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Original Articles

PDS5B regulates cell proliferation and motility via upregulation of Ptch2 in pancreatic cancer cells

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ABSTRACT

Pds5b (precocious dissociation of sisters 5B) is involved in both tumorigenesis and cancer progression; however, the functions and molecular mechanisms of Pds5b in pancreatic cancer (PC) are unknown. Several approaches were conducted to investigate the molecular basis of Pds5b-related PC progression, including transfection, MTT, FACS, western blotting, wound healing assay, transwell chamber invasion assay, and immunohistochemical methods. Pds5b overexpression inhibited cell growth and induced apoptosis, whereas the inhibition of Pds5b promoted growth of PC cells. Moreover, Pds5b overexpression inhibited cell migration and invasion, while the downregulation of Pds5b enhanced cell motility. Furthermore, reduced Pds5b expression was associated with survival in PC patients. Mechanistically, Pds5b positively regulated the expression of Ptch2 to influence the Sonic hedgehog signaling pathway. Consistently, Ptch2 downregulation enhanced cell growth, migration, and invasion, while inhibiting cell apoptosis. Notably, the downregulation of Ptch2 abolished Pds5b-mediated anti-tumor activity in PC cells. Strikingly, Pds5b expression was positively associated with levels of Ptch2 in PC patient samples, suggesting that the Pds5b/Ptch2 axis regulates cell proliferation and invasion in PC cells. Our findings indicate that targeting Pds5b and Ptch2 may represent a novel therapeutic approach for PC.

1. Introduction

Pancreatic cancer (PC) has significant mortality and poor prognosis compared to other types of human malignancies [1]. It is predicted that 56,770 new cases of PC will be diagnosed in the United States in 2019 and that approximately 45,750 patients will die of PC during this time [1]. Due to presentation of non-specific symptoms, PC patients are typically diagnosed at advanced stages, leading to fewer than 10% of patients experiencing curative surgical resection [2]. Therefore, the 5-year survival rate is only 10% for locally advanced cases, and 2% for metastatic patients who have this devastating disease [3]. Currently, computed tomography (CT), magnetic resonance imaging (MRI), and PET/CT are used to help diagnosis of PC [4]. CA19-9 (cancer antigen 19–9) is also useful for diagnosis [5,6]. The combination of Gemcitabine and synergistic chemotherapeutic agents (cisplatin, 5-FU, oxaliplatin, paclitaxel) fails to yield improved outcomes due to intrinsic and extrinsic drug resistance and therapy inefficacy [7,8]. Thus, it is urgent to develop novel therapeutic agents for the treatment of this deadly disease.

It has been reported that 5–10% of PC patients have a family history of this disease [9]. Aberrant gene expression and deregulation of signaling pathways are involved in PC development and progression. For example, the KRAS oncogene, TP53, cyclin-dependent kinase inhibitor 2A (CDKN2A), Smad family member 4 (SMAD4), and Notch-1 are commonly dysregulated in PC [10–14]. Recently, the hedgehog pathway is identified to play a critical role in pancreatic tumorigenesis and progression [15,16]. There are three hedgehog genes, sonic, desert and Indian. The hedgehog ligands bind to the Patched (Ptch) transmembrance protein, leading to the repression of the Smoothened receptor and subsequent activation of Gli zinc finger transcription factors [17]. The Hedgehog pathway is activated in pancreatic cancer cells and in Pdx1-Cre, LsL-Kras (G12D), and Ink4a/Arf (Lox/Lox) transgenic mice

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[18]. Moreover, Sonic hedgehog is upregulated in pancreatic cancer stem cells ($CD44^+$ CD24 + ESA+), suggesting that targeting sonic hedgehog could be of particular benefit for eradication of PC stem cells [19,20]. These reports reveal that the hedgehog pathway plays a critical role in PC development and progression. However, the regulatory mechanisms of the hedgehog pathway have not been fully elucidated in PC cells.

Multiple studies have demonstrated that precocious dissociation of sisters 5B (Pds5b), also called APRIN, is involved in DNA damage, sepsis, and carcinogenesis [21–23]. Topologically associating domains, chromatin loops, and sister-chromatid cohesion are partly regulated by Pds5b protein [24–26]. It has been reported that $Pds5b^{-/-}$ mice are embryonic lethal, suggesting that Pds5b is necessary in embryonic development [27]. Several studies have demonstrated that Pds5b is dysregulated in human cancers and associated with patient prognosis [21,28]. However, the physiological function and molecular mechanism of Pds5b in PC have not been determined. In the current study, we explored the role of Pds5b in PC cell growth, apoptosis, migration and invasion. We also identified Ptch2 as a target of Pds5b in PC cells. Moreover, we determined whether Pds5b exerts its biological function via regulation of Ptch2 in PC cells, revealing that Pds5b exhibits its antitumor activity via upregulation of Ptch2, one target gene of the hedgehog signaling pathway, in PC cells. Our study provides the rational for targeting Pds5b as a novel therapeutic target in PC.

2. Materials and methods

2.1. Cell culture, reagents and antibodies

Human pancreatic PaTu-8988 and Panc-1 cells were cultured in DMEM (Gibco, Gaithersburg, MD, USA), supplemented with 10% fetal bovine serum (FBS) in 5% CO_2 at 37 °C. 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma (St. Louis, Mo, USA). Transwell inserts and Matrigel were bought from BD Biosciences. *Anti*-Pds5b (Cat#: A300-537A) and *anti*-Vinculin antibodies were obtained from Bethyl laboratories. *Anti*-Ptch1 (#2468), *anti*-Ptch-2 (#2464), *anti*-Vinculin (#4650), and secondary antibodies were purchased from Cell Signaling Technology.

2.2. Transfection and luciferase assay

PC cells were seeded into six-well plates and transfected with the Pds5b cDNA constructor, or empty vector or Pds5b siRNA (GenePharma, Shanghai, China) using DharmaFect Transfection Reagent (Dharmacon, CO) following the manufacture's protocol [29]. The sequence is: siPds5b-1: 5′-GAA CAA UCA AUA GAU GGA-3'; siPds5b-2: 5′-GAG ACG ACU CUG AUC UUG U-3'; Pds5B complete cDNA was subcloned into an expression construct pOTENT-1. Cells were analyzed as described in Results section. HEK293 cells (5×10^4) were cultured in 24-well plates with DMEM overnight. Then, cells were transfected with 1 μg of each plasmid construct by Lipofectamine 3000. A Renilla luciferase plasmid (psiCHECK2-Ptch2-promoter) was co-transfected. After 24 h, cells were washed and harvested for Dual-luciferase reporter assay (Promega). Ptch2 promoter activity in the cells was evaluated with the luciferase reporter assay system, and luciferase activity was calculated in relative light units.

2.3. MTT assays

PC cells were cultured in 96-well plates (5×10^3 cells/well) overnight. Then, cells were transfected with Pds5b cDNA construct or Pds5b siRNA for different lengths of time. MTT assay was conducted as previously described [30].

2.4. Apoptotic death assay

PC cells were transfected with Pds5b cDNA construct or Pds5b siRNA as mentioned above. Then, cells were subjected to Annexin V-FITC/PI staining, and apoptosis was assessed by flow cytometry as previously described [31].

2.5. Transwell migration and invasion assays

Transwell migration and invasion assays were applied for measuring cell motility using 24-well plates with 8-μm pore size chamber inserts (Corning, New York, NY, USA) as previously described [32]. Migrated and invaded cells on the bottom surface of chambers were stained with Giemsa solution and were imaged and counted in 5 random fields for quantification.

2.6. Wound healing assays

Transfected PC cells were cultured in 6-well plates. When cells reached greater than 90% confluence, a pipette tip was used to create the scrape/wound. The generated floating cells were washed away using PBS. Wound healing was imaged at 0 and 16 h as previously described [29].

2.7. Real-time RT-PCR (RT-PCR)

Total cellular RNA was obtained using TRIzol Reagent (Invitrogen, CA, USA) following the manufacturer's protocol. Expression of PTCH1 and Ptch2 was determined using the SYBR green RT-PCR assay (Takara, Dalian, China) and normalized to GAPDH as previously described [33]. Primers used in PCRs are as follows: PTCH1, forward primer, 5′TGT GCG CTG TCT TCC TTC TG-3′, reverse primer, 5′ ACG GCA CTG AGC TTG ATT C- 3'; Ptch2, forward primer, 5′TGA TGA CCT CCA CTG CCC A-3′, reverse primer, 5′ATC AGC AAG AAG GTG CTC TG- 3′, GAPDH, forward primer, 5′CAG CCT CAA GAT CAG CA 3′, reverse primer, 5′TGT GGT CAT GAG TCC TTC CA 3'.

2.8. Western blotting analyses

Transfected PC cells were lysed using RIPA buffer supplemented with protease inhibitors. The protein concentrations were measured using a BCA protein assay. Proteins were separated by SDS–PAGE electrophoresis and transferred to PVDF membranes, which were blocked in 5% nonfat milk and immunoblotted with antibodies as previously described [34].

2.9. Human PC samples

Samples of human PC tissues and matched adjacent noncancerous tissues were obtained from Outdo Biotech Company (Shanghai, China). Immunohistochemical studies were performed to determine expression of Pds5b and Ptch2 in tumors.

2.10. Statistical analyses

Statistical comparisons were analyzed by Student's *t-test* for comparing two groups, and ANOVA was used for comparing multiple groups using GraphPad Prism 4.0 (Graphpad Software, La Jolla, CA) for cell growth, apoptosis, migration and invasion. The R^2 statistic was used to assess correlation between Pds5b and Ptch2 in clinical PC tumor samples. The error bars represent standard deviation (SD). $P < 0.05$ was considered statistically significant.

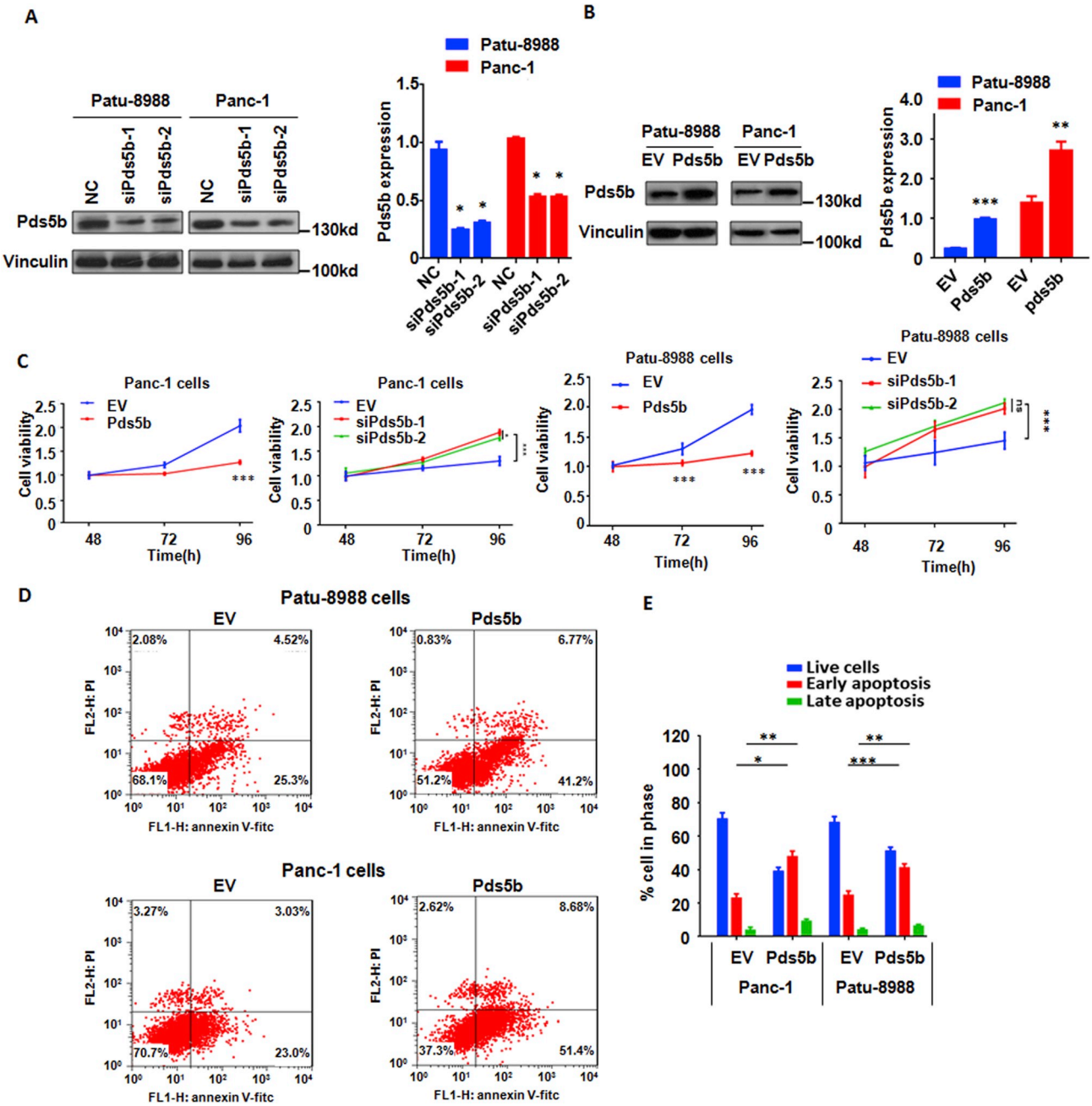

Fig. 1. Pds5b overexpression inhibits cell growth and induces cell apoptosis in PC cells.

A, Right panel: Western blotting analysis was performed to measure the expression of Pds5b in PC cells in response to Pds5b siRNA transfection. Left panel: Quantitative results are shown for top panel. *P < 0.001 vs control. NC: Negative control siRNA. siPds5b: Pds5b siRNA.

B, Right panel: Western blotting analysis was used to detect the expression of Pds5b in PC cells in response to after Pds5b overexpression. Left panel: Quantitative results are shown for top panel. **P < 0.05; ***P < 0.001 vs empty vector. vs control. EV: empty vector. Pds5b: Pds5b vector.

C, MTT assay was conducted in PC cells after Pds5b overexpression or inhibition. ***P < 0.001 vs control.

D, Apoptosis was measured in PC cells after Pds5b overexpression. E, Quantitaive data are shown for apoptosis in panel D.

3. Results

3.1. *Pds5b overexpression inhibits cell growth and induces apoptosis*

To investigate whether Pds5b regulates cell growth, Pds5b siRNAs were transfected into PC cells to downregulate the expression of Pds5b. Western blotting results revealed that downregulation of Pds5b by siRNAs reduced expression of Pds5b in PC cells (Fig. 1A). We also transfected a Pds5b cDNA construct into PC cells, observing elevated Pds5b protein abundance in response to Pds5b cDNA transfection in PC cells (Fig. 1B). Next, MTT assay was conducted to measure cell growth in PC cells after Pds5b cDNA or siRNA transfections. We found that upregulation of Pds5b suppressed cell growth, whereas downregulation of Pds5b promoted cell growth in PC cells (Fig. 1C). Consistent with these observations, Pds5b overexpression stimulated apoptosis in PC cells (Fig. 1D). Notably, Pds5b overexpression induced apoptotic cell death primarily in early apoptosis (Fig. 1E). Consistently, down-regulation of Pds5b inhibited cell apoptosis in both Panc-1 and Patu-8988 cells (Supplementary Fig. 1). Taken together, our results suggest that Pds5b overexpression inhibits cell growth and induces apoptosis in PC cells.

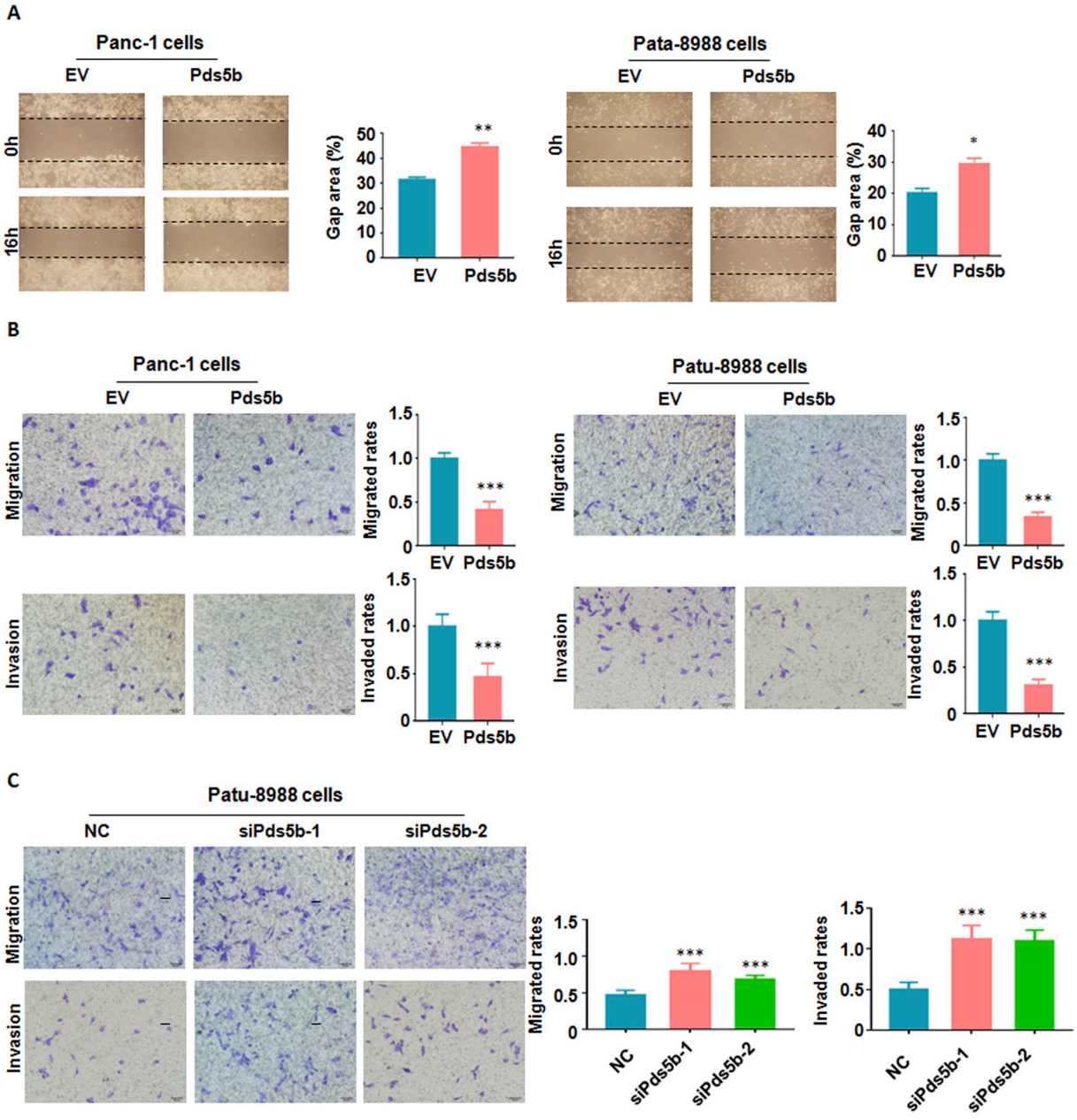

Fig. 2. Pds5b downregulation enhances cell motility in PC cells.
A, Left panel: Wound healing assay was performed to measure the migratory capacity in PC cells in response to Pds5b overexpression. Right panel: Quantitative results are shown for left panel. *P < 0.05; **P < 0.01 vs empty vector. EV: Empty vector. Pds5b: Pds5b vector.
B, Left panel: Migration and invasion assays were performed to measure the migratory capacity in PC cells in response to Pds5b overexpression. Right panel: Quantitative results are shown for left panel. ***P < 0.001 vs empty vector.
C, Left panel: Migration and invasion assays were performed to measure the migratory capacity in Patu-8988 cells in response to after Pds5b inhibition. Right panel: Quantitative results are shown for left panel. ***P < 0.001 vs control.

3.2. Pds5b downregulation enhances cell motility

To further dissect whether Pds5b governs the cell motility, a wound healing assay was tested in PC cells in response to the overexpression or downregulation of Pds5b. Our wound healing assay data revealed that overexpression of Pds5b inhibited cell migration, while downregulation of Pds5b increased the number of migrated cells in PC cells (Fig. 2A and Supplementary Fig. 2). To validate the function of Pds5b in migration and invasion of PC cells, a Transwell chamber assay was utilized to measure cell migration and invasion. We observed that Pds5b downregulation promoted cell migration and invasion, whereas Pds5b overexpression inhibited cell motility in PC cells (Fig. 2B and C). Taken together, these data show that Pds5b controls cell motility in PC cells, which could enhance tumor metastasis in the absence of Pds5b.

3.3. Pds5b expression is relatively lower in PC patients

To further examine the role of Pds5b in PC, immunohistochemical staining was used to detect expression of Pds5b in PC patients. We found that Pds5b was primarily localized to the nucleus, but not the cytoplasm or membrane of pancreatic cancer cells (Fig. 3A). Moreover, Pds5b expression in the nuclei of non-tumor tissues was observed. Consistently, most pancreatic adenocarcinomas expressed relatively low levels of Pds5b (Fig. 3B). The correlation between Pds5b protein

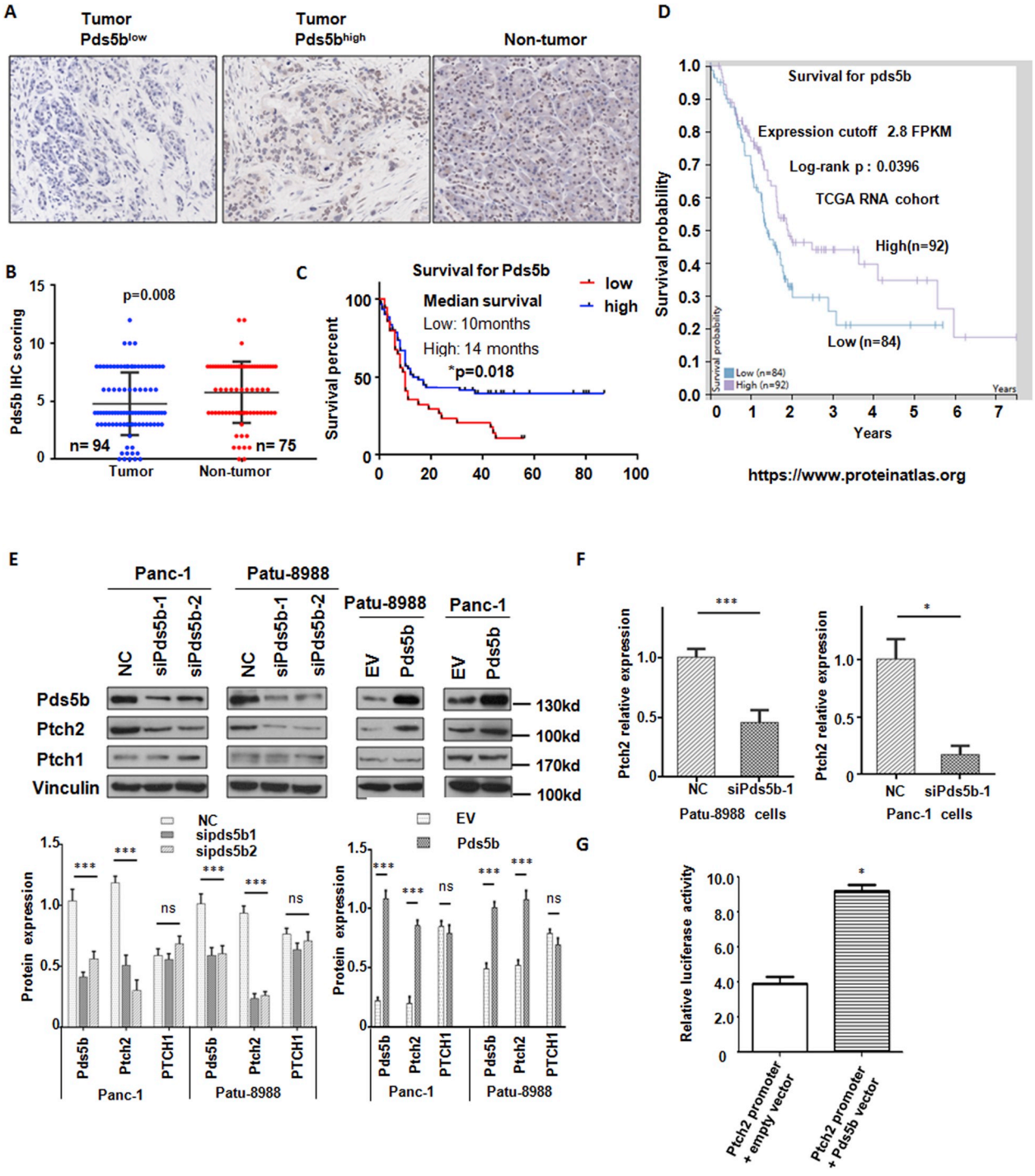

Fig. 3. Pds5b controls Ptch2 expression in PC cells.
A, Immunohistochemical staining of Pds5b protein in PC tissues.
B: Pds5b IHC scores in PC tumors and non-tumors tissues.
C: Survival curve for Pds5b in PC patients.
D, Pds5b is associated with patient survival in PC. The image was downloaded from the ProteinAtlas database.
E, Top panel: Western blotting analysis was used to detect the expression of Ptch2, Ptch1 and Pds5b in PC cells after Pds5b inhibition or overexpression. Bottom panel: Quantitative results are illustrated for top panel. ***P $<$ 0.001 vs control. NC: Negative control siRNA. EV: Empty vector. Pds5b: Pds5b vector.
F, Real-time RT-PCR was used to measure the mRNA level of Ptch1 and Ptch2 in PC cells after Pds5b siRNA transfection. G, Result of promoter report assay. Dual luciferase activity was measured by a luminometer at 24 h after transfection. *p $<$ 0.05 vs control.

expression and clinicopathological features of PC was then examined. We found that reduced expression of Pds5b was significantly associated with tumor metastasis. Specifically, among 56 patients with tumor metastasis, 42 cases had lower expression of Pds5b; whereas 20 tissues exhibited lower level of Pds5b from 38 tissues without metastasis. Notably, Pds5b expression is associated with survival in PC patients (Fig. 3C). This result is consistent with data derived from the protein atlas database (Fig. 3D). However, Pds5b protein expression was not associated with other clinicopathological features such as age, sex, tumor size, or tumor stage (data not shown). Our findings indicate that

Pds5b may represent a biomarker for tumor metastasis in PC patients.

3.4. Pds5b controls Ptch2 expression

Next, we mechanistically explored whether Pds5b regulates other cellular signaling pathways in PC cells to influence apoptosis and migratory abilities. Hedgehog signaling has been reported to be critically involved in pancreatic cancer development and progression [35]. Thus, we investigated whether there is a link between Pds5b and the hedgehog pathway. To this end, we measured expression of Ptch1 and Ptch2, two key molecules in the hedgehog pathway, in PC cells in response to Pds5b overexpression or inhibition. We found that Pds5b positively regulated Ptch2 expression (Fig. 3E). Inhibition of Pds5b reduced the protein abundance of Ptch2, but not Ptch1, in PC cells (Fig. 3E). In support of this notion, overexpression of Pds5b increased protein levels of Ptch2, but not Ptch1, in PC cells (Fig. 3E). Furthermore, our RT-PCR results showed that Pds5b downregulation reduced mRNA levels of Ptch2, but not Ptch1, in PC cells (Fig. 3F and supplementary 3). Furthermore, luciferase reporter activity of Ptch2 gene promoter was significantly increased in cells co-transfected with Pds5b (Fig. 3G). This result suggests that Pds5b may be a positive regulator of the Ptch2 promoter. Our findings provide evidence for the notion that Pds5b is a critical node influencing Ptch2 expression levels that potentially governs downstream activity of the hedgehog signaling pathway. In summary, our findings indicate that Pds5b governs Ptch2 expression in PC cells.

3.5. Downregulation of Ptch2 promotes cell growth and invasion in PC cells

To explore the role of Ptch2 in PC cell growth, MTT assay was performed in Ptch2 siRNA-transfected PC cells. We found that downregulation of Ptch2 enhanced cell growth in PC cells (Fig. 4A). We further observed that Ptch2 downregulation inhibited apoptosis in PC cells (Fig. 4B). Results from the wound healing assay revealed that downregulation of Ptch2 inhibited cell migration in both Panc-1 and Patu-8988 cells (Fig. 4C). Moreover, transwell chamber assay data demonstrated that Ptch2 downregulation retarded cell migration and invasion in PC cells (Fig. 4D). Altogether, our data indicate that Ptch2 is a tumor suppressor in PC.

3.6. Downregulation of Ptch2 rescues Pds5b-mediated tumor suppressive effects in PC cells

To further determine whether Pds5b exerts its tumor suppressive function via upregulation of Ptch2, PC cells were transfected with Pds5b cDNA in combination with Ptch2 siRNA. We found that downregulation of Ptch2 abrogated Pds5b-induced cell growth inhibition (Fig. 5A). Moreover, we observed that Ptch2 downregulation abolished Pds5b-triggered cell apoptosis (Fig. 5B). Our western blotting data revealed that Ptch2 downregulation was rescued by Pds5b overexpression in PC cells (Fig. 5C). Consistently, the downregulation of Ptch2 also abrogated Pds5b-mediated suppression of Smo expression (Fig. 5C). Strikingly, the downregulation of Ptch2 rescued Pds5b-triggered inhibition of cell migration in PC cells (Fig. 5D). Similarly, Ptch2 inhibition abolished Pds5b-mediated inhibition of cell invasive activity (Fig. 5D). Altogether, these results coherently demonstrate that Pds5b exerts its anti-tumor activity in part via regulation of Ptch2 in PC cells.

3.7. Ptch2 expression is reduced in PC patients

To further define the role of Ptch2 in PC, immunohistochemical staining was performed to measure levels of Ptch2 in PC tumor tissues. We found that Ptch2 levels were reduced in tumor tissues, which also have reduced Pds5b expression (Fig. 6A and B). We also found that Pds5b expression was associated with Ptch2 levels in PC tissues (data not shown). Importantly, we found that Ptch2 levels were associated with patient survival (Fig. 6C), which is consistent with results from the Protein Atlas database (Fig. 6D). These results demonstrate that Pds5b might be a major upstream regulator governing Ptch2 activity in PC cells.

4. Discussion

Accumulating evidence has demonstrated that Pds5b is involved in the development and progression of human cancers. Pds5b expression levels are associated with histological grade and chemotherapeutic outcome in breast cancer [36]. Reduced Pds5b expression levels are associated with a better survival in ovarian cancer patients [21]. Moreover, depletion of Pds5b sensitizes cells to Olaparib, a poly ADP-ribose polymerase (PARP) inhibitor, in xenograft zebrafish [21]. In line with this, high expression of Pds5b is observed and correlates with unfavorable survival in gastrointestinal stromal tumor [28]. Notably, the loss of Pds5b is identified in gastric and colorectal cancers due to frameshift mutations or high microsatellite instability [22]. In addition, loss of Pds5b disrupts stem cell programs in embryonal carcinoma, comprising a novel mechanism of Pds5b in tumor suppression [37]. Zhou et al. reported that overexpression of Pds5b inhibited proliferation and promoted apoptosis in embryonal carcinoma cells [38]. Interestingly, overexpression of Pds5b was observed in oral squamous cell carcinoma, but was not correlated with cell proliferation index by ki-67 or p53 [39]. Our study validate that reduced Pds5b expression is observed in PC and is associated with survival in PC patients, indicating that Pds5b may represent a biomarker for prognosis of PC patients.

One study demonstrated that overexpression of Pds5b suppressed cell proliferation and induced apoptosis in embryonal carcinoma cells [38]. Another study found that loss of Pds5b disrupted stem cell programs in embryonal carcinoma, leading to tumor suppression [37]. Interestingly, Pds5b stimulated the proliferation of multiple myeloma [40]. Since the function of Pds5b in PC has not been validated, we investigated the effects of Pds5b in PC cells. We found that the overexpression of Pds5b led to inhibition of cell growth and invasion, whereas the downregulation of Pds5b resulted in promotion of cell growth and motility, indicating that Pds5b functions as a tumor suppressor in PC. Our study provides new evidence for the antitumor activity of Pds5b in PC cells.

The precise mechanism for how Pds5b regulates cell growth and invasion still remains elusive. One study reported that miR-27a was directly upregulated by androgen receptor (AR), and repressed Pds5b in prostate cancer [41]. One study showed that miR-223 regulated cell proliferation and invasion via inhibition of Pds5b in pancreatic cancer cells [42]. Pds5b was also identified as a breast cancer 2 (BRCA2)-associated protein in breast cancer, a necessary element of the normal response to DNA damaging agents [36]. In this study, we validate that Pds5b upregulates Ptch2 in PC cells. Recent studies have highlighted the important role of Ptch2 in carcinogenesis. For instance, frameshift mutation in the Ptch2 gene causes nevoid basal cell carcinoma syndrome [43]. Ptch2 mutations are also observed in pancreatic neuroendocrine neoplasms [44]. Moreover, Ptch2 loss drives myeloproliferation and myeloproliferative neoplasm progression [45]. Recently, one study showed that Ptch2 modulates tumorigenesis linked to a Ptch1 mutation and is likely correlated with the congenital onset of rhabdomyosarcoma [46]. Herein, we found that Pds5b positively regulated Ptch2 in PC cells. Targeting the hedgehog pathway has been reported to be useful in clinical trials. For example, GDC-0449, IPI-926 and XL139, which target the smoothened receptor, have been shown tumor efficacy in various types of cancer cells. GANT inhibitors, which target Gli-mediated gene transcription, include GANT 58 and GANT 61. Due to Pds5b regulating Ptch2, targeting Pds5b could be useful for the treatment of PC patients with dysregulated hedgehog signaling. Taken together, we report that the Pds5b/Ptch2 axis is important for PC progression. Targeting this axis may represent a useful approach for treating PC. For example, using miR-223 inhibitor to upregulate Pds5b

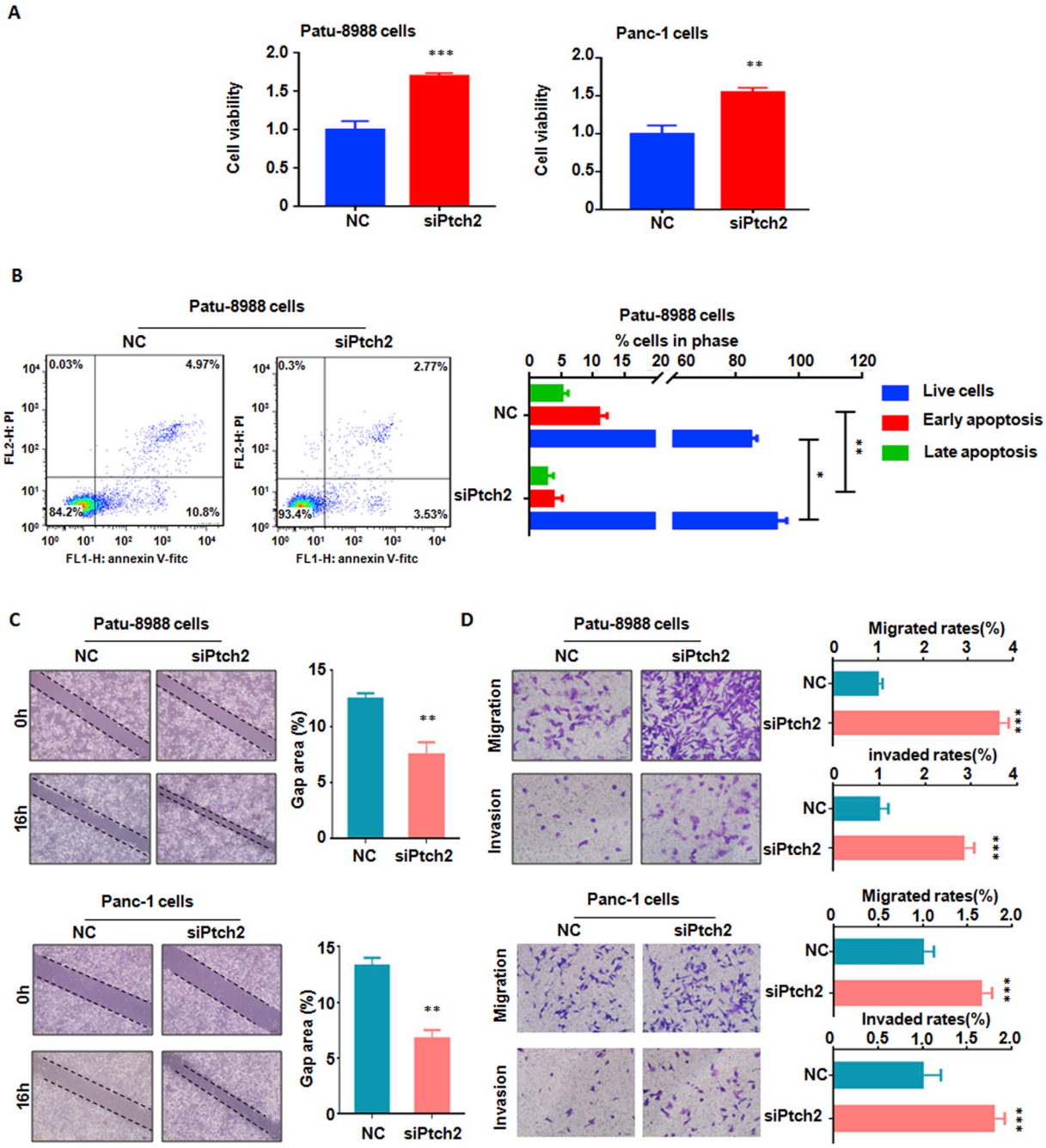

Fig. 4. Ptch2 downregulation enhances cell growth and invasion of PC cells.
A, MTT assay was conducted in PC cells after Ptch2 siRNA transfection. **P < 0.01, ***P < 0.001 vs control. NC: Negative control siRNA. siPtch2: Ptch2 siRNA.
B, Apoptosis analysis was performed in PC cells after Ptch2 siRNA transfection. *P < 0.05, **P < 0.01 vs control.
C, Left panel: Wound healing assay was performed to measure the migratory capacity in PC cells in response to Ptch2 siRNA transfection. Right panel: Quantitative results are shown for left panel. **P < 0.01 vs control.
D, Left panel: Migration and invasion assays were performed to measure the migratory capacity in PC cells after Ptch2 siRNA transfection. Right panel: Quantitative results are shown for left panel. ***P < 0.001 vs control.

is a potential strategy for PC treatment. In summary, our findings unravel new molecular mechanism of Pds5b-mediated tumor suppression in PC cells. This study further indicates that the upregulation of Pds5b and Ptch2 may represent a novel therapeutic strategy for treating PC.

Author contributions

J.M. conceived of the work, designed and performed the experiments, analyzed the data, and wrote the manuscript. Y.C., T.C., H.X. and Y.S. designed and performed experiments and analyzed the data. J.X. and Y.T. analyzed data. P.W. wrote the manuscript and critically viewed and supervised the study.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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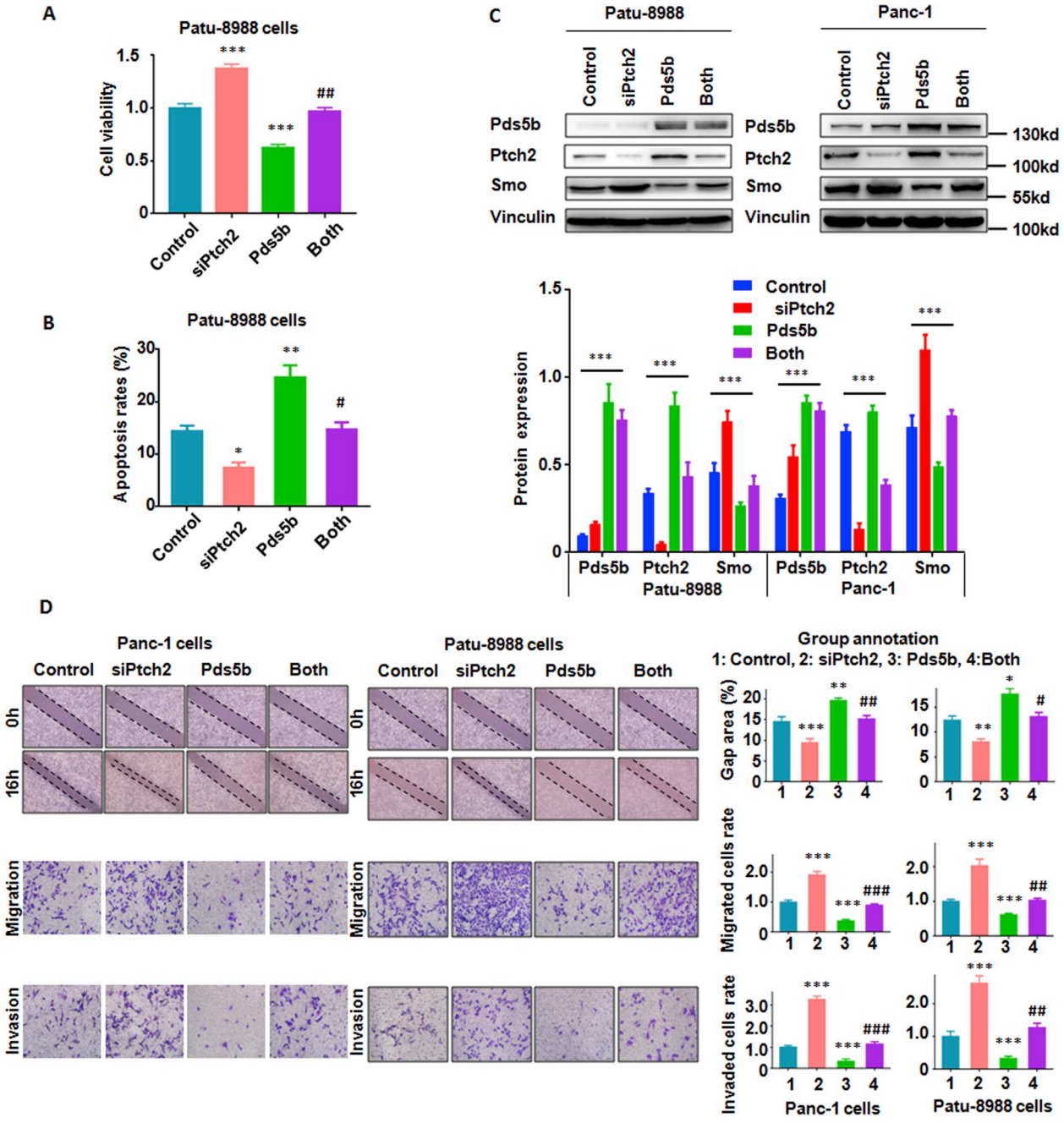

Fig. 5. Downregulation of Ptch2 rescues Pds5b-mediated tumor suppressive effects on PC cells.

A, MTT assay was conducted in PC cells after both Ptch2 siRNA transfection and Pds5b overexpression. ***P < 0.001 vs control; ##P < 0.01 vs Ptch2 siRNA transfection or Pds5b overexpression alone. Control: Negative control siRNA plus empty vector. siPtch2: Ptch2 siRNA; Pds5b: Pds5b vector; Both: Ptch2 siRNA plus Pds5b vector.

B, Apoptosis assay was performed in PC cells after Ptch2 siRNA transfection plus Pds5b overexpression. *P < 0.05; **P < 0.01 vs control; #P < 0.05 vs Ptch2 siRNA transfection or Pds5b overexpression alone.

C, Top panel: Western blotting analysis was performed to measure the expression of Pds5b, Ptch2, Smo in PC cells in response to Pds5b siRNA transfection and Pds5b overexpression. Bottom panel: Quantitative results are shown for top panel. ***P < 0.001 vs control.

D, Top-left panel: Wound healing assay was conducted in PC cells after both Ptch2 siRNA transfection and Pds5b overexpression. Middle-left: Migration assay was done in PC cells after both Ptch2 siRNA transfection and Pds5b overexpression. Bottom-left: Invasion assay was performed in PC cells after both Ptch2 siRNA transfection and Pds5b overexpression. Right panel: Quantitative results are shown for left panel. *P < 0.05; **P < 0.01 vs control; #P < 0.05, ##P < 0.01, ###P < 0.001 vs Ptch2 siRNA transfection or Pds5b overexpression alone.

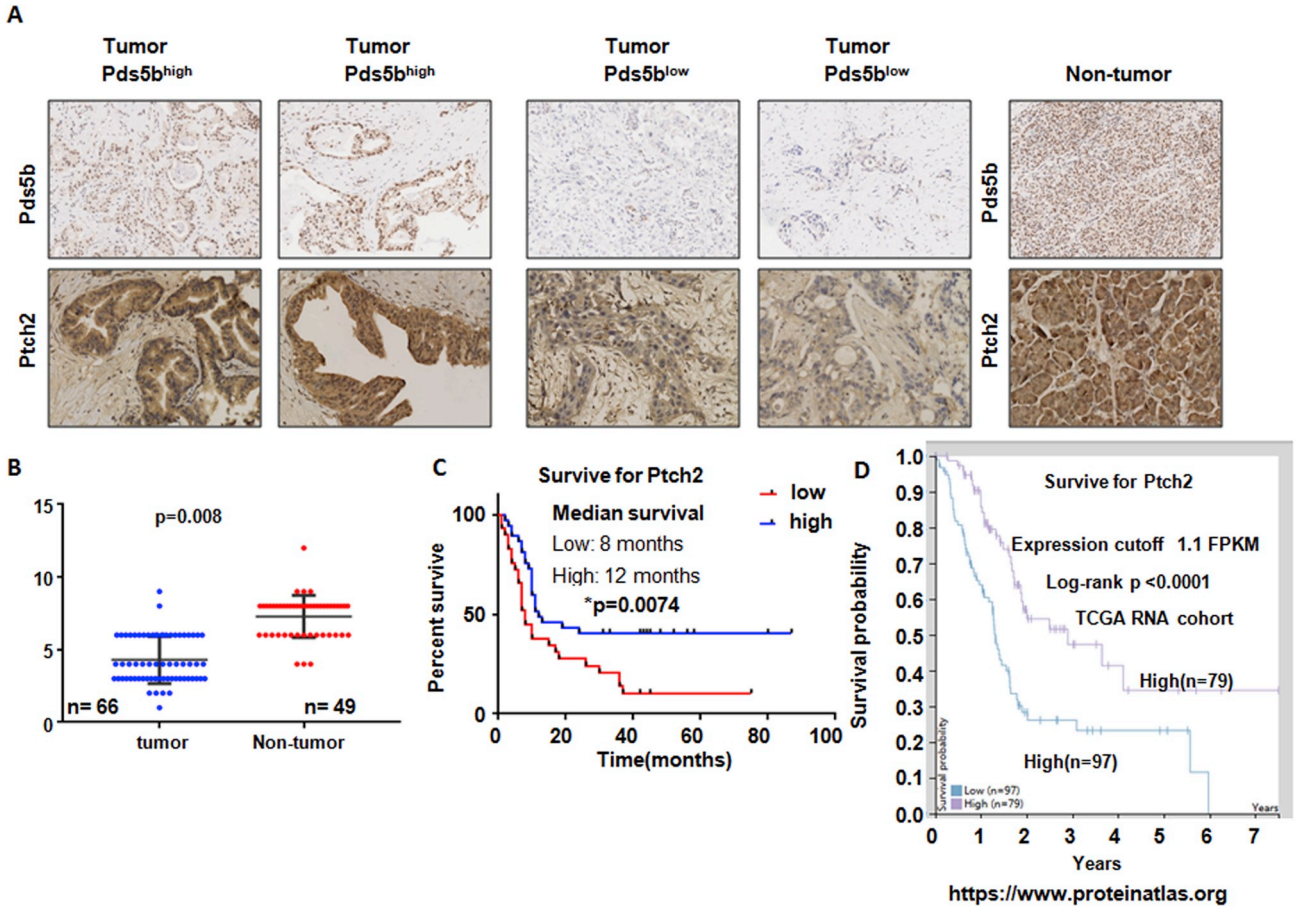

Fig. 6. Ptch2 expression in PC patients.
A, Immunohistochemical staining images of Pds5b and Ptch2 in PC tissues.
B, Ptch2 IHC scores in PC tissues and non-tumor tissues.
C, Survival curve for Ptch2 in PC patients.
D, Ptch2 is associated with patient survival in PC. The image was downloaded from the ProteinAtlas database.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.06.014.

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