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The long non-coding RNA PVT1/miR-145-5p/ITGB8 axis regulates cell proliferation, apoptosis, migration and invasion in non-small cell lung cancer cells

C. M. WEI#, X. F. ZHAO#, H. B. QIU, Z. MING, K. LIU, J. YAN*

Department of Oncology, Zigong First People's Hospital, Ziliujing District, Zigong, Sichuan Province, China

**Correspondence: yanjun1963629@163.com*
#Contributed equally to this work.

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Lung cancer is one of the leading causes of death worldwide and non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancer. Long noncoding RNAs (lncRNAs) are closely associated with the development and progression of various cancers, including lung cancer. The purpose of this study was to explore the potential role and molecular mechanism of lncRNA plasmacytoma variant translocation 1 (PVT1) in regulating the proliferation, apoptosis, migration, and invasion of NSCLC cells. The expressions of PVT1, integrin β-8 (ITGB8), and miR-145-5p were detected by quantitative real-time polymerase chain reaction (qRT-PCR). The protein levels of ITGB8, MEK, p-MEK, ERK, and p-ERK were measured by western blot analysis. Cell proliferation, apoptosis, migration, and invasion were determined by MTT assay, flow cytometry, and transwell assay, respectively. The potential binding sites between miR-145-5p and PVT1 or ITGB8 were predicted by online software and verified by luciferase reporter assay. A xenograft tumor model was established to confirm the effect of PVT1 on NSCLC in vivo. We found out that the expression levels of PVT1 and ITGB8 were upregulated in NSCLC tissues and cells. Knockdown of PVT1 or ITGB8 suppressed cell proliferation, migration, invasion and promoted apoptosis in NSCLC cells, which could be reversed by ITGB8 overexpression in NSCLC cells. Moreover, PVT1 could regulate ITGB8 expression via direct binding to miR-145-5p. Furthermore, PVT1 regulated the MEK/ERK pathway by affecting ITGB8 expression. In addition, knockdown of PVT1 inhibited tumor growth, ITGB8 expression, MEK/ERK signaling pathway, and increased miR-145-5p expression *in vivo*. In conclusion, the knockdown of PVT1 inhibited proliferation, migration, and invasion but induced apoptosis of NSCLC cells by regulating miR-145-5p/ITGB8 axis and inhibiting MEK/ERK signaling pathway, providing a novel avenue for the treatment of NSCLC.

Key words: non-small cell lung cancer, PVT1, ITGB8, miR-145-5p, MEK/ERK pathway

Lung cancer is one of the most common causes of cancer deaths with rising incidence worldwide and non-small cell lung cancer (NSCLC) accounts for approximately 80% lung cancer [1, 2]. Despite the diagnosis and treatment of NSCLC have made some progress over the past few decades, the prospect for patients with NSCLC remains unsatisfactory, with an overall 5-year survival rate for patients of all stages still only at 16.8% [3]. Therefore, the searching for new molecular targets and therapeutic strategies are critical for NSCLC treatment.

Long non-coding RNAs (lncRNAs) are a class of more than 200 nucleotides in length with no protein-coding potential [4]. It has been reported that some lncRNAs play critical roles in various cellular and physiological processes, including cell proliferation, apoptosis, and autophagy [5–7]. Recent evidence has shown that aberrant expression of lncRNAs is closely associated with tumorigenesis and the development of cancers, including NSCLC [8, 9]. Human plasmacytoma variant translocation 1 (PVT1) is located on chromosome region 8q24 and adjacent to the oncogene c-myc [10]. A previous report showed that highly expressed PVT1 was tightly linked to the pathophysiology of many cancers, such as breast cancer, ovarian cancer, and lung cancer [11, 12]. However, the precise biological role and underlying mechanism of PVT1 in NSCLC remain largely unknown.

Integrins are present on the surface of cancer cells and they contribute to the metastasis by mediating cell-cell adhesion and invasion in cancer cells [13]. The role of integrins also exists in the signal transduction process, including regulation of cell cycle and motility [14, 15]. Different members of

the integrin subfamily are determined by the beta subunit and integrin β-8 (ITGB8) can only bind to the αv subunit and plays key roles in many types of cancers [16]. It has been reported that miR-513a-3p was confirmed as a novel element targeting ITGB8 to control lung tumor cell migration [17]. However, there is no evidence to support the interaction between PVT1 and ITGB8.

MicroRNAs (miRNAs) are short noncoding RNA molecules with approximately 22 nucleotides and regulate a variety of physiological and pathological processes, including cell proliferation and apoptosis [18, 19]. Previous documents showed that miR-145-5p was involved in the progression of many cancers, such as prostate cancer and gastric cancer [20, 21]. In addition, it has been reported that miR-145-5p has some targets, such as c-Myc, mucin 1, and was downregulated in NSCLC [22]. In spite of these findings, the exact roles of miR-145-5p in the progression of NSCLC and its precise mechanism remain poorly understood.

In this study, we examined the expression of PVT1 and ITGB8 in NSCLC tissues and cells. Furthermore, we investigated the effect of PVT1 on cell proliferation, apoptosis, migration, invasion, and explored the regulatory network of PVT1/miR-145-5p/ITGB8.

Patients and methods

Clinical samples and cell culture. Thirty pairs of NSCLC tissues and adjacent normal tissues were collected from Zigong First People's Hospital during the period from January 2018 to June 2018. Informed consent was obtained from all patients and the study was approved by the Research Ethics Committee of Zigong First People's Hospital. All samples were immediately frozen in liquid nitrogen and stored in a refrigerator at –80 °C until experiments were carried out.

Normal human lung epithelial cells BEAS-2B and NSCLC cell lines (A549, PC-9, H157, and H460) were purchased from the Cobioer (Nanjing, China). All cell lines were cultured in Dulbecco's modified eagle medium (DMEM; Hyclone, Logan, Utah, USA) containing 10% FBS (Gibco, Carlsbad, CA, USA) at 37 °C in a humidified air supplemented with 5% CO_2.

Reagent and cell transfection. Lentivirus-mediated shRNA interference targeting PVT1 (sh-PVT1) and ITGB8 (sh-ITGB8), and empty lentiviral vector (sh-NC) were constructed by GenePharma (Shanghai, China). PVT1 expression plasmid (pcDNA-PVT1), ITGB8 overexpression plasmid (pcDNA-ITGB8), empty vector (pcDNA-control), miR-145-5p mimic (miR-145-5p), miR-control (miR-control), miR-145-5p inhibitor (anti-miR-145-5p), and inhibitor control (anti-control) were purchased from Ribobio Co. (Guangzhou, China). A549 and PC-9 cells were transfected with these oligonucleotides or plasmids by Lipofectamine3000 (Invitrogen, Carlsbad, CA, USA) according to the instructions of the manufacturer.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR) assay. Total RNA was isolated from tissues or cells utilizing the Trizol reagent (Invitrogen) following the manufacturer's instructions. The first strand complementary DNA (cDNA) was synthesized using Reverse Transcription Kit (Takara, Dalian, China) or TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). After reverse transcription, qRT-PCR was performed using the quantitative SYBR Green PCR kit (Toyobo, Tokyo, Japan) on a CFX96 real-time PCR system (Bio-Rad; Hercules, CA, USA) following the amplification instructions. The primers were purchased from Sangon Biotech (Shanghai, China) and listed below: PVT1 (Forward, 5'-TGAGAACTGTCCTTACGTGACC-3'; Reverse, 5'-AGAGCACCAAGACTGGCTCT-3'); ITGB8 (Forward, 5'-CGTGACTTTCGTCTTGGATTTGG-3'; Reverse, 5'-TCCTTTCGGGGTGGATGCTAA-3'); miR-145-5p (Forward, 5'-CAGGAATCCCTTAGATGCTA-3'; Reverse, 5'-CCATGACCTCAAGAACAGT-3'); U6 snRNA (Forward, 5'-CTCGCTTCGGCAGCACA-3'; Reverse, 5'-AACGCTTCACGAATTTGCGT-3'); GAPDH (Forward, 5'-CACCCACTCCTCCACCTTTG-3'; Reverse 5'-CCACCACCCTGTTGCTGTAG-3'). The relative expression of PVT1, ITGB8, and miR-145-5p was calculated using the $2^{-\Delta\Delta Ct}$ method and normalized by GAPDH or U6 snRNA.

Western blot assay. Total proteins were isolated from tissues and cells by RIPA lysis buffer (Sigma, St. Louis, MO, USA) containing protease inhibitor cocktail (Sigma) for 30 min at 4 °C. Subsequently, centrifugation was carried out at 12,000 rpm for 10 min to collect the protein supernatant. Total protein concentration was determined by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). Equivalent quantities of proteins were separated by 10% SDS-PAGE and then transferred to PVDF membrane (Millipore Corp, Atlanta, GA, USA). After blocking with 5% (w/v) non-fat milk in Tris-buffered saline and 0.1% Tween 20 (TBST) buffer for 2 h, the membranes were incubated for 12 h at 4 °C with primary antibodies against ITGB8 (#88300, 1:1000), MEK (#4694, 1:1000), phospho-MEK1/2 (Ser217/221, #3958, 1:1000), ERK (#4965, 1:1000), phospho-ERK1/2 (Thr202/Tyr204, #4370, 1:1000) or GAPDH (#5174, 1:1000), all purchased from Cell Signaling Technology, Beverly, MA, USA. After washing the membranes 3 times with TBST, membranes were incubated by horseradish peroxidase-conjugated secondary antibodies (Sangon Biotech) at 1:4000 dilutions for 2 h at room temperature. Finally, the protein bands were examined by enhanced chemiluminescence (ECL; Tanon, Shanghai, China) and imaged using a chemiluminescent gel imaging system (Tanon).

Cell proliferation assay. Cell proliferation was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay. Briefly, exponentially growing cells (2×10^4 cells/ml) were seeded in 96-well plate and cultured overnight. Then cells were transfected with sh-PVT1, sh-ITGB8, sh-PVT1+pcDNA-ITGB8 or their matched controls for 0 h, 24 h, 48 h or 72 h. After transfection, 20 μl MTT solution at a concentration of 5 mg/ml was added to each well, then the plates

were incubated for 4 h at 37 °C. The culture medium mixture was discarded and 150 µl dimethyl sulfoxide (DMSO; Sigma) was added to each well to dissolve the formazan crystals. Cell proliferation was measured at 490 nm by microplate reader (Bio-Teck, Winooski, VT, USA).

Apoptosis assay. The percentage of apoptotic cells was examined using flow cytometry with Annexin V-FITC/PI apoptosis kit (BD Pharmingen, San Diego, CA, USA). A549 and PC-9 cells were sowed in 6-well plates and treated with different transfection reagents. After 48 h, cells were collected and washed with cold PBS (pH 7.4) three times. Next, the cells were stained with Annexin V-FITC and PI in the dark environment for 15 min. Finally, cell apoptosis was examined by flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) and the apoptosis rate was analyzed by the flow cytometer software.

Transwell assay. Transwell assay was used to measure cell migration and invasion. For the migration assay, A549 and PC-9 cells (2×10^4 cells/well) in 100 µl of serum-free DMEM medium were seeded into the upper chambers (Corning Costar, NY, USA) with 8 µm porous membrane, and 600 µl of DMEM with 10% FBS medium was added to the lower chambers. After incubation for 24 h, A549 and PC-9 cells remaining on the top surface of the membranes were removed with a cotton-tipped swab. Migrated cells were fixed with 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet (Sigma) for 1 h, which were counted by a microscope (Leica, Solms, GER). For cell invasion, the transwell chambers were pre-coated with Matrigel (BD, San Jose, CA, USA), and the remaining steps were similar to the migration experiment.

Luciferase reporter assay. The possible binding sites of miR-145-5p and PVT1 or ITGB8 were predicted by online software MiRcode online tool (http://www.mircode.org/) or Target Scan online tool (http://www.targetscan.org/vert_71/). The sequences of wild type PVT1 (WT-PVT1), mutant PVT1 (MUT-PVT1), wild type 3'untranslated regions of ITGB8 (ITGB8 3'UTR-WT) or mutant type 3'untranslated regions of ITGB8 (ITGB8 3'UTR-MUT) were amplified and cloned into pGL3 plasmids (Promega, Madison, WI, USA). The miR-145-5p, anti-miR-145-5p, miR-145-5p+pcDNA-PVT1, or their matched controls were co-transfected with WT or MUT luciferase reporter plasmids into A549 and PC-9 cells according to the protocols of the manufacturer. After the cells were transfected for 48 h, luciferase activities were measured by using the Dual-Luciferase Assay Kit (Promega).

***In vivo* experiment.** The experiments were carried out with the approval of the committee of Animal Research of Zigong First People's Hospital and performed in strict accordance with the institutional guide for the laboratory animals. Five-week-old female BALB/c nude mice (5 per group) were obtained from Henan Experimental Animals Centre (Zhengzhou, China). A549 cells (1×10^7) transfected with sh-PVT1or sh-NC were injected subcutaneously into the flank of the nude mice. The tumor volumes were gauged every three days with a caliper and calculated as the formula: volume = length $\times$ width^2 $\times$ 0.5. After 24 d, all mice were sacrificed and tumors were weighed and harvested for subsequent qRT-PCR and western blot analysis.

Data analysis. In the present study, more than three individual experiments were accomplished for each experiment. All data were presented as the mean ± standard deviation. The significant differences between groups (usually $p<0.05$ means statistically significant) were analyzed by Student's t-test using GraphPad Prism version 6.0 software (GraphPad Software, San Diego, USA). Spearman rank correlation was employed to analyze the association between PVT1 and ITGB8.

Results

PVT1 and ITGB8 were upregulated in human NSCLC tissues. To confirm the potential roles of PVT1 and ITGB8 in NSCLC, qRT-PCR and western blot analysis were performed to explore the expression of PVT1 and ITGB8 in the tissues. Analysis of qRT-PCR indicated that expression levels of both PVT1 and ITGB8 were increased in NSCLC tissues, compared with the corresponding normal tissues (Figures 1A, 1B). Similarly, western blot analysis showed that the protein level of ITGB8 was increased in NSCLC tissues versus matching adjacent normal tissues (Figure 1C). Moreover, we also analyzed the relationship between PVT1 and ITGB8 and found that expression of PVT1 was positively correlated with ITGB8 in NSCLC tissues (Figure 1D). These data revealed that PVT1 and ITGB8 might play an oncogenic role in NSCLC.

Knockdown of PVT1 inhibited cell proliferation, migration, invasion, and promoted apoptosis in NSCLC cells. To explore the effect of PVT1 in NSCLC, the expression of PVT1 in NSCLC cells (A549, PC-9, H157, and H460) and normal human lung epithelial cells (BEAS-2B) was measured by qRT-PCR. The result showed that the expression level of PVT1 was elevated in NSCLC cells (A549, PC-9, H157, and H460) compared to that in BEAS-2B cells (Figure 2A), especially in A549 and PC-9 cells. Therefore, A549 and PC-9 cells were chosen and transfected with sh-PVT1 or sh-NC for further analysis. Next, the qRT-PCR assay revealed that the transfection of sh-PVT1 led to an obvious reduction of PVT1 expression in A549 and PC-9 cells (Figure 2B). MTT assay suggested that the proliferation of A549 and PC-9 cells was inhibited at different time points in the sh-PVT1 group relative to the sh-NC group (Figures 2C, 2D). Flow cytometry analysis indicated that the apoptosis rate was increased about 3-fold after the transfection of sh-PVT1 in A549 and PC-9 cells (Figure 2E). In addition, transwell assay revealed that PVT1 knockdown inhibited migration and invasion ofA549 and PC-9 cells (Figures 2F, 2G). Taken together, these results showed that knockdown of PVT1 suppressed cell proliferation, migration, invasion and enhanced cell apoptosis in NSCLC cells.

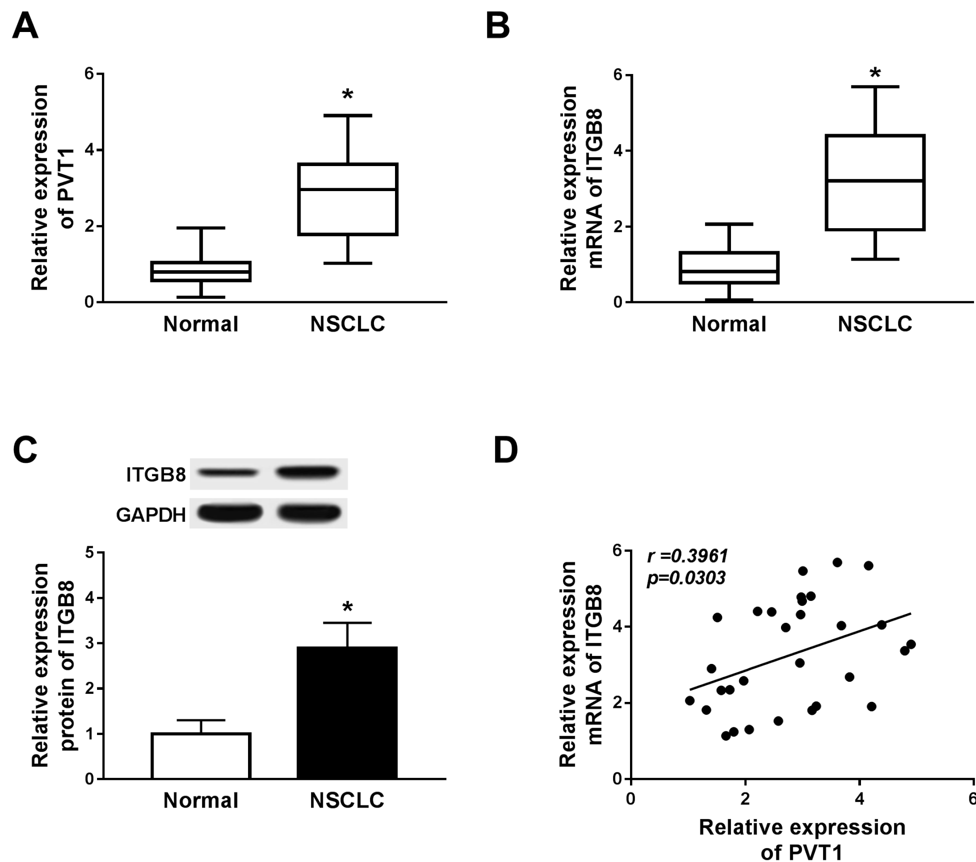

Figure 1. The expression levels of PVT1 and ITGB8 were enhanced in NSCLC tissues. A, B) The expression levels of PVT1 and ITGB8 were detected in NSCLC tissues and adjacent normal samples by qRT-PCR. C) The protein level of ITGB8 was measured by western blot assay. D) The correlation between PVT1 expression and ITGB8 mRNA level in NSCLC tissues was analyzed (r=0.3961, p=0.0303). *p<0.05

Interference of ITGB8 suppressed cell proliferation, migration, invasion and induced apoptosis in NSCLC cells. To investigate the role of ITGB8 in NSCLC cells, sh-ITGB8 or sh-NC was transfected into A549 and PC-9 cells. The qRT-PCR and western blot assays exhibited that the mRNA and protein expression levels of ITGB8 were increased approximately 3-fold in A549 and PC-9 cells compared to BEAS-2B cells (Figures 3A, 3B). Moreover, qRT-PCR and western blot analysis measured the mRNA and protein levels of ITGB8 in A549 and PC-9 cells transfected with sh-ITGB8 or sh-NC. Results showed that the mRNA and protein expression levels of ITGB8 were decreased by about 70% in A549 and PC-9 cells transfected with sh-ITGB8 relative to the sh-NC group (Figures 3C, 3D). Then cell proliferation and apoptosis were evaluated by using MTT and flow cytometry analysis, respectively. MTT assay proved that interference of ITGB8 inhibited cell proliferation at different time points in A549 and PC-9 cells (Figures 3E, 3F). Flow cytometry analysis revealed that, compared with the sh-NC group, ITGB8 knockdown resulted in a significant increase of apoptosis rate in A549 and PC-9 cells (Figure 3G). Furthermore, transwell assay proved that migration and invasion abilities in A549 and PC-9 cells with knockdown of ITGB8 were significantly weakened (Figures 3H, 3I). In a word, these data suggested that the silencing of ITGB8 restrained cell proliferation, migration, and invasion but contributed to cell apoptosis in NSCLC cells.

ITGB8 overexpression reversed the effects of PVT1 knockdown on cell proliferation, apoptosis, migration, and invasion in NSCLC cells. To further explore the relationship between PVT1 and ITGB8 in NSCLC cells, sh-PVT1, pcDNA-PVT1, sh-PVT1+pcDNA-ITGB8 or their matched controls were transfected into A549 and PC-9 cells. As shown in Figures 4A and 4B, the qRT-PCR assay revealed that silencing PVT1 inhibited the expression of ITGB8, and transfection of pcDNA-PVT1 showed an opposite effect in A549 and PC-9 cells. Moreover, the protein level of ITGB8 was detected by western blot analysis. The result showed that knockdown of PVT1 resulted in the notable reduction of ITGB8 level, whereas the overexpression of PVT1 induced ITGB8 expression in A549 and PC-9 cells (Figures 4C, 4D). In addition, cells were co-transfected with sh-PVT1 and pcDNA-ITGB8 to explore their effects on cell proliferation and apoptosis. The findings disclosed that knock-

down of PVT1 inhibited proliferation in a time-dependent manner, and promoted apoptosis in A549 and PC-9 cells, whereas the overexpression of ITGB8 abolished these effects (Figures 4E–4H). Furthermore, transwell assay suggested that deficiency of PVT1 decreased the number of migrated or invasive cells in A549 and PC-9 cells, while the upregulation of ITGB8 reversed these effects (Figures 4I–4L). Our data demonstrated that ITGB8 overexpression attenuated the effects of PVT1 knockdown on cell proliferation, apoptosis, migration, and invasion in NSCLC cells.

PVT1 regulated ITGB8 expression by targeting miR-145-5p in NSCLC cells. By using an online database MiRcode Tools, we found that miR-145-5p was a potential target of PVT1 (Figure 5A). Then, a dual-luciferase reporter assay was performed to further demonstrate the interaction between PVT1 and miR-145-5p. Results showed that the transfection of miR-145-5p resulted in a conspicuous reduction in relative luciferase activity of WT-PVT1 reporter, and knockdown of miR-145-5p caused an opposite effect on luciferase activity of WT-PVT1 reporter, whereas no obvious effect was found on relative luciferase activity of MUT-PVT1 reporter in A549 and PC-9 cells (Figures 5B, 5C). Interestingly, online software TargetScan showed that miR-145-5p might bind to 3'-UTR of ITGB8 (Figure 5D). To confirm the prediction, we cloned ITGB8 3'-UTR-WT into the pGL3-luciferase reporter vector and mutated the putative miR-145-5p binding sites in the ITGB8 3'-UTR-MUT. These results showed co-transfection of miR-145-5p and ITGB8 3'-UTR-WT limited the luciferase activity in A549 and PC-9 cells, which was abrogated by transfection of pcDNA-PVT1 (Figures 5E, 5F). However, there was no significant difference in luciferase activity in cells with ITGB8 3'-UTR-MUT. The mRNA and protein expression levels of ITGB8 were determined by qRT-PCR and western blot assays. As displayed

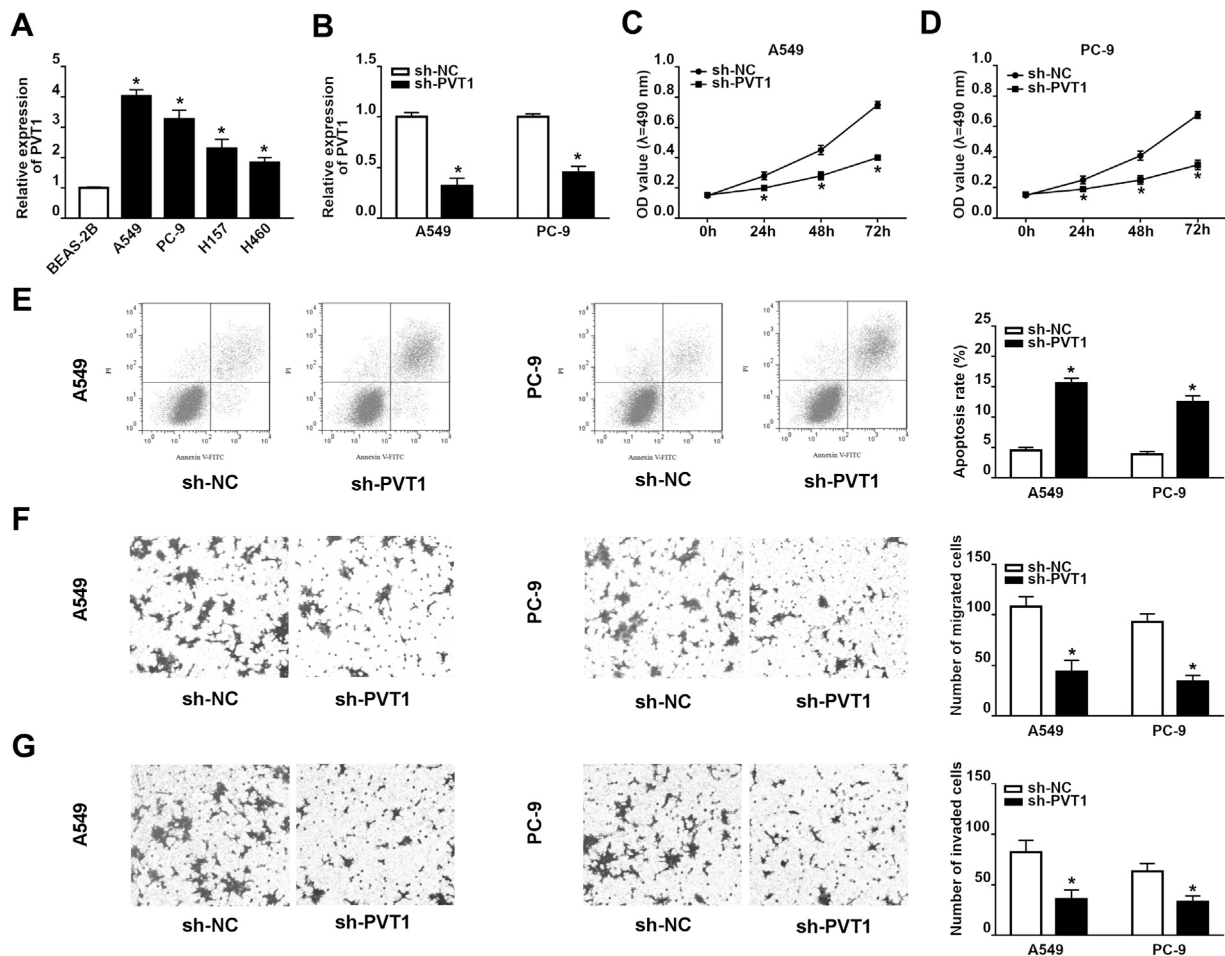

Figure 2. The knockdown of PVT1 inhibited the progression of NSCLC cells. A) The expression of PVT1 was measured in NSCLC cells (A549, PC-9, H157, and H460) and normal human lung epithelial cells (BEAS-2B) by qRT-PCR. B) The expression of PVT1 was determined in A549 and PC-9 cells transfected with sh-PVT1or sh-NC by qRT-PCR. The effects of PVT1 on cell proliferation (C, D), apoptosis (E), migration (F) and invasion (G) were detected in A549 and PC-9 cells transfected with sh-PVT1 or sh-NC by MTT assay, flow cytometry, and transwell assay, respectively. *p<0.05

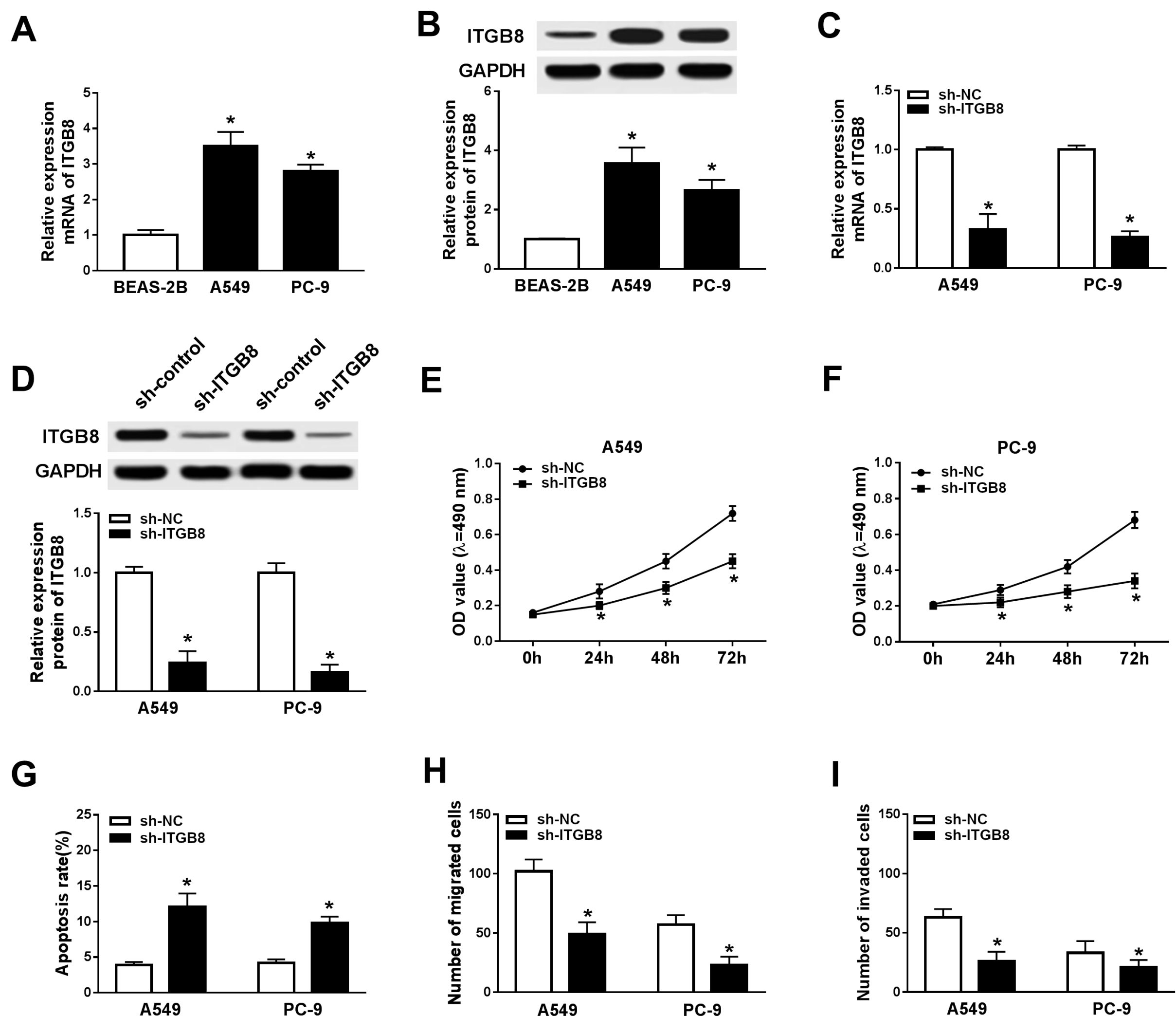

Figure 3. The knockdown of ITGB8 suppressed the progression of NSCLC cells. A, B) The mRNA and protein expression of ITGB8 were detected in NSCLC cells (A549 and PC-9) and BEAS-2B cells by using qRT-PCR and western blot assays, respectively. C, D) The mRNA and protein expression of ITGB8 were analyzed in A549 and PC-9 cells transfected with sh-ITGB8 or sh-NC by qRT-PCR and western blot assays, respectively. The effects of ITGB8 on cell proliferation (E, F), apoptosis (G), migration (H) and invasion (I) were examined in A549 and PC-9 cells transfected with sh-ITGB8 or sh-NC by MTT assay, flow cytometry, and transwell assay, respectively. *p<0.05

in Figures 5G–5J, overexpression miR-145-5p limited the expression of ITGB8, which was abated by the transfection of pcDNA-PVT1. Our data suggested that PVT1 regulated ITGB8 expression by targeting miR-145-5p.

PVT1 regulated the MEK/ERK pathway by affecting ITGB8. MEK/ERK pathway plays key roles in diverse physiological processes in many human cancers. To further investigate the molecular mechanism of PVT1 in the process of NSCLC, the expression levels of proteins related to the MEK/ERK signaling pathway in A549 and PC-9 cells were detected by western blot assay. Results suggested that the transfection of pcDNA-PVT1 resulted in a prominent increase of phosphorylation of MEK and ERK, whereas the effects were reversed by the transfection of sh-ITGB8, but there were no changes of total MEK and ERK proteins in A549 and PC-9 cells (Figures 6A, 6B). These data demonstrated PVT1 could regulate and activate the MEK/ERK pathway by affecting ITGB8.

Knockdown of PVT1 decreased xenograft tumor growth *in vivo*. To further determine the tumorigenicity of PVT1, we subcutaneously injected A549 cells transfected with sh-PVT1 or sh-NC into nude mice (N=5). The tumor volume was measured once every 3 days, and tumor weight and volume were measured after 24 days post-injection. The results disclosed that the transfection of sh-PVT1 suppressed tumor growth (including the average tumor volume and weight) *in vivo* compared with the sh-NC group (Figures 7A, 7B). In addition, the expression levels of miR-145-5p and ITGB8

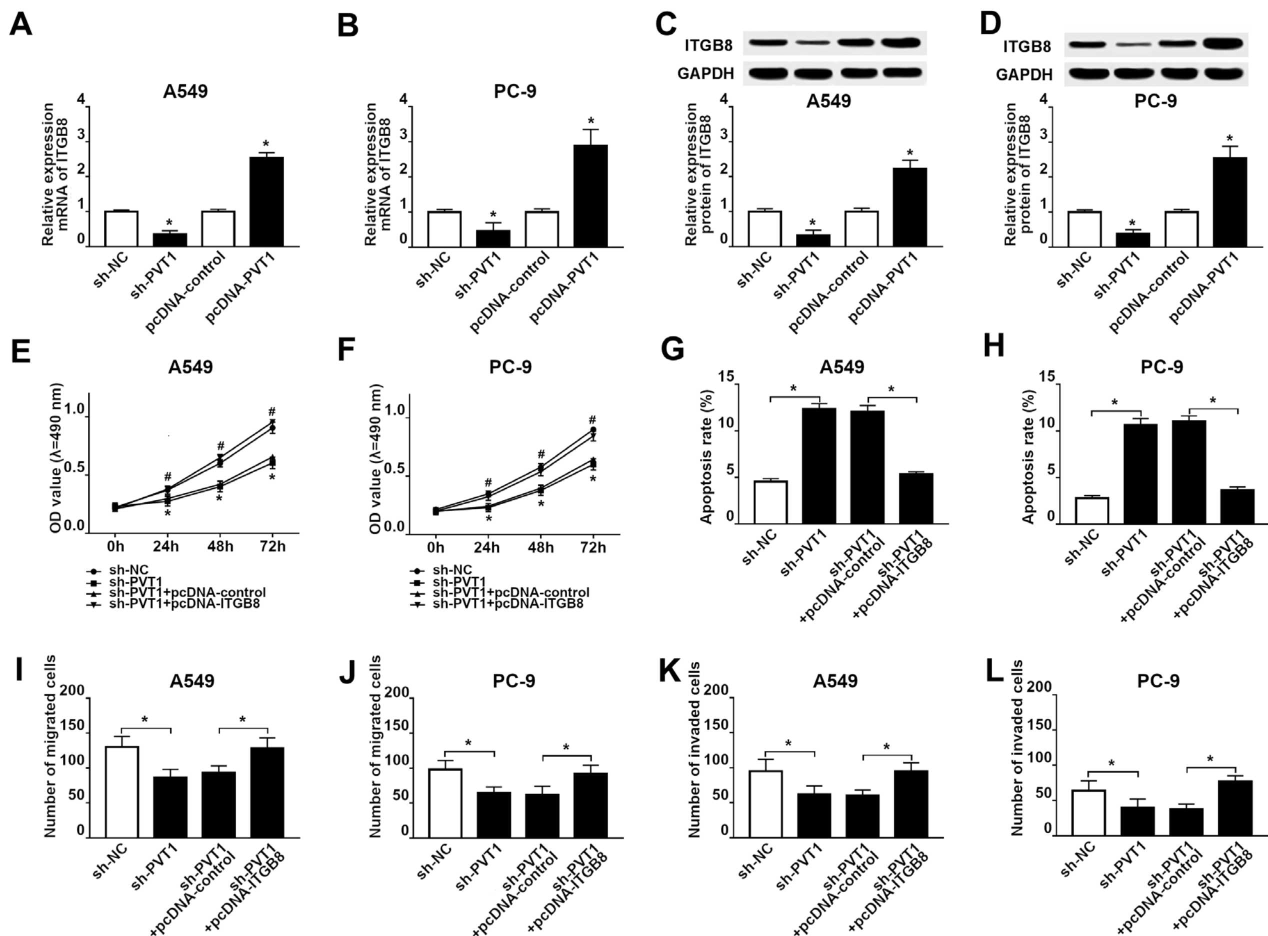

Figure 4. ITGB8 overexpression reversed the effect of PVT1 knockdown on progression in NSCLC cells. A-D) The mRNA and protein expression of ITGB8 in A549 and PC-9 cells transfected with sh-PVT1, pcDNA-PVT1 or their matched controls were detected by qRT-PCR and western blot assays. The effects of ITGB8 and PVT1 on cell proliferation (E, F), apoptosis (G, H), migration (I, J) and invasion (K, L) in A549 and PC-9 cells transfected with sh-PVT1, pcDNA-ITGB8 or their matched controls were measured by MTT assay, flow cytometry, and transwell assay, respectively. *p<0.05

in xenograft tissues were detected by qRT-PCR. The results exhibited that knockdown of PVT1 upregulated miR-145-5p expression and downregulated ITGB8 expression (Figures 7C, 7D). Furthermore, western blot analysis showed that PVT1 knockdown suppressed ITGB8, p-MEK and p-ERK protein levels in tumor tissues (Figure 7E). Collectively, these findings disclosed that knockdown of PVT1 blocked tumor growth, elevated expression of miR-145-5p, inhibited expression of ITGB8 and inactivated the MEK/ERK pathway *in vivo*.

Discussion

Lung cancer is one of the leading causes of cancer death, owing to its high incidence and lack of effective treatment [23]. Recently, a growing number of studies demonstrated that lncRNAs could act as a tumor suppressor or an accelerator, and thus play key regulatory roles in tumorigenesis and tumor progression [24, 25]. In this study, we focused on the role of PVT1, miR-145-5p, and ITGB8 in NSCLC.

It has been reported that PVT1 was upregulated in many carcinomas and its overexpression was closely related to the low survival rate of cancer patients [26]. For example, PVT1 was upregulated in colon cancer samples and promoted metastasis and proliferation of colon cancer [27]. In addition, Guo et al. demonstrated that knockdown of PVT1 suppressed cell viability, invasion, and promoted apoptosis in NSCLC cells via regulating miR-497 expression [28]. Consistent with this study, the PVT1 expression was increased in NSCLC tissues and cells, and knockdown of PVT1 inhibited cell proliferation, migration, and invasion but induced cell apoptosis in NSCLC cells. Furthermore, the downregulation of PVT1 also inhibited tumors growth.

The emerging evidence suggested that integrins played an important role in the regulation of normal and cancer cell migration and survival [29]. In addition, ITGB8 has been

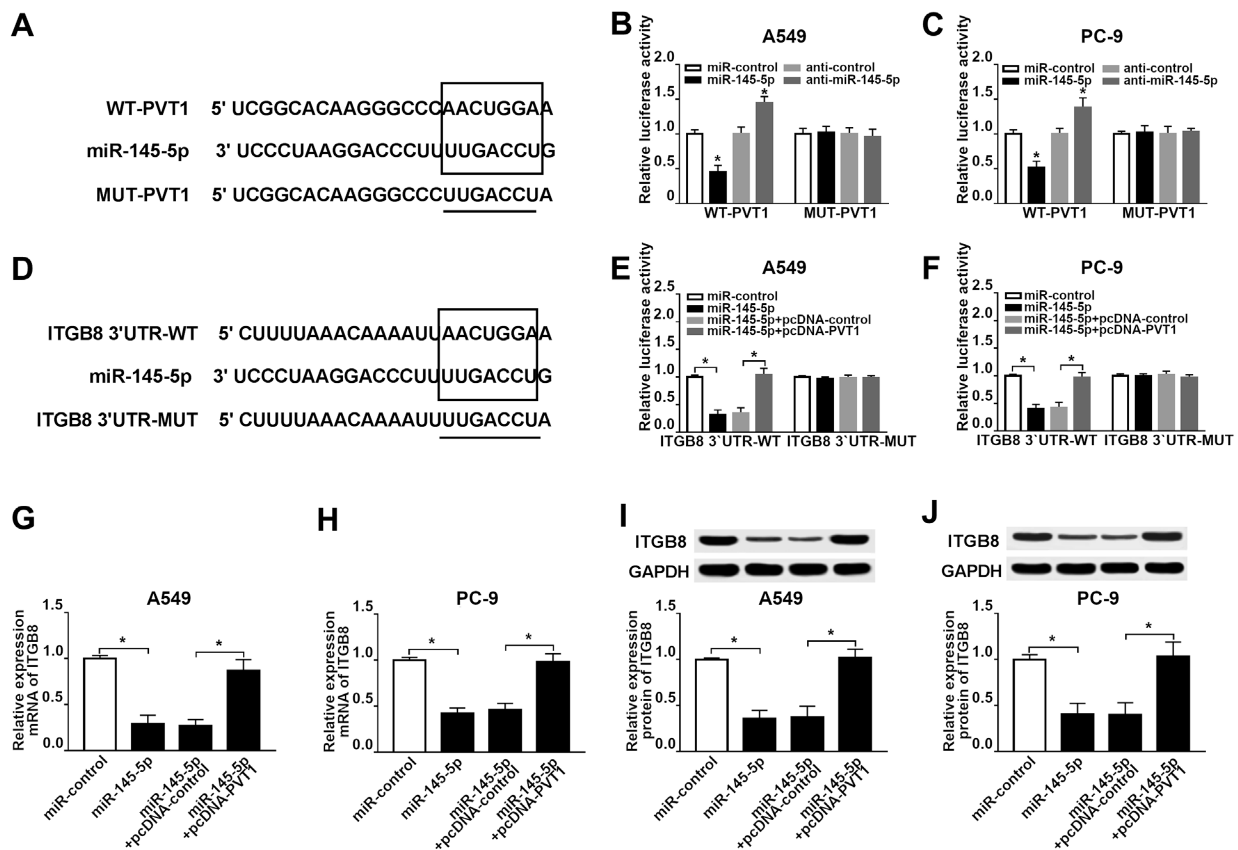

Figure 5. PVT1 regulated ITGB8 expression by targeting miR-145-5p in NSCLC cells. A) The possible binding sites of PVT1 and miR-145-5p were predicted by MiRcode Tools. B, C) A549 and PC-9 cells were co-transfected with WT-PVT1 or MUT-PVT1 reporter and miR-145-5p, miR-control, anti-miR-145-5p, or anti-control, followed by the detection of relative luciferase activity. D) The possible binding sites of miR-145-5p and ITGB8 were predicted by TargetScan Tools. E, F) A549 and PC-9 cells were co-transfected with ITGB8 3'-UTR-WT or ITGB8 3'-UTR-MUT reporter plasmids and miR-145-5p, miR-145-5p+pcDNA-PVT1 or their matched controls, followed by the detection of relative luciferase activity. G-J) The mRNA and protein expression of ITGB8 in A549 and PC-9 cells transfected with miR-145-5p, miR-145-5p+pcDNA-PVT1 or their matched controls were determined by qRT-PCR and western blot assays. *p<0.05

shown to be a tumor promoter and upregulated in various types of cancer, including lung cancer [30, 31]. Furthermore, Xu et al. found that knockdown ITGB8 might reduce the adhesion and invasion of lung cancer cells [32]. Similarly, our results showed that the expression level of ITGB8 was elevated in NSCLC tissues and cells, silencing ITGB8 inhibited cell proliferation, migration, and invasion but promoted cell apoptosis in NSCLC cells. Moreover, ITGB8 overexpression reversed the effects of PVT1 knockdown on cell proliferation, apoptosis, migration, and invasion in NSCLC cells. These data suggested that PVT1 and ITGB8 were important regulatory factors in NSCLC.

A previous document indicated that miRNAs and lncRNAs could target each other and share some common signaling pathways [33]. Moreover, the elucidation of miRNA targets has become an important research area [34]. It has been demonstrated that miR-145-5p could regulate oncogenes in lung adenocarcinoma pathogenesis [35]. Therefore, the targeting relationship between miR-145-5p, PVT1, and ITGB8, and their interaction in lung cancer cells were explored. Here, we found the miR-145-5p could be directly targeted by PVT1 and directly targeted ITGB8, and overexpression of miR-145-5p limited the expression of ITGB8, while these effects were alleviated by transfection of pcDNA-PVT1. Hence, it was speculated that PVT1 exerted its function via the miR-145-5p/ITGB8 axis (Figure 8).

The mitogen-activated protein kinase/extracellular signal-regulating kinase (MEK/ERK) signaling pathway regulates cell growth, apoptosis and cell cycle in diverse types of cells [36]. The highly evolutionary conserved MEK1/2 is known to phosphorylate the upstream protein kinases of ERK1/2 [37]. ERK can regulate a variety of cellular processes, such as survival, migration, proliferation, differentiation, transcription, and metabolism by phosphorylation of a large number of target proteins [38]. The previous report demonstrated that miR-221 affected the MEK/ERK signaling pathway via targeting PAK1 to suppress the proliferation of endothelial progenitor cells [39]. Additionally, quercetin-induced

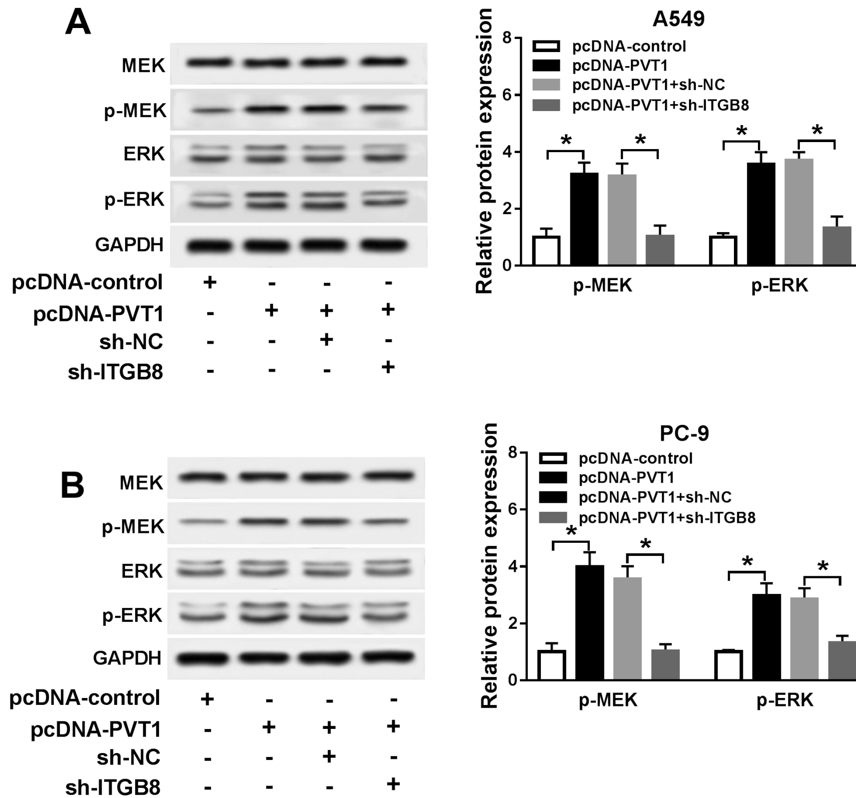

Figure 6. PVT1 regulated the MEK/ERK pathway by affecting ITGB8. A, B) The protein expression of MEK, p-MEK, ERK, and p-ERK was evaluated in A549 and PC-9 cells transfected with pcDNA-PVT1, pcDNA-PVT1+sh-ITGB8 or their matched controls by western blot assay. *p<0.05

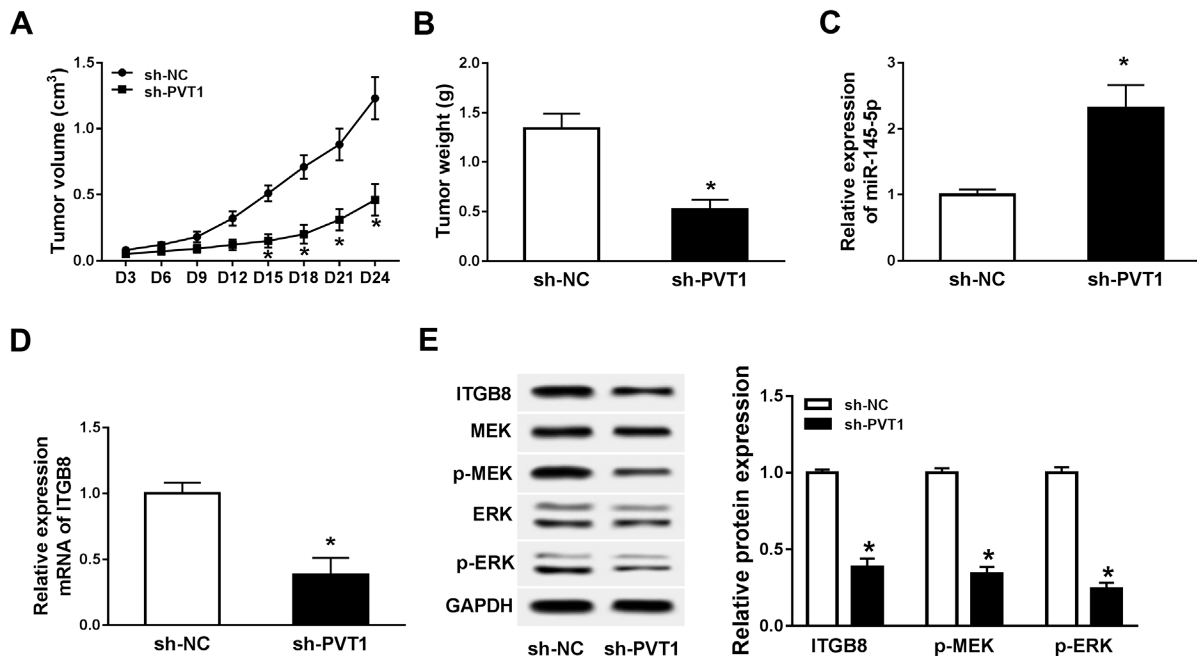

Figure 7. The knockdown of PVT1 inhibited tumor growth *in vivo*. A) The A549 cells introduced with sh-PVT1 or sh-NC were injected subcutaneously into nude mice, and tumor volumes were examined every 3 days. B) The resected tumors were weighed after 24 d injection. C) The expression of miR-145-5p was detected by qRT-PCR. D) The expression of ITGB8 in the resected tumors was measured by qRT-PCR. E) The protein expression of ITGB8, MEK, p-MEK, ERK, and p-ERK was evaluated by western blot analysis. *p<0.05

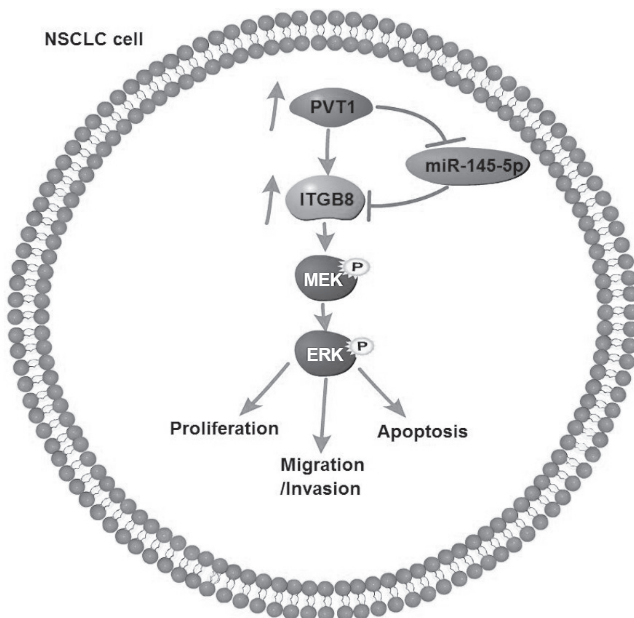

Figure 8. Mechanism diagram of lncRNA PVT1/miR-145-5p/ITGB8 axis in NSCLC progression. ↑ indicated that the expression was increased in NSCLC cells. PVT1 regulated cell proliferation, migration, invasion, and apoptosis of NSCLC cells by regulating miR-145-5p/ITGB8 axis and MEK/ERK signaling pathway.

apoptosis in A549 lung cancer cells required activation of the MEK/ERK signaling pathway [40]. However, the effects of PVT1 and ITGB8 on the MEK/ERK pathway in NSCLC have not been reported previously. In our study, overexpression of PVT1 resulted in a significant increase in phosphorylation of MEK and ERK, whereas the effects were reversed by downregulating ITGB8 in NSCLC cells. Furthermore, knockdown of PVT1 suppressed the MEK/ERK signaling pathway *in vitro* and *in vivo*. Thus, these data indicated that PVT1 could regulate the MEK/ERK pathway and was affected by ITGB8 expression (Figure 8).

In conclusion, the expression levels of PVT1 and ITGB8 were upregulated in NSCLC tissues and cells. Inhibition of PVT1 or ITGB8 suppressed cell proliferation, migration, invasion and promoted apoptosis in NSCLC cells, which could be abated by upregulating ITGB8 in NSCLC cells. Moreover, PVT1 could regulate ITGB8 expression via directly binding to miR-145-5p. PVT1 regulated the MEK/ERK pathway by affecting ITGB8 expression. In addition, silencing PVT1 inhibited tumor growth, ITGB8 expression, and MEK/ERK signaling pathway, but increased miR-145-5p expression *in vivo*. Taken together, knockdown of PVT1 inhibited progression of NSCLC through downregulating ITGB8, upregulating miR-145-5p, and inactivating the MEK/ERK pathway, elucidating the possible molecular mechanism of PVT1/miR-145-5p/ITGB8 regulatory axis in NSCLC, providing a theoretical basis for the exploration on lncRNA-directed target for NSCLC.

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