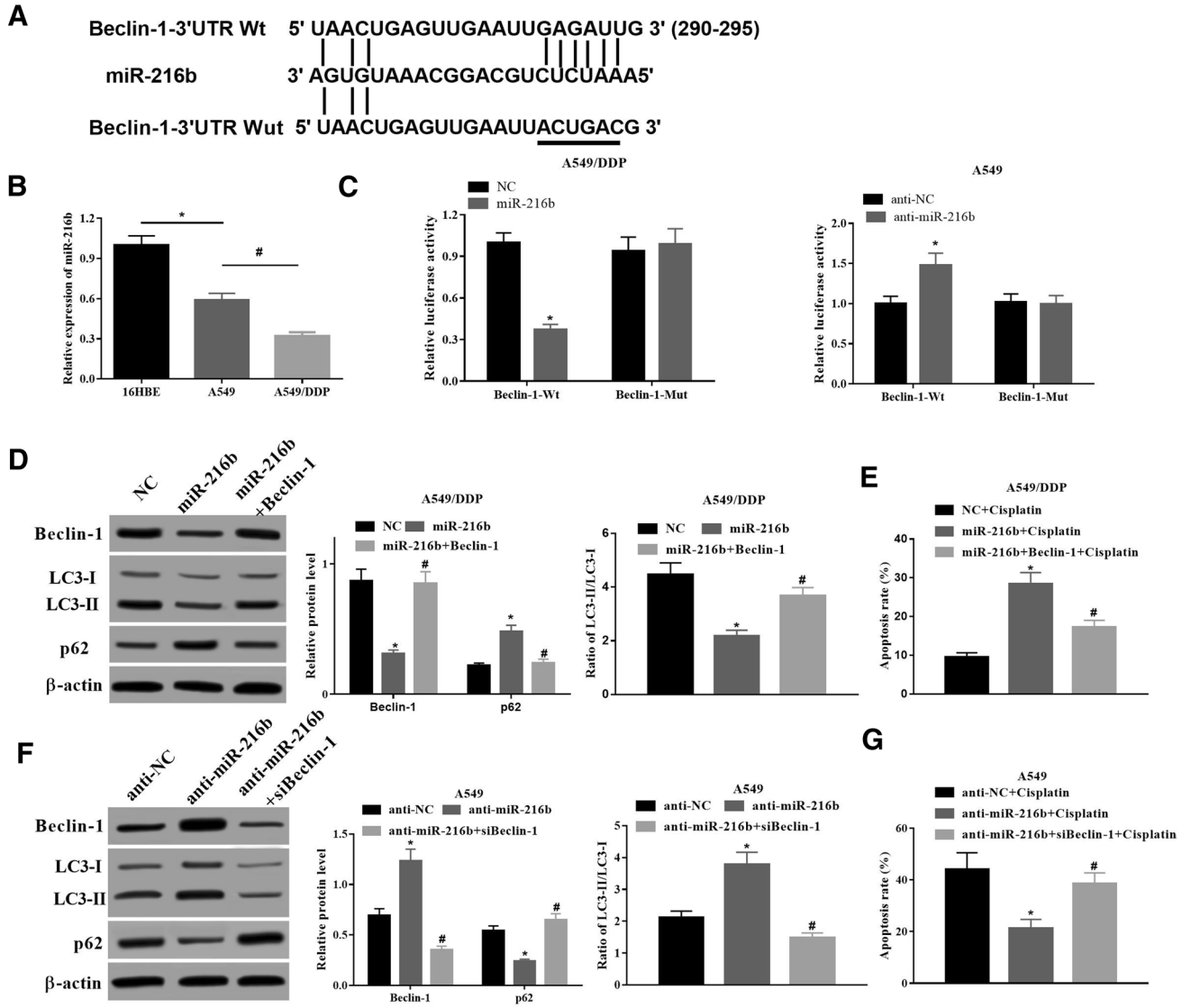


**Fig. 5** miR-216b inhibits cisplatin resistance in NSCLC cells by directly targeting Beclin-1. **a** Putative miR-216b targeting sequences of 3′-UTR of Beclin-1 were predicted by TargetScan and MiRanda. **b** The expression of miR-216b was detected in NSCLC cells by qRT-PCR. **c** Analysis of luciferase activity was performed in miR-216b-transfected A549/DDP cells or anti-miR-216b-transfected A549 cells. **d, e** The effect of miR-216b or Beclin-1 on autophagy-related proteins expression or apoptosis was investigated in A549/DDP cells transfected with NC, miR-216b, miR-216b and Beclin-1. **f, g** The expressions of autophagy-related protein or apoptosis were investigated in A549 cells transfected with anti-NC, anti-miR-216b, anti-miR-216b and siBeclin-1. $^{*}P < 0.05$, $^{\#}P < 0.05$

the interaction between PVT1 and miR-216b. In the present study, we first provided that PVT1 could sponge miR-216 by luciferase reporter and RIP assays. Furthermore, miR-216b overexpression inhibited autophagy but promoted cisplatin-induced apoptosis, suggesting miR-216b as a sensitizer of cisplatin, which is also consistent with former efforts which revealed that miR-216b could trigger drug sensitivity by regulating autophagy and apoptosis [23, 24]. Previous studies demonstrated that PVT1 or miR-216b could address autophagy by regulating Beclin-1 [25, 26]. Additionally, Beclin-1 was suggested to contribute to cisplatin resistance in cancer cells, including NSCLC [27, 28]. In this study, we found that up-regulation of Beclin-1 reversed miR-216b-mediated promotion of cisplatin sensitivity, reflecting that miR-216b played an important role in enhancing cisplatin sensitivity by targeting Beclin-1. Consistent to miR-216b, knockdown of PVT1 could suppress the target of miR-216b (Beclin-1) expression, and it was abated by miR-216b deficiency, indicating that PVT1 might function as a ceRNA for miR-216b to regulate Beclin-1 expression in NSCLC cells. Since the processes of autophagy and apoptosis are varying

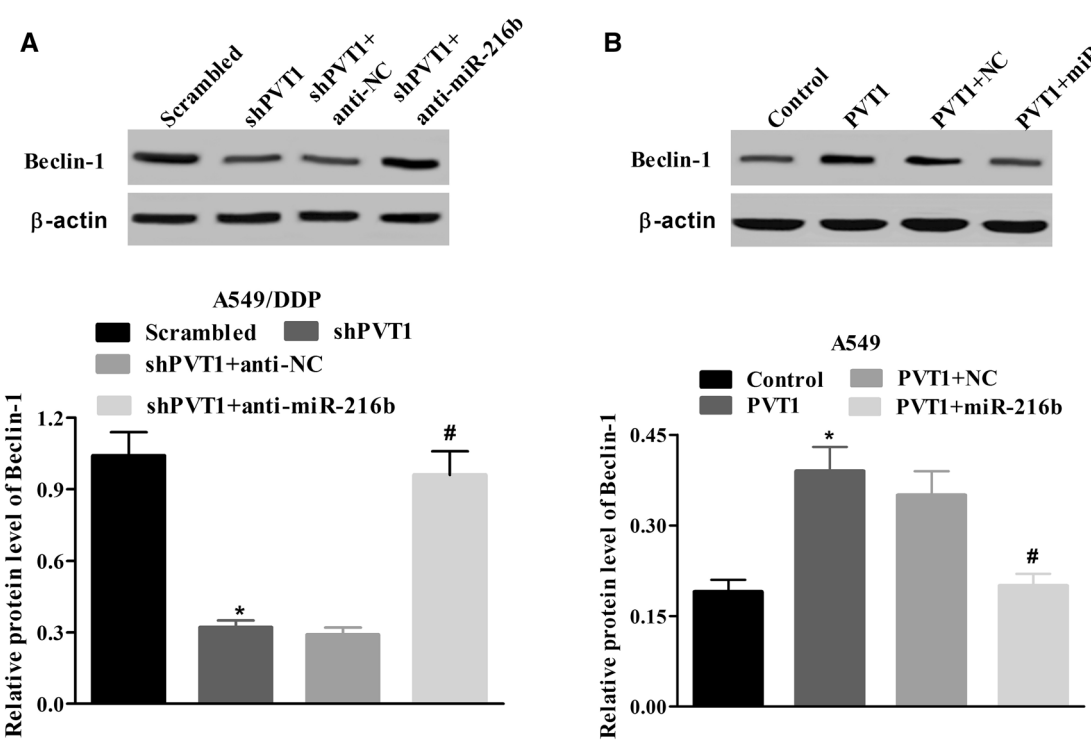


**Fig. 6** Beclin-1 was regulated by PVT1 and miR-216b in NSCLC cells. **a** The effect of shPVT1 and anti-miR-216b on Beclin-1 expression was investigated in A549/DDP cells. **b** The effect of PVT1 and miR-216b on Beclin-1 expression was examined in A549 cells. $^{*}P < 0.05$, $^{\#}P < 0.05$

in different conditions or periods, more detail remains to be investigated in future.

In conclusion, PVT1 indicated worse outcome of NSCLC patients and enhanced cisplatin resistance via autophagy and anti-apoptosis through acting as a sponge for miR-216b and regulating Beclin-1. It was promising that PVT1 could be exploited in a novel prognostic and therapeutic avenue for NSCLC treatment by regulating chemo-sensitivity.

**Author contributions** LC designed and performed the experiments, wrote the manuscript. XH and ZH contributed to experimental work and data analysis. LC and LC conducted the experiments and revised the manuscript. All authors have read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest** Author Liangfeng Chen declares that he has no conflict of interest. Author Xiaobing Han declares that she has no conflict of interest. Author Zhongzhou Hu declares that he has no conflict of interest. Author Liangxin Chen declares that he has no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All animal experiments were performed in accordance with the Guidelines for Care and Use of Laboratory Animal.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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