Long non-coding RNA PVT1 promotes epithelial-mesenchymal transition via the TGF-β/Smad pathway in pancreatic cancer cells

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Abstract. Recent studies have revealed that overexpression of long non-coding RNA (lncRNA) PVT1 is correlated with several types of cancer. However, its role in pancreatic cancer development remains to be clarified. In the present study, we found that PVT1 promoted the growth and the epithelial-mesenchymal transition (EMT) of pancreatic cancer cells. We first determined that PVT1 was upregulated in pancreatic cancer tissues compared with adjacent normal tissues. Knockdown of PVT1 inhibited viability, adhesion, migration and invasion. Furthermore, PVT1 knockdown reduced the expression of mesenchymal markers including Snail, Slug, β-catenin, N-cadherin and vimentin, while it increased epithelial marker expression of E-cadherin. Finally, knockdown of PVT1 inhibited the TGF-β/Smad signaling, including p-Smad2/3 and TGF-β1 but enhanced the expression of Smad4. In contrast, overexpression of PVT1 reversed the process. These findings revealed that PVT1 acts as an oncogene in pancreatic cancer, possibly through the regulation of EMT via the TGF-β/Smad pathway and PVT1 may serve as a potential target for diagnostics and therapeutics in pancreatic cancer.

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Introduction

Pancreatic cancer is one of the major human cancers with extremely poor prognosis compared with other leading cancers (1). In the USA, the mortality rate of pancreatic cancer is ranked fourth among all malignancies, and the overall 5-year survival rate of patients with pancreatic cancer is less than 5% (2,3). It is estimated that by the year 2030, pancreatic cancer may become the second leading cause of cancer-related deaths in the USA (4). The primary reason for this poor prognosis is a failure to diagnose the disease at an early stage (5). Furthermore, apart from surgery, chemotherapy and radiation therapy, there are no effective therapies for the treatment of pancreatic cancer (6). Hence, novel biomarkers and therapeutic strategies are urgently required.

Previous studies have suggested that epithelial-mesenchymal transition (EMT) contributes to early-stage dissemination of cancer cells and is pivotal for invasion and metastasis of pancreatic cancer (7). EMT results in the loss of E-cadherin expression and the acquisition of mesenchymal markers including fibronectin or vimentin (8). Long non-coding RNAs (lncRNAs) are RNA molecules (>200 nucleotides in length) lacking coding capacity (9). Increasing evidence suggests that lncRNAs play important role in diverse cellular biological processes. Plasmacytoma variant translocation 1 (PVT1) is an oncogenic lncRNA (10) and was initially found in the translocations occurring in variant Burkitt's lymphoma and murine plasmacytoma (11). PVT1 is a downstream target of the well-known Myc oncogene (12). Recent studies have indicated that PVT1 can regulate a series of human tumors, such as breast and ovarian (13,14), prostate (15), lung (16) and gastric cancer (17). In ovarian and breast cancer, the PVT1 gene was revealed to be overexpressed and to contribute independently to ovarian and breast cancer pathogenesis and inhibit apoptosis (13). Yang *et al* revealed that the expression level of PVT1 was significantly upregulated in non-small cell lung carcinoma (NSCLC) tissues and cell lines (16). Using a genome-wide screening strategy, PVT1 was identified as a regulator of gemcitabine sensitivity in pancreatic cancer cells,

and upregulation of PVT1 was negatively associated with gemcitabine sensitivity (18). Finally, PVT1 may serve as an independent prognostic factor for poor overall survival rate in patients with pancreatic ductal adenocarcinoma (PDAC) (19). However, the role of PVT1 in pancreatic cancer cells remains to be clarified.

In the present study, we found that PVT1 had a significantly higher expression in tumor tissues than that in adjacent normal tissues, and was critical for cell viability, the ability of adhesion, migration and invasion in pancreatic cancer cells. Moreover, it was demonstrated that PVT1 promoted EMT in pancreatic cancer cells possibly via TGF-β/Smad signaling. All these findings demonstrated for the first time that PVT1 may be a facilitator in pancreatic cancer progression.

Materials and methods

Tissue samples. Human tumor tissue samples and adjacent normal controls were obtained by surgical resection from nine patients with pancreatic cancer, at the Department of General Surgery, Changhai Hospital of Shanghai, Second Military Medical University. All samples were snap-frozen and stored in liquid nitrogen (-176˚C). Signed informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the hospital.

Cell cultures. The human pancreatic cancer cell lines (PANC1, PaTu8988 and SW1990) were kindly provided by the Second Military Medical University of Shanghai. The cell lines were cultured with DMEM (Hyclone Laboratories; GE Healthcare Life Sciences, Shanghai, China) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) in a humidified 5% CO_2 incubator at 37˚C.

Quantitative RT-PCR. Total RNA was extracted from tissues and cultured cells with Trizol Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. RNA concentration and integrity were determined by spectrophotometry. Reverse transcription was performed using the PrimeScript™ RT reagent kit (Takara Bio, Inc., Otsu, Japan). Quantitative RT-PCR was carried out using the SYBR® Premix Dimmer Eraser kit (Takara Bio, Inc.). GAPDH was used as an internal control. The primer pair used for the amplification was as follows: PVT1 forward, 5'-TGAGAACTG TCCTTACGTGACC-3' and reverse, 5'-AGAGCACCAAGA CTGGCTCT-3'; GAPDH forward, 5'-TTGGTATCGTGG AAGGACTCA-3' and reverse, 5'-TGTCATCATATTTGG CAGGTT-3'. PCR reactions were performed on the ABI7300 system (Applied Biosystems; Thermo Fisher Scientific, Inc., Sunnyvale, CA, USA). The relative expression fold change of mRNAs was calculated using the $2^{-\Delta\Delta Ct}$ method.

PVT1-siRNA and plasmid construction. The specific sequences of small interfering RNAs for PVT1 were: siPVT1-1 sense, GCUUGGAGGCUGAGGAGUUTT and antisense, AAC UCCUCAGCCUCCAAGCTT; siPVT1-2 sense, CCCAAC AGGAGGACAGCUUTT and antisense, AAGCUGUCCUCC UGUUGGGTT. They were synthesized by GE Healthcare Dharmacon (Lafayette, CO, USA). A non-specific siRNA

sequence was used as a negative control. For ectopic expression, we amplified the entire PVT1 sequence with RT-PCR using the following primers: Forward, TGCTCTAGACTC AAGATGGCTGTGCCTGTCAGCT and reverse, CCGGAA TTCAGTAGAAAAAGAATTTAATAGACACG, and then cloned it into pCD513B-1 (SBI Pharmaceuticals, Co., Ltd., Tikyo, Japan) at *Xba*I and *Eco*RI sites. All PCR products were confirmed by DNA sequencing.

Cell transfection. Cells in logarithmic growth phase were diluted and seeded at 2.5×10^5 cells/well into 6-well plates. The siRNAs or plasmid were transfected into pancreatic cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The cells were incubated in a humidified chamber for 48 h before use in the following assays.

Cell viability assay. A cell viability assay was performed using a CCK-8 kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) following the manufacturer's protocol. Cells were seeded in 96-well plates at a density of 5,000 cells/well and cultured with medium containing 10% FBS for 10, 24, 48 and 72 h. After the cells were washed twice with cold PBS, 10 μl CCK-8 and 100 μl serum-free medium were added into each well and the plates were incubated for a further 45 min at 37˚C. The absorbance was assessed at a wavelength of 450 nm using Wellscan MK-3 ELISA (Labsystems Dragon, Helsinki, Finland).

Western blotting. Protein was extracted from transfected cells with RIPA lysis buffer at 4˚C for 10 min. Equal amounts of protein were loaded and separated by 10% SDS-PAGE and transferred to PVDF membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat dried milk in TBST for 1 h at room temperature and then incubated with primary antibodies at 4˚C overnight. After washing with TBST, the membranes were further incubated for 1 h at room temperature with corresponding horseradish peroxidase-conjugated secondary antibody in an appropriate dilution (goat anti-mouse; 1:5,000; cat. no. A0216; and goat anti-rabbit; 1:10,000; cat. no. A0208) and then washed three times with the same buffer. The immunoreactive protein bands were visualized using an ECL kit (Pierce; Thermo Fisher Scientific, Inc.). The antibodies used were: rabbit anti-TGF-β (cat. no. 3712; Cell Signaling Technology, Inc., Danvers, MA, USA), rabbit anti-MMP2 (cat. no. YT2798) and rabbit anti-MMP9 (cat. no. YT1892; both from ImmunoWay Biotechnology Co., Plano, TX, USA), an EMT antibody sampler kit (cat. no. 9782), a Smad2/3 antibody sampler kit (cat. no. 12747), mouse anti-β-Tubulin (cat. no. 4466), and rabbit anti-β-actin (cat. no. 4970; all from Cell Signaling Technology, Inc.) and all above antibodies diluted with 1:1,000. β-actin and β-tubulin were used as internal controls. Signals were visualized using an enhanced chemiluminescence system.

Transwell migration and invasion assay. Briefly, migration assays were conducted using 8.0-μm culture insert chambers (EMD Millipore). Invasion assays were performed using the Corning® Matrigel® Invasion Chamber (Corning Inc., Corning, NY, USA). FBS-containing medium (10%) was placed in the
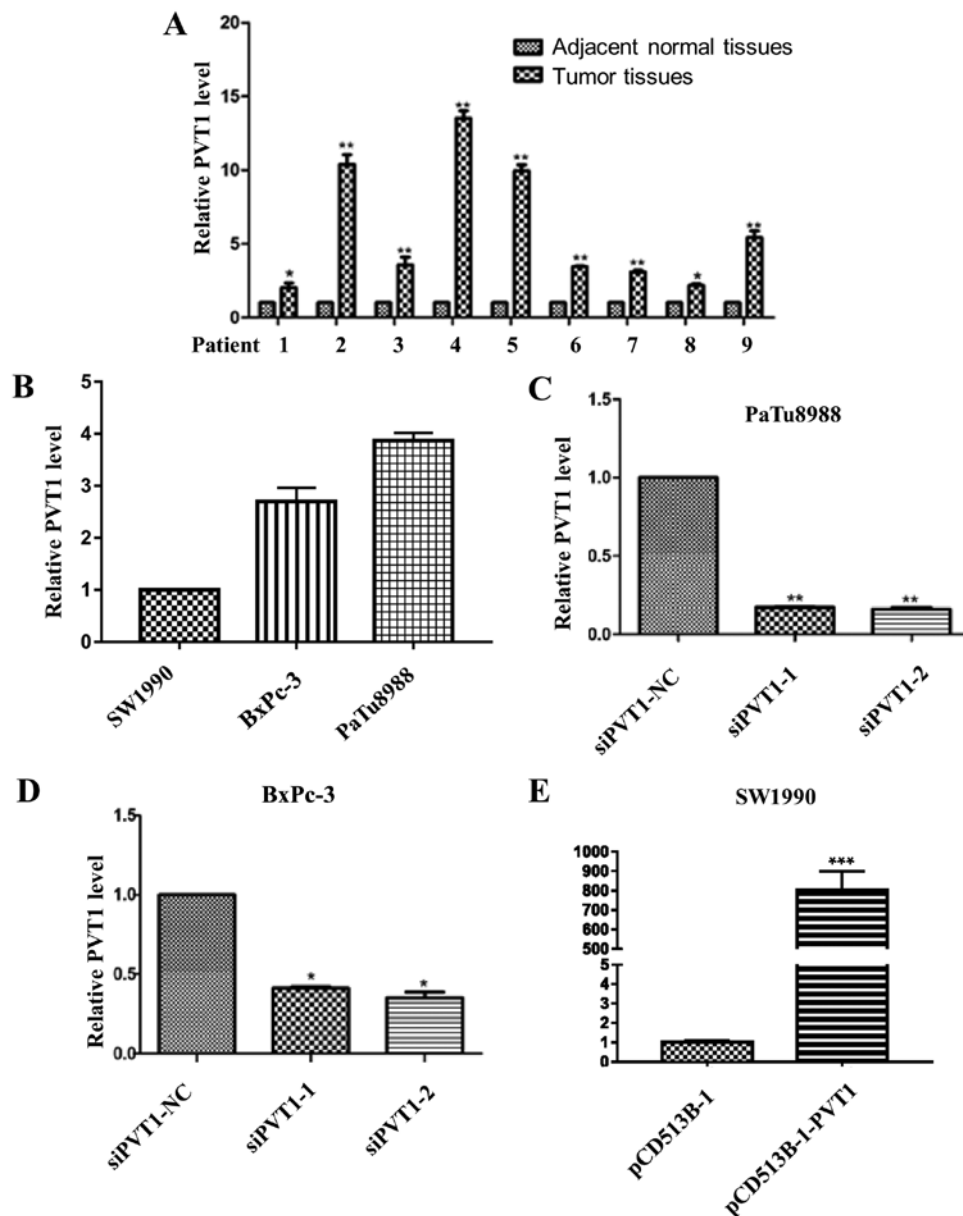
Figure 1. Detection of PVT1 expression in human pancreatic cancer tissues and cell lines. (A) The expression of PVT1 in 9 pairs of matched human pancreatic cancer tissues, as detected by qRT-PCR. (B) The mRNA expression of PVT1 in three human pancreatic cancer cell lines, SW1990, BxPc-3 and PaTu8988. (C and D) Identification of siRNA-mediated PVT1 knockdown in PaTu8988 and BxPc-3 cells by real-time-PCR analysis. GAPDH was used for normalization. (E) The mRNA level of PVT1 was detected in SW1990 cells transfected with pCD513B-1 and pCD513B-1-PVT1. Data are the mean ± SD from three independent experiments. *P<0.05, **P<0.01, ***P<0.001.

bottom chamber to act as a chemoattractant. Then, 5x10⁴ cells in a 100-μl volume of serum-free medium were placed in the upper chamber. Invasive cells on the lower surface of the membrane were those that had invaded the Matrigel or had migrated through the polycarbonate membrane. After incubation at 37˚C for 24 h, the cells were fixed in paraformaldehyde solution and stained with crystal violet. The top surface was gently scrubbed with a cotton bud and the remaining cells at the bottom surface were counted under a microscope (Olympus GX41; Olympus Corp. Tokyo, Japan) in five randomly selected fields at a magnification of x20.

Wound healing assay. Transfected cells were grown to 95% confluence. A wound was scratched in the monolayer with a 200-μl pipette tip. Suspended cells and debris were washed with PBS three times, then the cells were incubated in medium containing 2% FBS at 37˚C. At 0 and 24 h the wound image was captured using an OLYMPUS inverted microscope at a magnification of x100, respectively, to evaluate the cell migratory potential.

Adhesion assay. Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was diluted in serum-free media to a concentration of 0.04 μg/μl, and then 50 μl of this Matrigel was transferred into 96-well plates. Transfected cells (4x10⁴) in a 100-μl volume of serum-free medium were plated onto the Matrigel-precoated 96-well plates in triplicate. After incubation at 37˚C for 4 h, the medium was then carefully removed, and the plate was washed with PBS to remove the non-adherent cells. The Matrigel-precoated plate without cells served as the negative
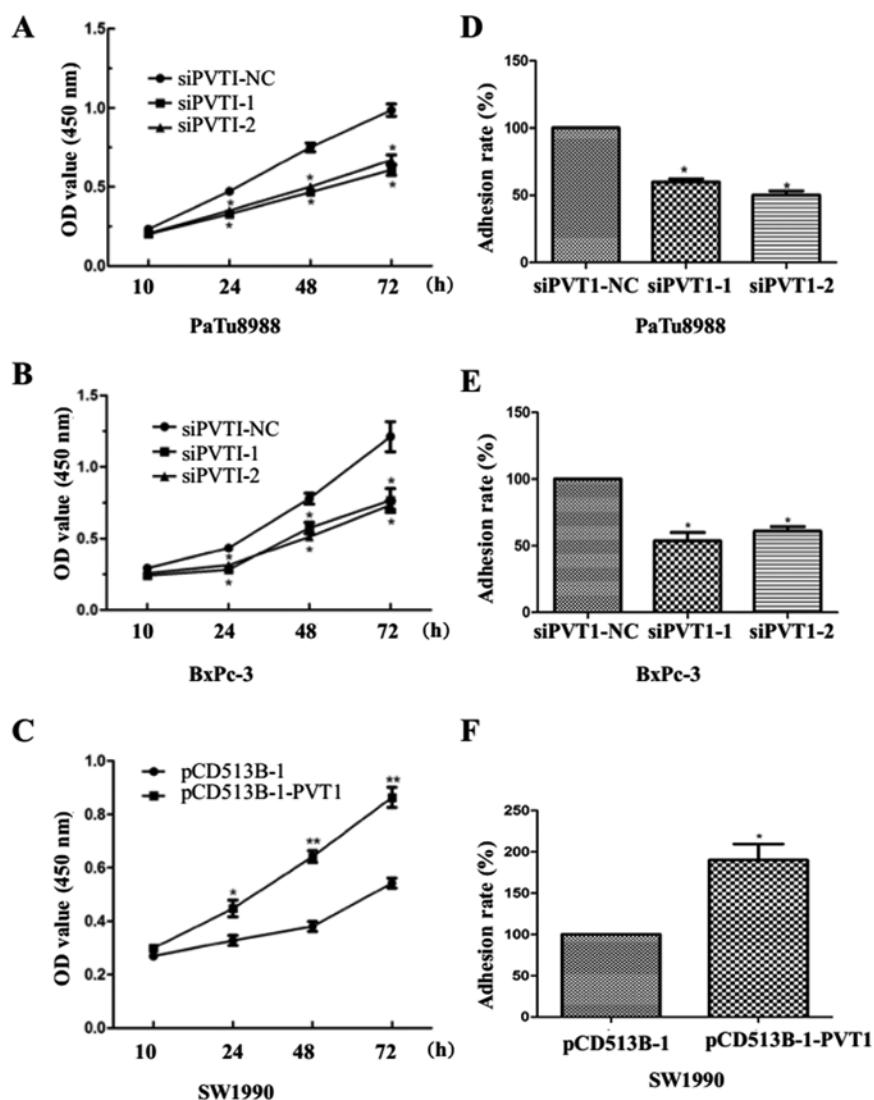
Figure 2. PVT1 promotes the viability and adhesion abilities of pancreatic cancer cells. (A and B) Cell viability was detected by the CCK-8 assay in PaTu8988 and BxPc-3 cells transfected with siPVT1-NC, siPVT1-1, siPVT1-2, respectively. (C) Cell viability was detected by the CCK-8 assay in SW1990 cells transfected with pCD513B-1 and pCD513B-1-PVT1. An adhesion assay was used to analyze the cell adhesion ability of (D) PaTu8988, (E) BxPc-3 and (F) SW1990 cells. Data are expressed as the mean ± SD from three independent experiments. *P<0.05, **P<0.01. The data were obtained from at least three independent experiments.

control. After washing, adherent cells were assessed with a CCK-8 kit (Dojindo Molecular Technologies, Inc.) following the manufacturer's protocol. The relative optical density (OD) value was determined at 490 nm using Wellscan MK-3 ELISA. The OD values reflected the proportion of cells in the Matrigel-coated 96-well plate.

Statistical analysis. All the presented data and results were confirmed in at least three independent experiments and were expressed as the mean ± SD. Statistical comparisons were performed using Student's t-test and one-way ANOVA. The data was analyzed using GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of PVT1 is profiled in human pancreatic cancer tissues and cell lines. To confirm the role of PVT1 in

pancreatic cancer, we first examined its expression profile in 9 pairs of matched human pancreatic tumors and adjacent normal tissues by qRT-PCR. The results indicated that the expression level of PVT1 in tumor tissues was significantly higher than that in adjacent normal tissues (Fig. 1A). In addition, it was revealed that PaTu8988 cells expressed the highest level of PVT1, whereas SW1990 cells expressed the lowest level of PVT1 among the 3 cell lines (Fig. 1B). Since PaTu8988 and BxPc-3 cells expressed a high level of PVT1, these two cell lines were selected to investigate the effect of specific depletion of PVT1 (Fig. 1C and D), and SW1990 was selected to study the effect of overexpression of PVT1 assays (Fig. 1E).

PVT1 promotes cell viability and the ability of adhesion in pancreatic cancer cells. To determine the role of PVT1 in pancreatic cancer cells, we utilized a CCK-8 assay to estimate cell viability. We found that PVT1 knockdown induced lower viability of PaTu8988 and BxPc-3 cells at 10, 24, 48 and 72 h after transfection (Fig. 2A and B). In contrast, PVT1
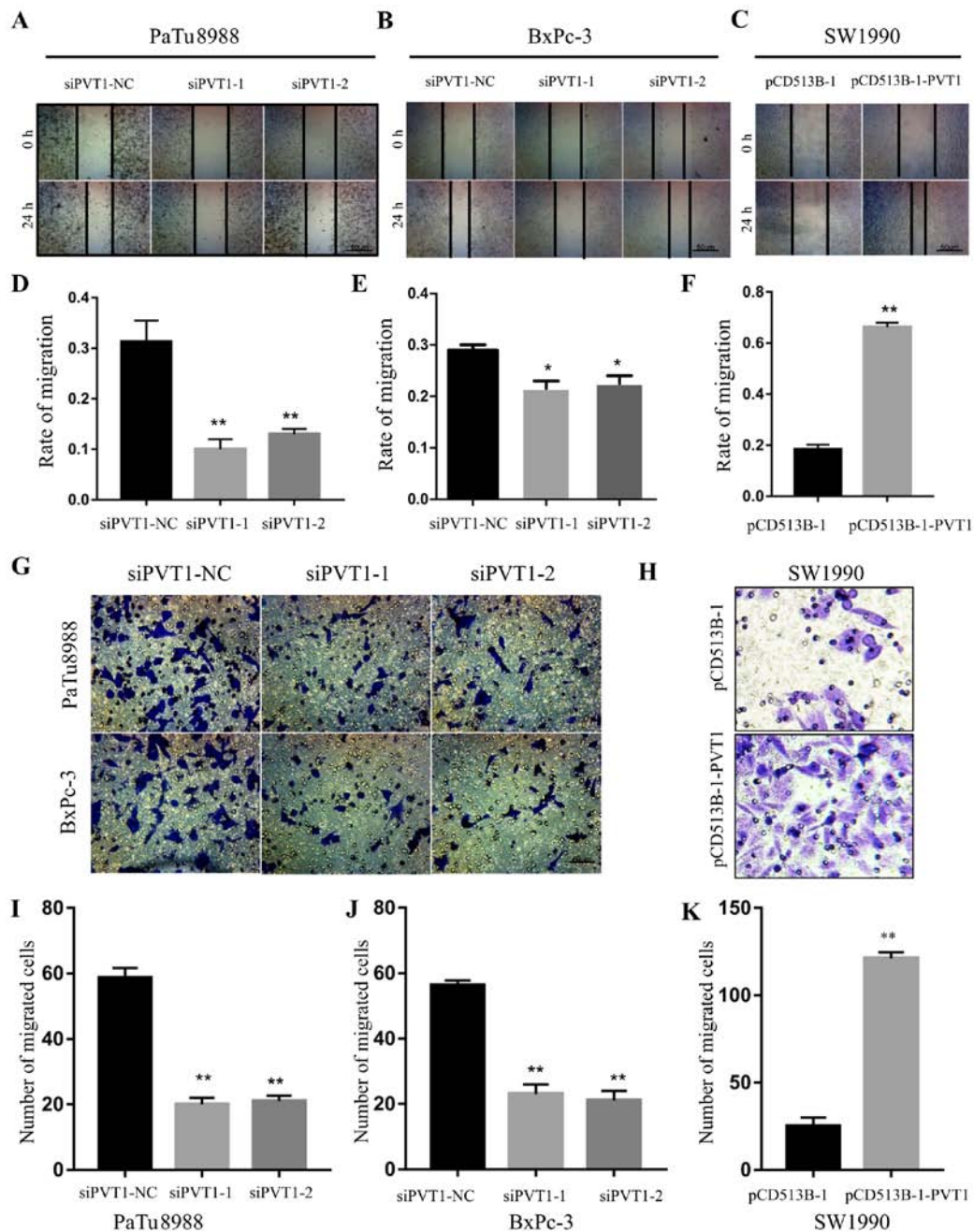
Figure 3. PVT1 promotes the ability of pancreatic cancer cell migration. A wound healing assay was used to evaluate the cell migration ability of (A) PaTu8988- and (B) BxPc-3 -transfected cells with siPVT1-NC, siPVT1-1, siPVT1-2 and (C) SW1990-transfected cells with pCD513B-1 and pCD513B-1-PVT1. Representative images of migrated cells are presented (x100). (D-F) The rate of migration was analyzed. *P<0.05, **P<0.01. (G) Effects of PVT1 knockdown on the migration of BxPc-3 and PaTu8988 cells was further examined by Transwell assay. (H) The migration of SW1990-transfected cells with pCD513B-1 and pCD513B-1-PVT1 was assessed by Transwell assay. Representative images of migrated cells are presented (magnification, x100). (I-K) The number of migrated cells was counted and analyzed. **P<0.01. The data were obtained from at least three independent experiments.

overexpression enhanced cell viability at 10, 24, 48 and 72 h in SW1990 cells (Fig. 2C). In addition, we found that PVT1 knockdown suppressed the ability of adhesion in PaTu8988 and BxPc-3 cells (Fig. 2D and E) while PVT1 overexpression enhanced the ability in SW1990 cells (Fig. 2F). Collectively, these results revealed that PVT1 may promote viability of pancreatic cancer cells and tumor cell adhererence to the extracellular matrix (ECM).

PVT1 enhances the migration ability of pancreatic cancer cells. Next, we examined the ability of

migration by wound healing assays in pancreatic cancer cells. The migration rate was 0.292±0.053, 0.125±0.012 and 0.143±0.005 (P<0.01) in siPVT1-NC, siPVT1-1 and siPVT1-2 PaTu8988 cells (Fig. 3A and D), and 0.291±0.002, 0.224±0.025 and 0.245±0.034 (P<0.05) in siPVT1-NC, siPVT1-1 and siPVT1-2 BxPc-3 cells, respectively (Fig. 3B and E), while 0.185±0.015 and 0.664±0.011 in pCD513B-1 and pCD513B-1-PVT1 (P<0.01) SW1990 cells (Fig. 3C and F). These results indicated that knockdown of PVT1 significantly decreased the migration ability of PaTu8988 and BxPc-3 cells. To confirm the aforementioned
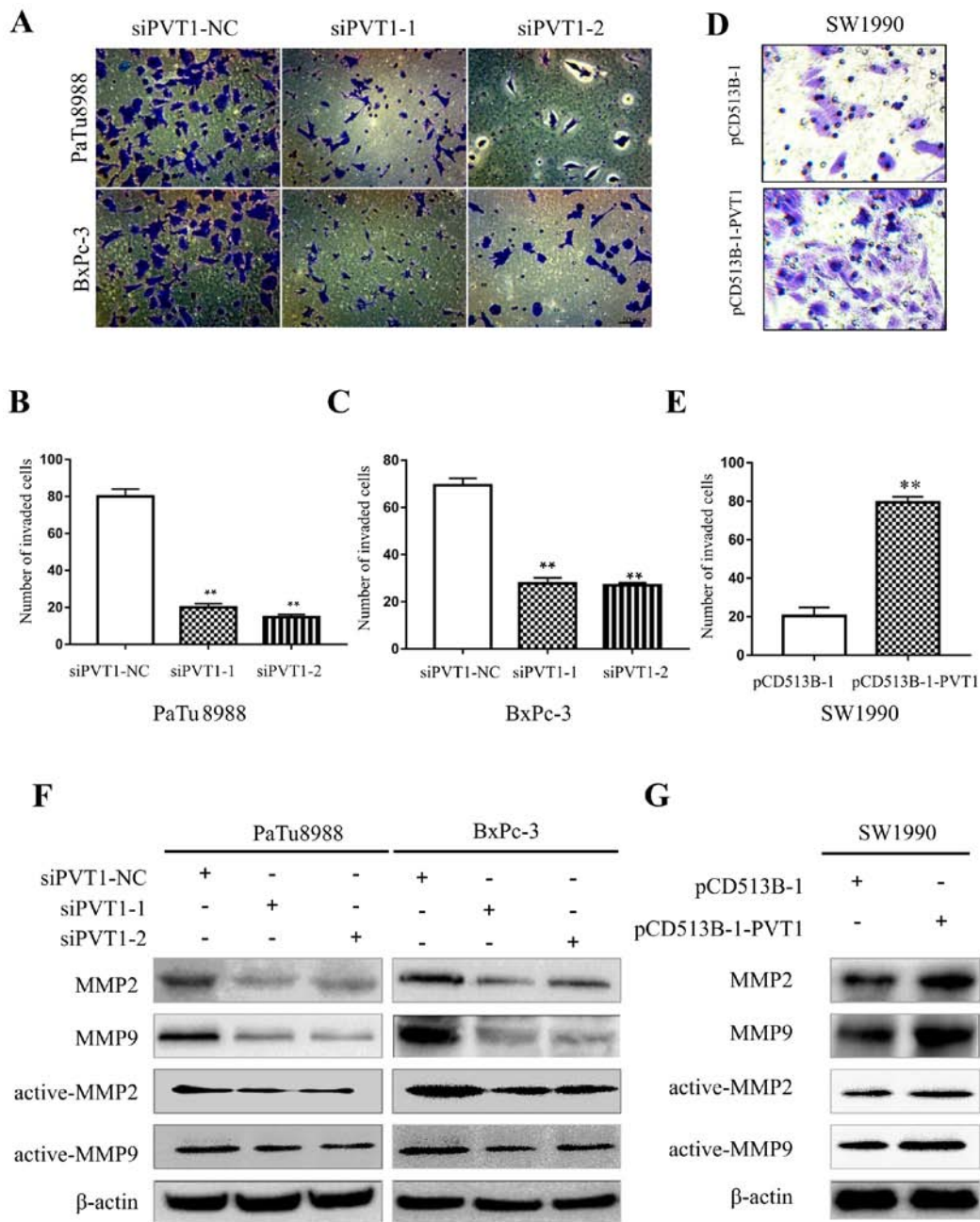
Figure 4. PVT1 enhances the invasion ability of pancreatic cancer cells. (A) The invasion ability was examined using BD Matrigel invasion assay in PaTu8988 and BxPc-3 cells transfected with siPVT1-NC, siPVT1-1, siPVT1-2. (D) SW1990-transfected cells with pCD513B-1 and pCD513B-1-PVT1. (B, C and E) Invasive cells were counted and analyzed. P<0.01. (F and G) MMP2 and MMP9 (active and total) were determined using western blotting. Data are expressed as the mean ± SD from three independent experiments.

results, we conducted Transwell assays to examine migration ability. The number of migrated cells was 58±12, 16±10 and 18±9 (P<0.01) in siPVT1-NC, siPVT1-1 and siPVT1-2 PaTu8988 cells, and 57±6, 20±8 and 18±9 (P<0.01) in siPVT1-NC, siPVT1-1 and siPVT1-2 BxPc-3 cells, respectively (Fig. 3G, I and J). Furthermore, the number of migrated cells was 20±10 and 127±12 (P<0.01) in pCD513B-1 and pCD513B-1-PVT1 SW1990 cells (Fig. 3H and K). All these data revealed that PVT1 promotes the ability of migration in pancreatic cancer cells.

PVT1 promotes the invasion ability of pancreatic cancer cells. Subsequently, we examined the ability of invasion

using BD Matrigel invasion assays in pancreatic cancer cells. PaTu8988 and BxPc-3 cells were transfected with siPVT1-NC, siPVT1-1 and siPVT1-2 plasmids, and SW1990 cells with pCD513B-1 and pCD513B-1-PVT1 for 72 h. The number of invasive cells was 82±5, 24±9 and 20±5 in siPVT1-NC, siPVT1-1 and siPVT1-2 PaTu8988 cells, and 68±3, 28±7 and 24±11 in siPVT1-NC, siPVT1-1 and siPVT1-2 BxPc-3 cells, respectively (Fig. 4A-C), demonstrating that downregulation of PVT1 significantly inhibited the invasion ability of PaTu8988 and BxPc-3 cells. In addition, the number of invasive cells was 20±5 and 80±8 in pCD513B-1 and pCD513B-1-PVT1 SW1990 cells, respectively (Fig. 4D and E) thus revealing that upregulation of PVT1 significantly promoted the
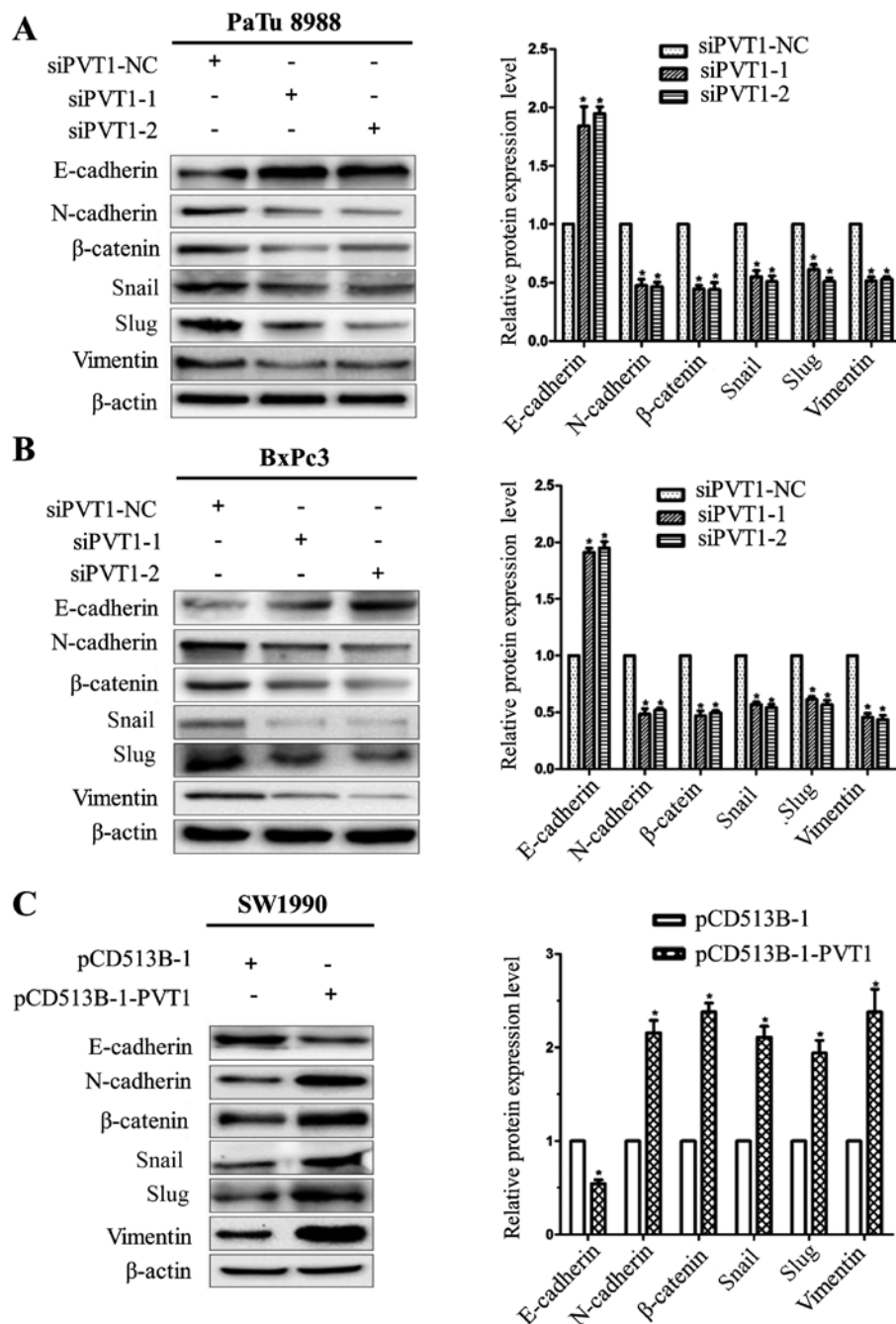
Figure 5. PVT1 promotes EMT in pancreatic cancer cells. (A and B) PVT1 knockdown in PaTu8988 and BxPc-3 cells reversed EMT, as detected by an increase in E-cadherin and decreases in vimentin, N-cadherin, β-catenin, Snail and Slug. (C) Treatment of SW1990 cells with pCD513B-1-PVT1 enhanced EMT compared to the control. β-actin was used as a loading control. Data are expressed as the mean ± SD from three independent experiments. *P<0.05.

invasion ability of SW1990 cells. Matrix-metalloproteinases (MMPs) have been confirmed to play pivotal roles in tumor cell invasion through adherence and degradation of the basement membrane and ECM (20). To determine whether PVT1 can regulate the activity of MMPs, we investigated the expression level of MMP2 and MMP9 through western blotting. In the PaTu8988 and BxPc-3 cells, knockdown of PVT1 suppressed MMP2 and MMP9 (active and total) protein levels (Fig. 4F). Conversely, in SW1990 cells overexpression of PVT1 increased MMP2 and MMP9 (active and total) protein levels (Fig. 4G). Collectively, these data revealed that PVT1 promotes the invasion ability of pancreatic cancer cells.

PVT1 enhances epithelial-mesenchymal transition in pancreatic cancer cells. During tumor development, tumor cells constantly communicate with the surrounding microenvironment, which can guide tumor cells to undergo a phenomenon termed EMT. EMT is regarded as a pivotal step for the promotion of tumor invasion and metastasis and plays a potential role in the progression of pancreatic cancer (21). Thus, we assessed whether PVT1 was involved in EMT to influence cancer metastasis. We examined the expression of EMT marker genes and EMT-related transcription factors using western blotting. PVT1-siRNAs increased E-cadherin expression and decreased vimentin, N-cadherin, β-catenin, Snail and Slug expression in PaTu8988 and BxPc-3 cells (Fig. 5A and B).
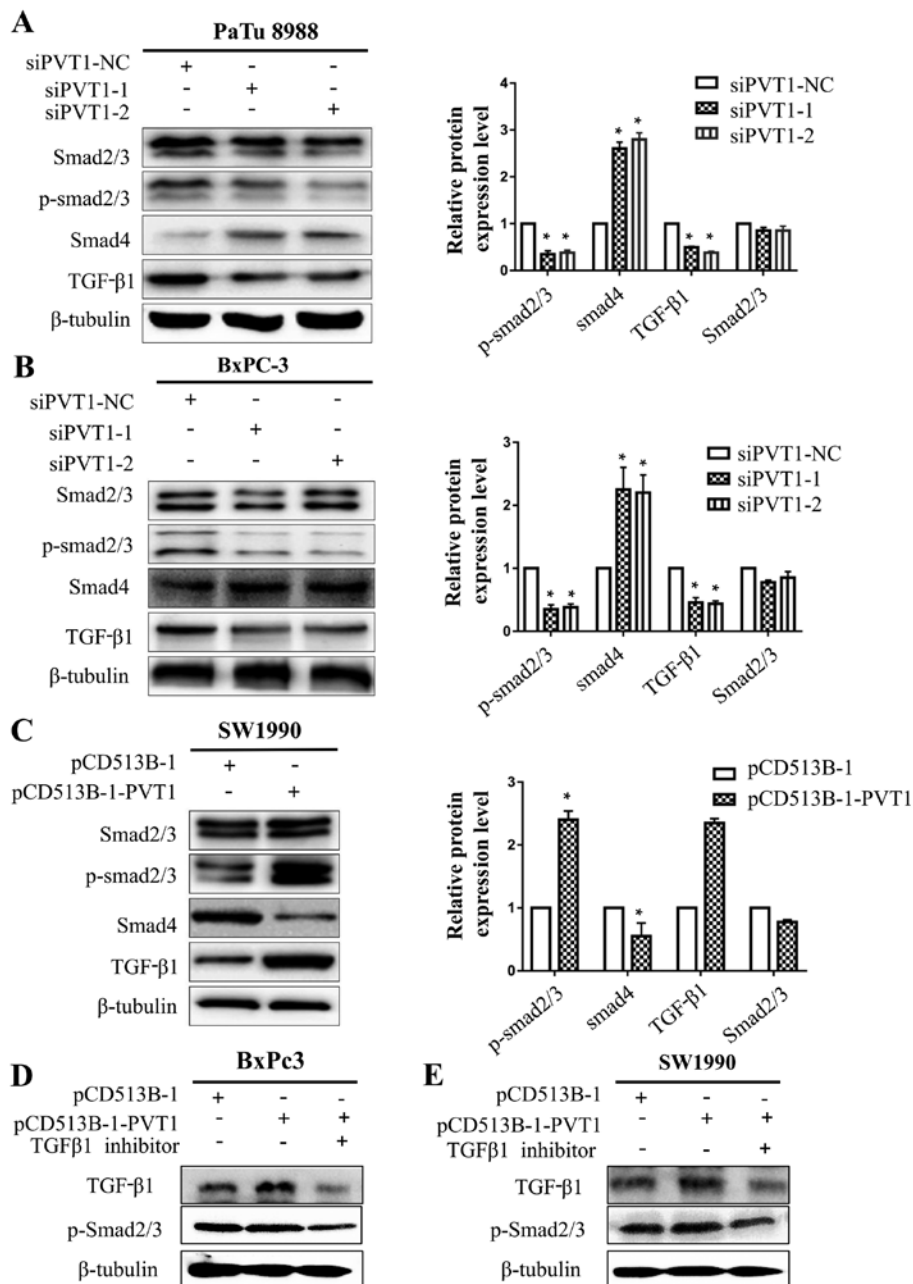
Figure 6. PVT1 enhances EMT via TGF-β/Smad signaling. (A and B) Immunoblotting of Smad2/3, p-Smad2/3, Smad4, TGF-β1 in PaTu8988 and BxPc-3 cells treated with siPVT1-NC, siPVT1-1, siPVT1-2. (C) Immunoblotting of Smad2/3, p-Smad2/3, TGF-β1 in SW1990 cells transfected with pCD513B-1-PVT1 or pCD513B-1. β-tubulin was used as a loading control. $^*P<0.05$. Data are expressed as the mean ± SD from three independent experiments. (D and E) TGF-β1 knockdown downregulated p-Smad2/3 after BxPc-3 and SW1990 cells were transfected with pCD513B-1-PVT1.

Conversely, ectopic expression of PVT1 decreased E-cadherin expression and increased vimentin, N-cadherin, β-catenin, Snail and Slug expression in SW1990 cells (Fig. 5C). These observations strongly demonstrated that PVT1 promoted a transition from epithelial to mesenchymal phenotype.

PVT1 activates EMT via TGF-β/Smad signaling in human pancreatic cancer cells. Multiple studies have suggested that the TGF-β/Smad signaling pathway is a central regulator in cancer cell viability, metastasis and the EMT process. Thus, we speculated that PVT1 could regulate TGF-β/Smad signal transduction in human pancreatic cancer cells, focusing on p-Smad2/3, Smad4 and TGF-β1 since they are pivotal signaling molecules in the

TGF-β/Smad signaling pathway. PVT1 knockdown increased Smad4 expression and decreased p-Smad2/3 and TGF-β1 expression in PaTu8988 and BxPc-3 cells (Fig. 6A and B). Conversely, SW1990 cells transfected with PVT1 expression plasmid decreased Smad4 expression and increased p-Smad2/3 and TGF-β1 expression (Fig. 6C). However, TGF-β1 knockdown downregulated p-Smad2/3 after we transfected BxPc-3 and SW1990 cells with pCD513B-1-PVT1 (Fig. 6D and E), indicating that PVT1 upregulates p-Smad2/3 mainly through TGF-β1 upregulation. Knockdown or overexpression of PVT1 had no influence on total Smad2/3 protein. These data revealed that PVT1 promoted EMT in pancreatic cancer cells possibly via TGF-β/Smad signaling.

Discussion

In the present study, we determined that PVT1 may function as an oncogene in pancreatic cancer. We revealed for the first time, to the best of our knowledge, that PVT1-induced expression of p-Smad2/3 represents a novel and critical role for controlling the growth and EMT in pancreatic cancer cells. All these findings revealed that PVT1 may be a potential candidate for the diagnosis of pancreatic cancer.

A previous study demonstrated that frequent mutation of the q24 band of chromosome 8(8q24) was associated with PVT1 expression in breast cancer (14). PVT1 expression is required for high Myc protein levels in 8q24-amplified human cancer cells (12). Our study confirmed that PVT1 expression was significantly increased in pancreatic cancer tissues compared to adjacent normal tissues, corresponding with the findings of Huang *et al* (19), which indicated that the reduced PVT1 expression may play an important role in the development of pancreatic cancer and may be a biomarker for the detection of cancer development and progression.

PVT1 can promote cervical cancer progression by epigenetically silencing miR-200b (22). Kong *et al* found that PVT1 promoted gastric cancer cell viability by epigenetically regulating p15 and p16 (23). In the present study, we confirmed that PVT1 stimulated oncogenic activities including the viability, adhesion, migration and the ability of invasion in pancreatic cancer cells consistent with previous studies in other tumors further supporting its oncogenic potential. Carcinoma cells can transition from an epithelial to mesenchymal differentiation state through a process known as EMT (24). The process of EMT is characterized by alterations in the pattern of gene expression, loss of epithelial cell junction proteins, such as E-cadherin, and an upregulation of mesenchymal markers, such as vimentin and N-cadherin (25). Loss of E-cadherin expression is considered as a key event during the induction of EMT (26). The present study confirmed that knockdown of PVT1 upregulated E-cadherin and downregulated vimentin, N-cadherin, β-catenin, Snail and Slug while PVT1 overexpression resulted in the downregulation of E-cadherin and upregulation of vimentin, N-cadherin, β-catenin, Snail and Slug. All these data revealed that PVT1 may drive typical epithelial phenotype cell transformation into spindle-shaped mesenchymal phenotype cells, promoting the ability of viability, migration and invasion in human pancreatic cancer cells.

It has been demonstrated that TGF-β acts as a potent driver of cancer progression through the induction of EMT (27). Upon stimulation by TGF-β1, Smad2/3 is activated by phosphorylation and along with Smad4, they are translocated into the nucleus to activate transcription of cancer cells, promoting EMT progression (28). Our results revealed that PVT1 promoted the growth and EMT through driving TGF-β/Smad signaling. As anticipated, PVT1 knockdown downregulated TGF-β1 and p-Smad2/3 while PVT1 overexpression upregulated active-TGF-β1, and then activated p-Smad2/3. Conversely, Smad4, a well-known tumor-suppressor gene and a major mediator of intracellular TGF-β signaling (29), was upregulated by knockdown of PVT1. These results demonstrated that knockdown of PVT1 promoted EMT via TGF-β signaling activation in pancreatic cancer cells. Notably, it is suggested that PVT1 plays an important role in treating cancer and may be a potential therapeutic target candidate in pancreatic cancer.

In conclusion, PVT1 plays a pivotal role in cell viability, adhesion, migration and invasion in pancreatic cancer cells. PVT1 may function as a key regulator of EMT in pancreatic cancer cells through the TGF-β/Smad signaling pathway. Therefore, PVT1 may prove to be clinically useful for developing a prognostic biomarker and therapeutic target for pancreatic cancer.

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

MX, XZ, WF and AG conceived, designed, coordinated the study and wrote the paper. MX, XZ and WF participated in all experiments. JZ performed and analyzed experiments shown in Fig. 1. LG, YZ, WP and DW performed and analyzed experiments in Figs. 2-4. WF and JZ performed and analyzed experiments shown in Figs. 5 and 6. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Signed informed consent was obtained from all patients, and the study was approved by the Ethics Committee of Changhai Hospital of Shanghai, Second Military Medical University.

Consent for publication

Not applicable.

Competing interests

The authors state that they have no competing interests.

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