PTPN3 inhibits the growth and metastasis of clear cell renal cell carcinoma via inhibition of PI3K/AKT signaling

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Abstract

Background: The underlying molecular mechanism driving clear cell renal cell carcinoma (ccRCC) progression is not fully understood. The significant downregulation of protein tyrosine phosphatase non-receptor type 3 (PTPN3) expression in the tumor tissues suggested its protective role in ccRCC progression.

Methods: Immunohistochemical analysis of PTPN3 protein in 172 ccRCC tissue revealed that PTPN3 expression was an independent, favorable prognostic factor for overall survival (P = 0.0343) and distant metastasis-free survival (P = 0.0166) of patients. The ccRCC cell lines SN12C, 1932, ACHN and Caki-1 were used to evaluate, both in vitro and in vivo , the biological roles of PTPN3.

Results: We observed that overexpression of PTPN3 significantly inhibited the proliferation, migration, and invasion of ccRCC cells. In contrast, the knocking down of PTPN3 elicited opposite effects. PTPN3 overexpression suppressed xenograft tumor growth and lung metastasis in vivo mice models. PTN3 inhibited tumor cell motility by suppressing the phosphorylation of AKT, and subsequently inactivating the PI3K/AKT signaling pathway of ccRCC cells. Further, the inhibition of phospho-AKT Thr308 and phospho-AKT Ser473 reversed PTPN3 induced-silencing in tumor cell migration. Our work revealed that the overexpression of PTPN3 could suppress kidney cancer progression by negatively regulating the AKT signaling pathway, and served as a favorable prognostic factor in ccRCC patients.

Conclusions: Our findings provided insight that PTPN3 could be a potential target for therapy aiming to inhibit the malignant behaviors of ccRCC.

Background

Kidney cancer represents about 4% of all new cancer diagnoses [1]. The most common form of kidney cancer arises from renal epithelium, termed as renal cell carcinoma (RCC) [2]. Clear cell renal cell carcinoma (ccRCC) is the most common histological subtype of RCC, accounting for 70%–75% of the cases [3]. The worldwide incidence and mortality rate of ccRCC has been estimated of rising by 2–3% per decade [4]. Although a higher incidence of small renal masses is being detected, approximately one-third of the patients still present with or will develop metastatic lesions during the course of their disease [5] but treating them still remains a challenge as the detailed mechanism(s) of metastasis is yet to be elucidated [6, 7].

The phosphoinositide 3-kinase (PI3K)/protein kinase B (also named AKT) signaling pathway has been shown to regulate a series of cellular processes, including cell cycle, proliferation, apoptosis, autophagy, and metastasis, in various types of cancer [8, 9], and several potential molecular targets of the PI3K/Akt signaling pathway have been proposed in cancer therapy [10], which have also been demonstrated to be highly activated and serving critical roles in ccRCC [11, 12].

Reversible tyrosine protein phosphorylation by protein tyrosine kinases and protein tyrosine phosphatases (PTPs) acts as a molecular switch that regulates a variety of biological processes [13]. PTPN3, a membrane-associated nonreceptor of PTP, contains an N-terminal FERM domain, a middle PDZ domain, and a C-terminal phosphatase domain [14], and these three domains have been implicated in the regulation of cell growth and proliferation [15]. As a tyrosine phosphatase, PTPN3 is capable of dephosphorylating several substrates, for instance, the epidermal growth factor receptor (EGFR) pathway substrate 15 [13]. However, its role as a receptor protein tyrosine kinase signaling is still unclear.

Accumulating evidence have suggested that PTPN3 plays an equivocal role in the progression of a variety of human cancers [13, 14, 16]. Li et al found that the ectopic expression of PTPN3 in non-small-cell lung cancer cells inhibited cell proliferation, migration, and tumor growth through the regulation of EGFR endocytic trafficking, degradation and signaling [13]. On the contrary, Hou et al showed that PTPN3 may cooperate with its substrate, p38γ, to increase malignant growth [16]. Gao et al also found that numerous activating mutations and high expression levels of PTPN3 were associated with intrahepatic cholangiocarcinoma recurrence [14]. Thereby, the exact role of PTPN3 in ccRCC remains undetermined.

In this study, through the findings of various investigations, we report that PTPN3 could suppress ccRCC progression by negatively regulating the AKT signaling pathway, providing insight that PTPN3 could be a potential target for therapy aiming to inhibit the malignant behaviors of ccRCC.

Methods

Cell lines and culture

Originally bought from ATCC, ccRCC cell lines SN12C, 1932, Caki–1, ACHN were routinely maintained in liquid nitrogen in our laboratory [17, 18]. The cell lines were cultured in DMEM or 1640 (Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Waltham, MA, USA) with 100 IU/mL of streptomycin and 100 ug/ml of penicillin in a humidified atmosphere of 5% CO_2 at 37 °C.

Patients tissue samples

A total of 12 ccRCC tissues and matched para-cancerous tissues were collected from the Department of Urology, Sun Yat-sen University Cancer Center (SYSUCC, Guangzhou, China) and stored at − 80 °C before use for quantitative real-time reverse-transcription PCR (qRT-PCR) analysis. Another cohort of 172 formalin-fixed, paraffin-embedded primary ccRCC specimens obtained from the Department of Pathology (SYSUCC) was used for immunohistochemical evaluation. Written informed consent was obtained from all patients and the study was approved by the ethics committees of SYSUCC.

RNA isolation and qRT-PCR

Total RNA was isolated using TRIzol reagent (Invitrogen, Waltham, MA, USA). Complementary DNA (cDNA) was synthesized using a reverse transcription kit (K1622, Thermo, Waltham, MA, USA) following the manufacturer's instructions. qRT-PCR analysis was performed using the SYBR Green PCR Kit (Novazym, Nanjing, China), and β-actin was used as the internal control. The sequences of real-time PCR primers for β-actin and PTPN3 were as follows: forward: 5'-CACCATTGGCAATGAGCGGTTC–3' and reverse: 5'-AGGTCTTTGCGGATGTCCACGT –3', and forward 5'-GGACATCTCAGAACACACGCATG–3' and reverse 5'-GAAGTCAGCAAATGAGCGGACAG–3', respectively.

MTS assay

Cells were seeded into 96-well plates at the density of 2000 cells/well in 200 μL normal culture medium. Cell growth was determined using MTS (Cell Titer 96 Aqueous One Solution Cell Proliferation Assay solution; Promega, Madison, Wisconsin, USA). Every day at the same time, 10 μL of the MTS reagents were added to 100 uL culture medium per well and incubated for 2–4 h at 37 °C. OD490 of these cells seeded into 96-well plates was determined with a microplate reader (Bio-Tek EPOCH2, Winooski, VT, USA). All experiments were performed in triplicates.

RNA interference

For transient knockdown of PTPN3, siRNAs targeting human PTPN3 were transfected into cells using the Lipofectamine RNAiMAX Reagent (Invitrogen; Carlsbad, California, USA). The siRNAs were made by Gene-Pharma Biological Technology (Shanghai, China). Their sequences targeting human PTPN3 were 5'-GCGUGGUACAGACCUUUAATT–3'(si1#) and 5'- GCUGAAUCCAGGGAACAUATT–3'(si2#), and the negative control sequence was 5'-CATTAATGTCGGACAAC–3'.

Clonogenic assay

Equal numbers of cells were seeded at a density of 500 cells/mL into 6-well plates and incubated for 10 days at 37°C. At the end of the experiments, the cells were stained with 0.1% crystal violet for 15 min. The number of positive colonies, those with > 50 cells, were counted under a microscope (NIKON ECLIPSE 80i; Tokyo, Japan).

Lentiviral transduction studies

PTPN3 overexpressing plasmid or vector were purchased from Genecopoeia Co. Ltd. (Guangzhou, China). Lentiviruses were produced in 293 T cells co-transfected with lenti-plasmid and packing plasmids using the X-treme GENE DNA transfection reagents (Roche; Basel, Switzerland). The cells were first incubated with infectious lentiviruses for 12 h and then exchanged to incubation with fresh medium for another

12h, after which they were selected with puromycin (2 ng/L) for 3 days and validated by qRT-PCR and immunoblotting.

Western blot analysis

The primary antibodies used were PTPN3 (#GTX54572, GeneTex, Southern California, USA), GAPDH (#60004–1-Ig, Proteintech, Chicago, USA), Phospho-Akt (Thr308) (#13038, Cell Signaling Technology, Boston, USA), Phospho- Akt (Ser473) (#4060P, Cell Signaling Technology, Boston, USA), AKT (#60203–2-Ig Proteintech, Chicago, USA). The HRP-conjugated goat anti-rabbit or anti-mouse secondary antibodies were purchased from Vazyme Biotech (Nanjing, China). Proteins were visualized with an enhanced chemiluminescence detection system (Bio-rad, California, USA).

Migration and invasion assays

5×10^4 cells of ACHN, 3×10^4 cells of Caki–1, 4×10^4 cells of SN12C or 2×10^4 cells of 1932 were seeded in a serum-free medium in the top chambers (Corning, Michigan, USA) with 700 μL of DMEM with 10% FBS in the lower chambers. After cultured for 24 h, the cells were fixed with methanol and stained with 0.1% crystal violet for 30 min at room temperature. For invasion assays, the upper chamber membranes were coated with matrigel (Corning, Life sciences, Michigan, USA). Three random fields per well were observed, and cells were counted under a microscope (NIKON ECLIPSE 80i; Tokyo, Japan). The inhibitor of AKT—MK–2206 (1uM) was purchased from Selleck (Houston, USA). Both experiments were repeated independently three times.

Wound healing assays

Cells were seeded into 6-well plates (Corning, Michigan, USA) and cultured to a 90% confluent followed by 24 h starvation in a serum-free medium. Artificial wounds were created using a sterile 200-μL tip in the cell monolayer and the floating cells were removed by washing with PBS. Respective images were captured at 0 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h using an inverted microscope (OLYMPUS IX73; Tokyo, Japan). The width of the scratch were quantified before and after cell migration.

Immunohistochemical (IHC) staining

Rabbit anti-PTPN3 antibody (1:100 dilution, #GTX54572, GeneTex, Southern California, USA) was used, and the immunohistochemical staining results were assigned a mean score considering both the intensity of staining and the proportion of tumor cells with an unequivocal positive reaction. Each section was independently assessed by two pathologists without prior knowledge of patient data. The percentage of stained cells was scored as follows: 0% as 0; 1%–10% as 1; 11%–50% as 2; 51%–80% as

3; 81%–100% as 4, and the intensity of staining: no staining as 0; weak staining as 1; moderate staining as 2; strong staining as 3. Then multiplying the two was the score of each tissue(score range: 0~12).

Animal experiments

Female athymic mice between 4 and 6 weeks of age were obtained from Beijing Vital River Laboratory Animal Center (Beijing, China). Mice were cared in accordance with the principles and procedures outlined in the Institutional Animal Care and Use Committee of SYSUCC. 8×10^6 /mL SN12C cells stably expressing scrambled shRNA control or shPTPN3 were injected subcutaneously into the mice. Ten days after the injection, the mice were sacrificed and the tumors were excised and measured. For lung metastasis, SN12C kidney cancer cells stably expressing scrambled shRNA control or shPTPN3 (1.5×10^6 cells per 0.1 mL PBS) were injected into the lateral tail vein of nude mice. Eight weeks after the injection, the lungs were surgically excised, weighed and photographed. The number of metastatic nodules on the surface of the lungs were visually counted after staining with picric acid. The lungs were paraformaldehyde-fixed for paraffin sections and stained by hematoxylin and eosin using a standard histological protocol.

Statistical analysis

The SPSS V.14 (Chicago, USA) software package was used to perform statistical data analyses. $P < 0.05$ was considered significant. Data are presented as the mean ± SD. Multivariate analyses were performed using the Cox proportional hazards model. Survival curves were constructed using the Kaplan-Meier method and compared using the log-rank test. T-test was used to compare PTPN3 mRNA levels between normal and tumor samples from the kidney renal clear cell carcinoma dataset of The Cancer Genome Atlas (TCGA).

Results

Reduced expression of PTPN3 correlate with poor prognosis in ccRCC patients

We investigated PTPN3 expression in ccRCC tissues. Quantitative real-time PCR analysis revealed that PTPN3 mRNA was significantly lower in tumoral tissues as compared to their corresponding adjacent normal renal tissues in the 12 matched human ccRCC tissues (Fig. 1a). To evaluate the expression level of PTPN3 in ccRCC tissue, we also performed IHC staining in 172 human ccRCC samples (Fig. 1b). The association between PTPN3 expression and clinicopathological features to survival outcomes are summarized in Table 1 and 2. Patients with tumors having high PTPN3 expression (cut off score > 6) had significantly longer overall survival (OS) and distant metastasis-free survival (DMFS) than those with low PTPN3 expression tumors (Fig. 1c; $P = 0.0343$ VS $P = 0.0166$). Multivariate analyses revealed that high

PTPN3 expression was an independent and favorable prognostic indicator for DMFS (P = 0.047) in all patients (Table 2).

We also analyzed the gene expression data of ccRCC patients in the TCGA database. PTPN3 mRNA was found to be significantly lower in all tumor samples than in paired normal tissues (Additional file 1: Figure S1a and b; $P < 0.05$). Kaplan–Meier survival analyses of TCGA data also showed that lower expression of PTPN3 was associated with poorer survival in ccRCC (Fig 1d; P < 0.0001). These observations suggest that low levels of PTPN3 may act as a novel prognostic factor for ccRCC patients.

Knocking down PTPN3 promotes ccRCC cell growth in vitro and in vivo

We transfected SN12C and 1932 cells with siRNA for PTPN3 (si1# and si2#) or negative control siRNA (Fig. 2a and b). PTPN3 suppression significantly increased renal cell carcinoma cell proliferation (Fig. 2c) and colony formation ability of these two cell lines (Additional file 1: Figure S2). Further, PTPN3 was stably overexpressed in SN12C, Caki–1 and ACHN cell lines (Fig. 2d) and the overexpression of PTPN3 effectively inhibited cell proliferation (Fig. 2e) and reduced colony formation ability (Fig. 2f). In addition, the overexpression of PTPN3 remarkably inhibited tumor growth in nude mice (Fig. 2g and f). Taken together, these results suggested that PTPN3 can act as a tumor suppressor in the development of renal cell carcinoma.

PTPN3 inhibits ccRCC cell migration, invasion, and metastasis

To examine whether PTPN3 influences renal cell carcinoma cellular mobility, we performed transwell migration, invasion, and wound healing assays. Findings from these assays revealed that the knockdown of PTPN3 with siPTPN3 increased the migration and invasion of renal cell carcinoma cells transfected (Fig. 3a, b and e). Accordingly, the overexpression of PTPN3 dramatically decreased the migration and invasion ACHN and SN12C cells (Fig. 3c d and f). Furthermore, the tail veins and lung metastatic *in vivo* experiments in nude mice showed that the number of lung metastatic nodules were significantly fewer in the SN12C-PTPN3 group than the control group (P < 0.05) (Fig. 3g and h). Taken together, these results show a role of PTPN3 in the downregulation of renal cell carcinoma cellular migration, invasion, and metastasis.

PTPN3 inhibits ccRCC cellular motility through suppression of PI3K/AKT signaling

Protein kinase B (also named AKT) is an important mediator of cell proliferation, apoptosis, autophagy, and metastasis. Here, we found that the expression of phospho-AKT^{Thr308} and phospho-AKT^{Ser473} were enhanced by silencing PTPN3 in the SN12C and 1932 cells (Fig. 4a). Conversely, the overexpression of

PTPN3 decreased expression of phospho-AKT^{Thr308} and phospho-AKT^{Ser473} in SN12C and 1932 cells (Fig. 4a). Furthermore, we found that treatment with MK–2206 decreased phospho-AKT^{Thr308} and phospho-AKT^{Ser473} in SN12C-siPTPN3 and 1932–1-siPTPN3 cells (Fig. 5b). These results suggest that PTPN3 suppressed the PI3K/AKT signaling pathway in renal cell carcinoma cells. In addition, we found that AKT inhibition by MK–2206 impaired the migration and invasion of SN12C-siPTPN3 and 1932-siPTPN3 cells (Fig. 5a and b), as well as the proliferation of SN12C-siPTPN3 and 1932-siPTPN3 cells (Fig. 5c and d). These results illustrate that the knockdown of PTPN3 stimulated renal cell carcinoma cells growth, migration, and invasion through the activation of the AKT pathway.

Discussion

Although metastases are the main reasons for death in ccRCC patients, the detailed mechanisms of metastasis remain unclear [6, 7]. In the present study, we showed for the first time that the expression of PTPN3 is downregulated in renal cell carcinoma cell lines and tissue samples. Also, the overexpression of PTPN3 inhibited renal cell carcinoma cell growth, migration, and invasion, and resulted in smaller subcutaneous tumors and fewer pulmonary metastatic tumors in nude mice. Further, lower expression of PTPN3 protein in ccRCC tissues could independently predict poor overall survival and progression-free survival in ccRCC. Data from this preliminary study suggests that PTPN3 could be a powerful conservator for anti-metastasis in ccRCC.

The PI3K/AKT pathway has been shown to play important roles in a variety of biological processes, and the dysfunction of the AKT signaling was associated with diseases such as cancer, diabetes, and autoimmune [19, 20]. PIP3 recruits several pleckstrin homology domain-containing proteins to the membrane of cells, including AKT and PDK1. AKT is phosphorylated at Thr308 by PDK1 and at Ser473 by the mTOR complex 2 (mTORC2) to increase its kinase activity [21]. AKT, directly and indirectly, phosphorylates many downstream proteins, including the GSKs, p27KIP1, FoxO transcription factors, MDM2, and BAD, to enhance cell survival and growth [22]. In this study, we discovered that PTPN3 attenuated AKT activity in renal cell carcinoma by downregulating both phospho-AKT^{Thr308} and phospho-AKT^{Ser473}.

Because AKT was found as an important regulator of cell proliferation, growth, and survival in many cancers [23], our findings may be applicable beyond renal cell carcinoma to other cancers which are driven by the increase of AKT signaling. Targeting AKT pathways through PTPN3 may be a major goal in the effort to develop therapeutics. Although our results suggested that PTPN3 could inhibit tumor progression in renal cell carcinoma, in other cancers different mechanisms could be involved [24–26]. In our study, we found that PTPN3 might inhibit the progression of renal cell carcinoma through the PI3K/AKT signaling pathway because PTPN3 could decrease the expression of both phospho-AKT^{Thr308} and phospho-AKT^{Ser473} in renal cell carcinoma cells.

The mechanism between AKT and cancer has been reported in many other studies. For instance, m6A methylation was reported to affect AKT activity in adult t-cell leukemia (AML) [27] and endometrial cancer

[24]; CITED2 promoted prostate cancer metastasis by activating the nucleolin-AKT pathway [28]; SMAD and AKT pathways interacted to confer pro-oncogenic responses to TGF-β and drove breast cancer metastasis [29]. Some studies further reported the potential mechanisms between AKT pathways and renal cell cancer. AKT was found to positively regulate rasfonin-enhanced autophagy and caspase-dependent apoptosis primarily through affecting the glycolytic pathway in renal cancer cells [30]. However, the upstream potential mechanism of AKT was rarely reported. Some researchers found that PIK3R1 down-regulation in RCC promoted the propagation, migration, EMT, and stem-like phenotype in renal cancer cells through the AKT/GSK3β/CTNNB1 pathway and may contribute to the progression and metastasis of RCC [31]. In this study, we observed that PTPN3 downregulates the AKT pathway to control cell proliferation, migration, and invasion in renal cell carcinoma.

Conclusion

PTPN3 inhibits renal cell carcinoma cells growth, migration, and invasion via downregulation of the AKT pathway.

Abbreviations

ccRCC: Clear cell renal cell carcinoma; PTPN3: Protein tyrosine phosphatase non-receptor type 3; RCC: Renal cell carcinoma; PI3K: The phosphoinositide 3-kinase; AKT: Protein kinase B; PTPs: Protein tyrosine phosphatases; EGFR: The epidermal growth factor receptor; SYSUCC: Sun Yat-sen University Cancer Center; qRT-PCR: Quantitative real-time reverse-transcription PCR; TCGA: The Cancer Genome Atlas; IHC: Immunohistochemical; OS: Overall survival; DMFS: Distant metastasis-free survival.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

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Authors' contributions

PXS and YJP analyzed the patient data regarding the ccRCC. PXS performed all the experiments in vitro and in vivo, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The animal research was approved by the institutional ethics committee of Sun Yat-sen University, Guangzhou, China (Exp. number: 19030J).

Availability of data and materials

The gene expression profiles and clinical data can be found at the GDC portal (https://portal.gdc.cancer.gov/). And all other data can be found in the manuscript and supplementary data.

Consent for publication

Not applicable.

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Supplemental Figure Legends

Fig S1. PTPN3 mRNA was significantly lower in all tumor samples than in paired normal tissues of the TCGA database. a-b. http://gepia.cancer-pku.cn/detail.php?gene=PTPN3

Fig S2. Suppression of PTPN3 increased the cell colony numbers. Representative micrographs and quantification of crystal violet stained cells from 3 independent experiments. Columns, average of three independent experiments; bars, SD. *P < 0.05, **P < 0.01, Student t test.

Tables

Table 1. Association between expression of PTPN3 and clinicopathological characteristics in 172 ccRCC patients

al factor	Cases (n=172)	*PTPN3* expression		*P* value
		High	Low	
er				
ale	47	37	10	0.428
	125	91	34	
	86	65	21	0.727
	86	63	23	
e				
	131	99	32	0.535
	41	29	12	
an score				
	33	23	10	0.243
	104	81	23	
	30	22	8	
	5	2	3	
matoid differentiation score				
	168	126	42	0.257
	4	2	2	
sis score				
	136	103	33	0.442
	36	25	11	
lar invasion score				
	164	123	41	0.429
	8	5	3	
invation score				
	172	128	44	-
	0	0	0	
ar collagens score				
	49	40	9	0.172
	123	88	35	
n nodes invasion score				
	172	128	44	-
	0	0	0	

Table 2. Univariate and multivariate analyses of different parameters for overall survival (OS) and progression-free survival (PFS) of ccRCC patients

Variables	Univariate analysis			Multivariate analysis		
	HR	CI	P	HR	CI	P
OS						
Fuhrman score	2.322	1.519~3.550	**<0.001**	2.322	1.519~3.550	**<0.001**
Sarcomatoid differentiation score	6.031	1.847~19.692	**0.001**	1.695	0.423~6.788	0.325
PTPN3 level	0.504	0.264~0.962	**0.034**	0.568	0.294~1.099	0.071
PFS						
Fuhrman score	2.13	1.420~3.194	**<0.001**	2.017	1.356~2.999	**0.001**
Sarcomatoid differentiation score	5.69	1.753~18.471	**0.001**	1.719	0.435~6.788	0.435
PTPN3 level	0.483	0.263~0.888	**0.017**	0.527	0.285~0.974	**0.047**

Abbreviation: CI confidence interval, HR hazard ratio. Statistical significance ($p < 0.05$) is shown in bold

Figures

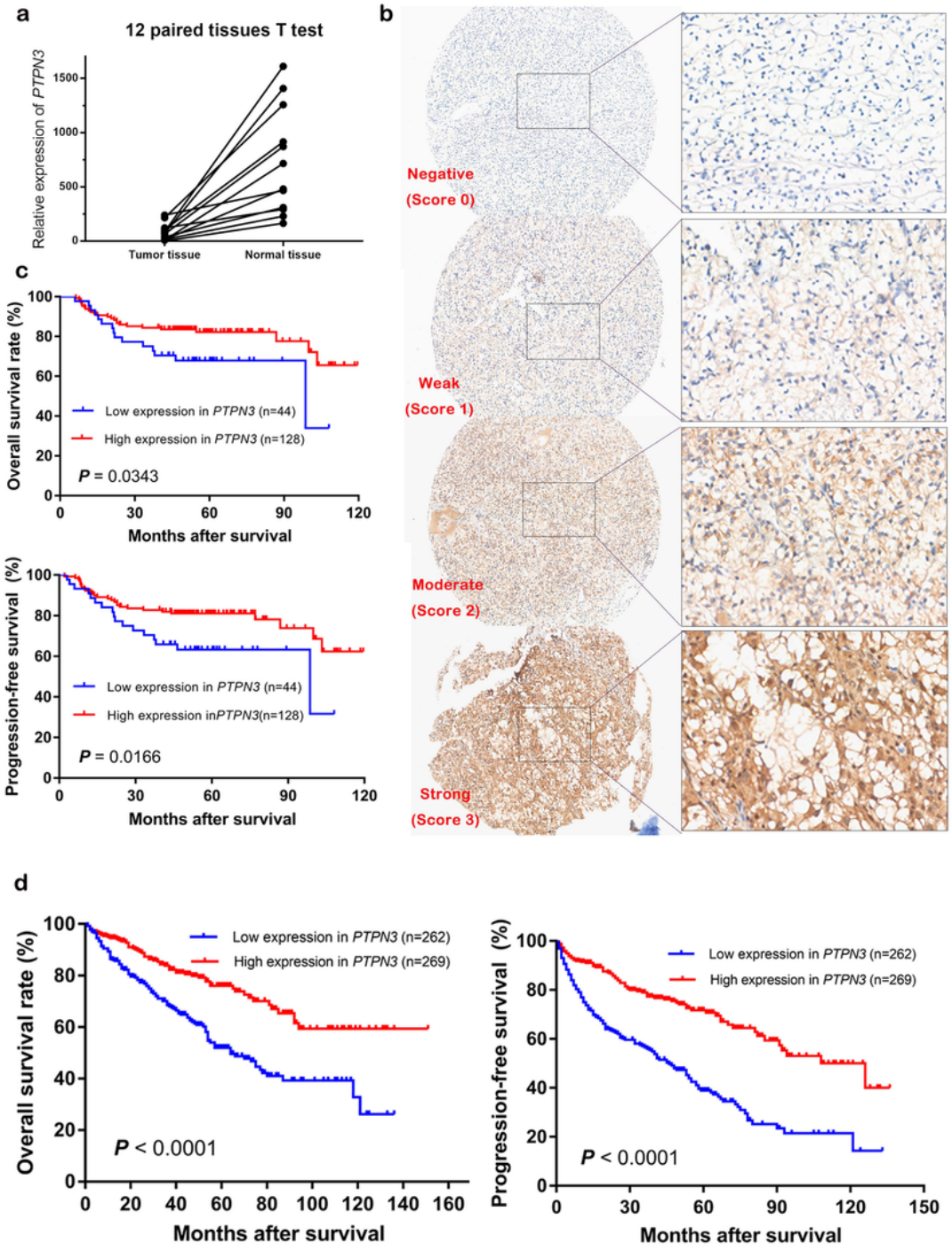

Figure 1

PTPN3 mRNA and protein expression levels in ccRCC tissues and cell lines. a. qRT-PCR analysis of PTPN3 mRNA expression levels in ccRCC matched tissues (n = 12), The mRNA levels of PTPN3 in ccRCC tissues were lower than the paired normal tissues, P = 0.0006, paired t test. b. Levels of PTPN3 protein expression in ccRCC tissue chip are shown under both low and high magnifications of a light microscope, low PTPN3 level correlated with shorter overall survival and progression-free survival in ccRCC patients. c.

In ccRCC tissue chip from our hospital, the overall survival (OS) and progression-free survival (PFS) rate were significantly higher in the higher PTPN3 group rate. d. Similarly, using data from the TCGA database, we observed that the overall survival (OS) and progression-free survival (PFS) rate was significantly higher in the higher PTPN3 group.

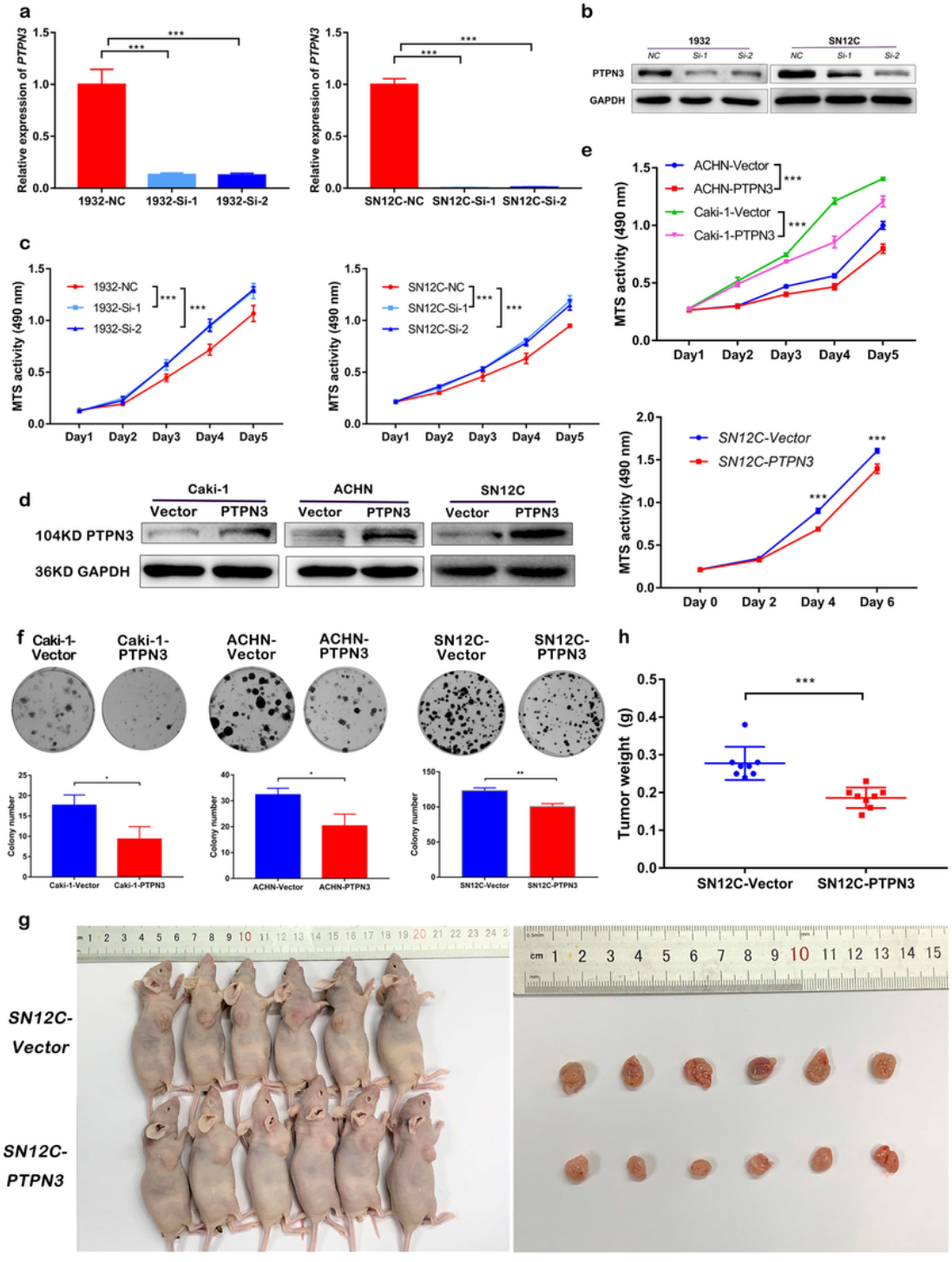

Figure 2

Suppression of PTPN3 promotes renal cell carcinoma cell growth in vitro and in vivo. a. Suppression of PTPN3 in renal cell carcinoma cells were determined by real-time quantitative PCR, normalized to β-actin. b. Suppression of PTPN3 in renal cell carcinoma cells were determined by immunoblotting analysis, GAPDH was used as a loading control. c. Suppression of PTPN3 promotes cells proliferation was determined by the MTS assay; ***P < 0.001, result of Student t test. d. Overexpression of PTPN3 in renal cell carcinoma cells were determined by immunoblotting analysis, GAPDH was used as a loading control. e. Overexpression of PTPN3 inhibits cells proliferation was determined by the MTS assay; ***P < 0.001, result of Student t test. f. Overexpression of PTPN3 decreased cell colony numbers; representative micrographs and quantification of crystal violet stained cells from 3 independent experiments. Columns, average of three independent experiments; bars, SD. *P < 0.05, **P < 0.01, Student t test. g. Overexpression of PTPN3 in SN12C cells and the vector control cells were subcutaneously injected into nude mice. h. The terminal tumor weights are decreased compared with the control group, ***P < 0.001, result of Student t test.

Figure 3

PTPN3 influences renal cell carcinoma cell migration, invasion and overexpressing PTPN3 impairs renal cell carcinoma metastasis in vivo. a. Suppression of PTPN3 in renal cell carcinoma cells could promote cell migration and cell invasion in SN12C and 1932 cells. b. Quantification of the effects of PTPN3 suppression on the migratory abilities and invasion ability in SN12C and 1932 cells as determined by transwell assays. All of the experiments were performed at least three times. Columns, average of three independent experiments; bars, SD. *P < 0.05, **P < 0.01, ***P < 0.001, result of Student t test. c. Overexpression of PTPN3 in renal cell carcinoma cells could inhibit cell migration and cell invasion in ACHN and SN12C cells compared with vector control cells. d. Quantification of the effects of PTPN3 overexpression on the migratory and invasion abilities in ACHN and SN12C cells as determined by transwell assays. All experiments were performed at least three times. Columns, average of three independent experiments; bars, SD. *P < 0.05, ***P < 0.001, result of Student t test. e. Representative images of the effects of PTPN3 suppression on the migratory abilities of SN12C and 1932 cells as determined by wound healing assays. All experiments were performed at least three times. f. Representative images of the effects of PTPN3 overexpression on the migratory abilities of ACHN and SN12C cells as determined by wound healing assays. The experiments were performed at least three times. g. Overexpression of PTPN3 decreased in vivo metastatic rate of SN12C cells. Histological image of lung metastasis in nude mice after tail vein injection of SN12C cells. Left, representative picture of lungs after picric acid staining; right, representative H&E staining of lungs; scale bar, 500 μm. g. The numbers of lung metastases (mean ± SD) were counted and summarized.

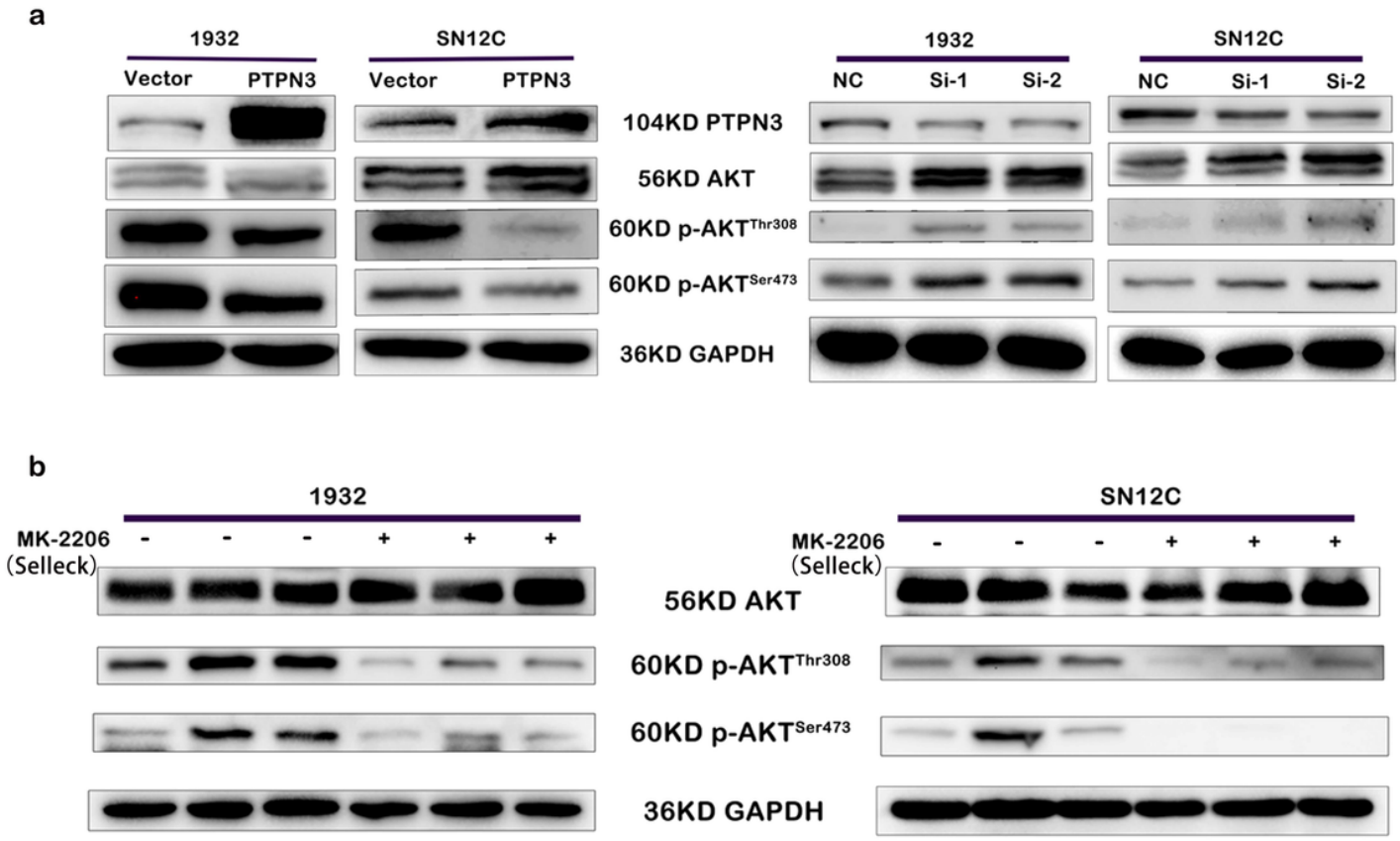

Figure 4

PTPN3 inhibits renal cell carcinoma cellular growth, migration, and invasion through suppression of the PI3K/AKT signaling. a. Western blot analysis of AKT, P-AKTThr308 and P-AKTSer473 expression levels after overexpression of PTPN3 and silencing of PTPN3. b. The expression of the PI3K/AKT signaling in PTPN3 silencing SN12C and 1932 cells after MK-2206 treatment was detected by immunoblotting. After treatment with 1 μM of MK-2206 or PBS for 24 h, cell lysates were harvested for western blot detection. Relative gradation corrected by GAPDH is shown below each band.

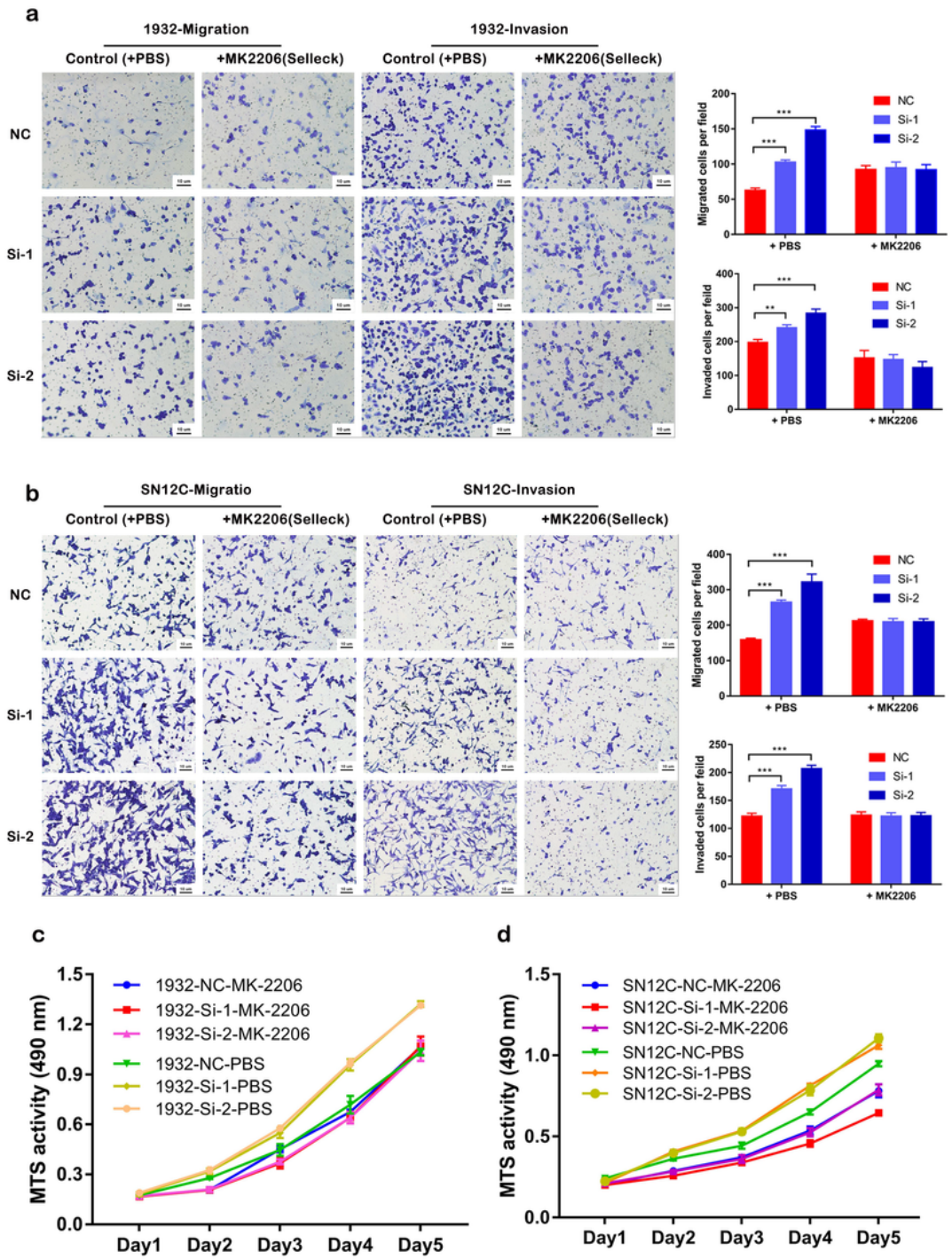

Figure 5

Inhibitors of P-AKTThr308 and P-AKTSer473 impair the migration, invasion, and growth of renal cell carcinoma cells induced by PTPN3 silencing. a-b. Cells were treated with 1 μM MK-2206 or PBS for 24 h and subjected to migration and invasion. Representative images and quantification of MK-2206 show the attenuating effects of PTPN3 silencing on the migratory abilities of SN12C and 1932 cells as determined by migration and invasion assays. Columns, average of three independent experiments; bars, SD. **P <

0.01, ***P < 0.001, Student t test. c-d. Cells were treated with 1 μM MK-2206 or PBS for 24h and subjected to and MTS assays. MK-2206 attenuate the accelerated effects of PTPN3 silencing on the growth abilities of SN12C and 1932 cells as determined by MTS assays. The difference is statistically significant in both the control and drug groups.

Supplementary Files

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- FigureS1LZW.tif
- FigureS2LZW.tif