

Basic Study

TBL1XR1 induces cell proliferation and inhibit cell apoptosis by the PI3K/AKT pathway in pancreatic ductal adenocarcinoma

Jian-Feng Gu, Wei Fu, Hai-Xin Qian, Wen-Xiu Gu, Yang Zong, Qian Chen, Long Lu

ORCID number: Jian-Feng Gu 0000-0002-8514-813X; Wei Fu 0000-0002-0153-937X; Hai-Xin Qian 0000-0003-2412-0747; Wen-Xiu Gu 0000-0003-4586-2838; Yang Zong 0000-0003-2831-4717; Qian Chen 0000-0001-7899-5552; Long Lu 0000-0001-7314-2972.

Author contributions: Gu JF and Fu W contributed equally to this work. Gu JF was involved in study concept and design, and acquisition, analysis and interpretation of data, drafted manuscript, critically revised the manuscript for important intellectual content, and provided statistical analysis and administrative, technical, or material support; Fu W performed data acquisition and drafted manuscript; Qian HX participated in data analysis and interpretation; Gu WX was involved in the drafting of the manuscript; Zong Y was involved in critically revising the manuscript for important intellectual content; Chen Q and Lu L took part in the statistical analysis.

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Institutional animal care and use

Jian-Feng Gu, Wen-Xiu Gu, Yang Zong, Qian Chen, Department of General Surgery, Changshu No. 1 People's Hospital Affiliated to Soochow University, Changshu 215500, Jiangsu Province, China

Wei Fu, Long Lu, Department of Oncology, Changshu No. 1 People's Hospital Affiliated to Soochow University, Changshu 215500, Jiangsu Province, China

Hai-Xin Qian, Department of General Surgery, the First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu Province, China

Corresponding author: Jian-Feng Gu, MD, Doctor, Department of General Surgery, Changshu No. 1 People's Hospital Affiliated to Soochow University, No.91, Yongjia Road, Changshu 215500, Jiangsu Province, China. jscsgjf@sina.cn

Abstract**BACKGROUND**

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest solid tumors. Identification of diagnostic and therapeutic biomarkers for PDAC is urgently needed. Transducin (β)-like 1 X-linked receptor 1 (TBL1XR1) has been linked to the progression of various human cancers. Nevertheless, the function and role of TBL1XR1 in pancreatic cancers are unclear.

AIM

To elucidate the function and potential mechanism of TBL1XR1 in the development of PDAC.

METHODS

Ninety patients with histologically-confirmed PDAC were included in this study. PDAC tumor samples and cell lines were used to determine the expression of TBL1XR1. CCK-8 assays and colony formation assays were carried out to assess PDAC cell viability. Flow cytometry was performed to measure the changes in the cell cycle and cell apoptosis. Changes in related protein expression were measured by western blot analysis. Animal analysis was conducted to confirm the impact of TBL1XR1 *in vivo*.

RESULTS

Patients with TBL1XR1-positive tumors had worse overall survival than those

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with TBL1XR1-negative tumors. Moreover, we found that TBL1XR1 strongly promoted PDAC cell proliferation and inhibited PDAC cell apoptosis. Moreover, knockdown of TBL1XR1 induced G0/G1 phase arrest. *In vivo* animal studies confirmed that TBL1XR1 accelerated tumor cell growth. The results of western blot analysis showed that TBL1XR1 might play a key role in regulating PDAC cell proliferation and apoptosis *via* the PI3K/AKT pathway.

CONCLUSION

TBL1XR1 promoted PDAC cell progression and might be an effective diagnostic and therapeutic marker for pancreatic cancer.

Key words: Pancreatic ductal adenocarcinoma; TBL1XR1; Proliferation; PI3K/AKT pathway

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Core tip: Transducin (β)-like 1 X-linked receptor 1 (TBL1XR1) has been linked to the progression of various human cancers. However, the function and role of TBL1XR1 in pancreatic cancers are unclear. Elucidation of the effect and potential molecular mechanism of TBL1XR1 in pancreatic cancer is important. This study showed that TBL1XR1 promoted pancreatic ductal adenocarcinoma (PDAC) cell proliferation, inhibited PDAC cell apoptosis and might regulate PDAC cell proliferation and apoptosis by the phosphatidylinositol 3-kinase/protein kinase B pathway. Therefore, TBL1XR1 might be a promising therapeutic marker for patients with advanced PDAC.

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INTRODUCTION

Pancreatic carcinoma is one of the deadliest solid tumors. Currently, pancreatic carcinoma is the fourth leading cause of cancer-related death in the United States, and there were approximately 48960 new cases confirmed and 40560 deaths in 2015^[1,2]. The best chance for survival is early surgical detection. However, due to the lack of effective screening tests for pancreatic carcinoma, local invasion and early metastasis, only 10%–20% of patients are diagnosed at a stage amenable to resection and curative treatment^[3,4]. Therefore, the overall 5-year survival rate is lower than 5%^[5]. Despite decades of efforts, there has been no significant improvement in the long-term survival of pancreatic carcinoma patients^[6]. Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic carcinoma. Therefore, identification of efficacious early tumor markers for the early diagnosis and treatment of PDAC has clinical value and significance^[7].

As a member of the TBL1 family, transducin (β)-like 1 X-linked receptor 1 (TBL1XR1) is the key element of the SMRT/N-CoR corepressor complex^[8]. TBL1XR1 is located on chromosome 3 at 3q26 and has a high degree of homology to the TBL1 protein. Recently, studies have demonstrated that TBL1XR1 could react to transcriptional regulators such as nuclear receptors by switching from gene inhibition to gene activation^[9]. Moreover, TBL1XR1 plays a vital role in cell growth, cell apoptosis, inflammation and transcriptional activation, which involve estrogen receptor, androgen receptor, thyroid hormone receptor β, peroxisome proliferator-activated receptor γ, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Notch and β-catenin^[10]. Currently, studies focus on the effect and mechanism of TBL1XR1 in carcinogenesis and tumor progression, including those of nasopharyngeal carcinoma, colon cancer, osteosarcoma cancer, prostate cancer, breast cancer, and hepatocellular carcinoma^[11-16]. Nevertheless, the role and molecular mechanism of TBL1XR1 in the progression of PDAC remain unclear.

In this study, we aimed to explore the effect of TBL1XR1 in PDAC. We found that

TBL1XR1 expression was closely associated with the clinicopathologic features of PDAC, which might provide a new diagnostic and therapeutic target for PDAC.

MATERIALS AND METHODS

Patients and tissue specimens

This study was performed in 90 PDAC patients. The patients all underwent radical pancreaticoduodenectomy and were histopathologically and clinically diagnosed at Changshu No. 1 People's Hospital Affiliated with Soochow University from 2011 to 2015. Our study complied with the ethical standards of the Declaration of Helsinki and was approved by the Ethics Committee of Changshu No. 1 People's Hospital. Ninety pairs of PDAC and normal pancreatic epithelial samples were promptly fixed in 4% formalin and embedded in paraffin for immunohistochemical (IHC) staining after removal.

Immunohistochemical analysis

After obtaining fresh isolated specimens, we immediately fixed the specimens in 4% formalin and then embedded them in paraffin. Next, we cut them into 5 mm sections and mounted them on slides. IHC and HE staining were performed to examine the expression of TBL1XR1^[17].

Immunohistochemical staining score

Immunohistochemical staining of TBL1XR1 expression in PDAC and normal pancreatic epithelial specimens was performed as described by Li *et al.*^[17]. The TBL1XR1 staining score was the summation of the staining strength and the ratio of positively stained cells. We defined TBL1XR1 staining strength as follows: 0% immunoreactive cells are scored 0; less than 5% immunoreactive cells are scored 1; 5%-50% immunoreactive cells are scored 2; and more than 50% immunoreactive cells are scored 3. Staining intensity was scored as follows: negative was scored 0; weak was scored 1; intermediate was scored 2; and strong was scored 3. For statistical convenience, we set intermediate and strong scores to be positive, and the negative and weak scores were considered to be negative^[18].

Cell lines and cell cultures

The PDAC cell lines (Panc1, MiaPaCa-2, Capan1 and Aspc-1) were purchased from the Cell Bank of the Chinese Academy of Science. Panc1 and Capan1 cells were cultured in DMEM, and MiaPaCa-2 and Aspc-1 cells were cultured in RPMI-1640 medium. Streptomycin (100 µg/mL) and penicillin (100 U/ml) and 10% fetal bovine serum were added to DMEM or RPMI-1640 medium. The cells were cultivated at 37 °C in a humidified incubator with 5% CO₂.

Cell transfection

Five hundred thousand cells were inoculated into 6-well plates, and after the cells attached, we performed a cell transfection assay according to the manufacturer's instructions. In short, 50 nmol siRNA and 6 µL of Lipofectamine 2000 were added to 200 µL of Opti-MEM medium and incubated for 5 min. Then, the two suspensions were mixed and incubated for 15 min. The suspension was added to 6-well plates and incubated for 4 h. After incubation, we replaced the medium and harvested fresh culture medium every 2 d. The siRNA sequences were as follows: CTRL siRNA: 5'-TTCTCCGAACGTGTCACGT-3'; TBL1XR1: 5'-GGAGUAGACAAGACUACAA-3'.

Quantitative real-time PCR

We performed the assay using the methods described by Li *et al.*^[17]. Total RNA was extracted from different treated cells with TRIzol reagent (Invitrogen) in accordance with the manufacturer's instructions. The expression of TBL1XR1 and GAPDH was determined by the primers shown below: TBL1XR1: 5'-GAG GTG TTT ATT TGT GCT TGG-3'; 5'-TGC ACT TAA TAT GAA GTT GCC-3'. GAPDH: 5'-GCCGCATCTTCTTTTGGCTCGC-3'; 5'-TCCCGTTCTCAGCCTTGACGGT-3'.

The expression of TBL1XR1 was determined by normalization to the expression of the housekeeping gene GAPDH.

Lentivirus-mediated RNA interference

The lentivirus vector was constructed by the Shanghai GeneChem Company.

Lentivirus-mediated RNA interference was performed in accordance with the manufacturer's instructions. After cell adherence, a considerable volume of virus was added to the cells for 6-8 h. Then, fresh complete medium was added, and the cells were harvested for 2 d. Cells were continuously cultured and used for subsequent studies.

Cell viability assay

A CCK-8 (Beyotime, Shanghai) assay was performed to determine the viability of PDCA cells. CCK-8 is widely used in the rapid and highly sensitive detection of cell proliferation and cytotoxicity based on WST-8. PDAC cells at 1000 cells/well were inoculated into 96-well plates after the corresponding treatment and incubated for the indicated times. Then, 10 μ L of CCK-8 was added to the medium and cultured for 2 h in the dark. A microplate reader (Bio-Tek, United States) was used to measure the absorbance of PDCA cells at 450 nm.

Colony formation assay

PDAC cells with different treatments (approximately 500 cells/well) were seeded in 6-well plates. The culture medium was changed every 3 d. After approximately 2 wk of culture, the cells were cleaned with PBS and fixed with 10% formalin for 20 min at ordinary temperature. Then, 0.1% crystal violet (Sigma-Aldrich) was used to stain the cells for 20 min at room temperature. After staining, the plates were washed and dried. The colonies (with more than 50 cells) were observed under a microscope (Leica, Germany).

Western blot analysis

Proteins were extracted using RIPA buffer mixed with 1% protease inhibitor cocktail (Beyotime, Shanghai, China). Ten percent sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was applied to separate the cell proteins, and polyvinylidene difluoride (PVDF) membranes were used for transfer. Five percent skim milk was used to block the membranes for 2 h. Then, the membranes were incubated with primary antibodies against TBL1XR1 (Abcam, ab228940), p-AKT (CST, 9271), p-PI3K (CST, 17366) and GAPDH (Abclonal, A19056) at 4 °C overnight. The second day, secondary antibodies were added and incubated with the PVDF membranes for 1 h at room temperature. A Gel Doc 2000 system (Bio-Rad, United States) was used to detect protein expression.

Cell cycle and apoptosis analyses

For cell cycle analysis, PDAC cells with different treatments were fixed with 75% ethanol at 4°C overnight. Then, the cells were centrifuged for precipitation and incubated with 5 μ L of RNase and 5 μ L of PI at room temperature. For cell apoptosis analysis, PDAC cells with different treatments were resuspended with 1 \times Annexin V binding buffer and Annexin V and PI at 37 °C. After incubation for half an hour, the apoptotic cell and cell cycle ratios were detected with flow cytometric analysis.

Animal study

Four- to six-week-old nu/nu nude mice were purchased from the Shanghai Laboratory Animal Centre of the Chinese Academy of Sciences (Shanghai, China). The mice were randomly divided into the Lv-shCTRL group and the Lv-shTBL1XR1 group. One hundred thousand Panc1 cells were resuspended in 0.1 mL of serum-free medium and hypodermically inoculated into the right axilla of the mice. After approximately 4 wk, the mice were sacrificed, and the subcutaneous tumors were measured and weighed. The volume of the tumors was calculated by the following formula: Volume = length (mm) \times width² (mm²)/2.

Statistical analysis

All experiments were repeated at least 3 times, and the results are expressed as the mean \pm standard deviation unless otherwise stated. Student's *t* test was used to compare differences between the treated groups and the corresponding control groups; *P* < 0.05 was considered statistically significant.

RESULTS

Clinicopathological significance of TBL1XR1 expression in patients with PDAC

The clinicopathological features of the 90 PDAC patients are shown in **Table 1**. As illustrated in **Table 1**, the overexpression of TBL1XR1 was associated with TNM stage ($P = 0.006$) but not with patient age ($P = 0.652$), gender ($P = 1.000$), histopathological subtype ($P = 0.929$), tumor size ($P = 0.465$), tumor location ($P = 0.065$) or lymph node metastasis ($P = 0.050$). To additionally confirm the importance of TBL1XR1 in PDAC, we analyzed the overall survival (OS) of 90 patients by the Kaplan-Meier method (**Table 2**). **Table 2** and **Figure 1A** show that patients with TBL1XR1-negative expression displayed a longer OS than those with TBL1XR1-positive expression ($P < 0.001$). Moreover, TNM stage ($P < 0.001$) and lymph node metastasis ($P = 0.047$) were obviously related to the average survival time. Furthermore, the Cox proportional hazards model was used in multivariate analysis. As shown in **Table 3**, TBL1XR1 expression, TNM stage and lymph node metastasis were found to be significant independent prognostic factors for patients with PDAC, suggesting that TBL1XR1 expression is a risk factor for PDAC.

Enhanced expression of TBL1XR1 in PDAC tissues and cell lines

To investigate the possible role of TBL1XR1 in PDAC, we used IHC staining to investigate the relationship between TBL1XR1 expression and the clinicopathological features of the patients with PDAC (**Figure 1B**). IHC staining revealed that TBL1XR1 was mainly localized in the PDAC cell nucleus. The positive rate of TBL1XR1 staining in the tumor cells was approximately 70% (63/90) of the PDAC patients. Only 30% (27/90) of the patients possessed positive staining in the corresponding control tissues (**Figure 1C**, $P < 0.001$).

To determine the expression of TBL1XR1 in the PDAC tumor tissues and the adjacent normal tissues, we performed quantitative RT-PCR (qRT-PCR) and western blot assays. As illustrated in **Figure 2A** and **B**, enhanced expression of TBL1XR1 was observed in the PDAC tissues compared with the adjacent tissues. Then, we used MiaPaCa-2, Panc1, Aspc-1, and Capan1 cell lines to evaluate the role of TBL1XR1 in PDAC cell lines. TBL1XR1 expression at the mRNA and protein levels was examined *via* qRT-PCR and western blotting. We observed that TBL1XR1 was overexpressed in the PDAC cell lines, particularly in the Aspc-1 and Panc1 lines, at the mRNA (**Figure 2C**) and protein (**Figure 2D**) levels. Taken together, these data suggest that TBL1XR1 is upregulated in PDAC.

Effects of TBL1XR1 on the proliferation and apoptosis of PDAC cells

We performed CCK-8 and colony formation assays to further evaluate the effect of TBL1XR1 on the proliferation of PDAC cells. We knocked down the expression of TBL1XR1 in the Aspc-1 and Panc1 cells, and the knockdown efficiency is shown in **Figure 3A** and **B**. The CCK-8 assay showed that the downregulation of TBL1XR1 prominently suppressed PDAC cell viability compared with that of the control cells ($P < 0.05$, **Figure 3C** and **D**). Moreover, colony formation analysis showed that the colony formation capacity was significantly reduced in the TBL1XR1-knockdown cells compared to that of the control group ($P < 0.05$, **Figure 3E**). These results suggested that TBL1XR1 may play a vital role in PDAC cell proliferation *in vitro*. Furthermore, we investigated whether TBL1XR1 played a role in the proliferation of PDAC cells *in vivo* using xenograft mouse models. As shown, the tumor volume and weight of the TBL1XR1-knockdown group were distinctly decreased compared to those of the control group (**Figure 3F** and **G**).

To explore the probable molecular mechanism of TBL1XR1 in PDAC cells, we detected the cell cycle and apoptotic profile of the Lv-shCTRL and Lv-shTBL1XR1 groups. As shown in **Figure 4A**, cell cycle analysis demonstrated that the cells treated with Lv-shTBL1XR1 exhibited cell arrest at the G0/G1 phase (**Figure 4A**). Moreover, we examined the protein expression of cell cycle-related regulatory genes to determine the impact of TBL1XR1 on the cell cycle by western blot assays. As depicted in **Figure 4B**, the protein expression levels of CDK2, CDC25A, or cyclin D1 in the Lv-shTBL1XR1 group were obviously lower than those in the control group. Therefore, we concluded that downregulation of TBL1XR1 induced PDAC cell cycle arrest at the G0/G1 phase by regulating the expression of cell cycle-related regulatory genes.

Furthermore, we performed an apoptosis assay to explore the effect of TBL1XR1 on apoptosis in PDAC cells. As shown in **Figure 4C**, an obvious increase in the percentage of apoptotic cells was observed in the TBL1XR1 knockdown group compared with the Lv-shCTRL group. Moreover, the knockdown of TBL1XR1 significantly enhanced the

Table 1 Association between transducin (β)-like 1 X-linked receptor 1 expression and the clinicopathological parameters of pancreatic ductal adenocarcinoma cells

Parameter	Category	Case number	TBL1XR1 expression		
			Number of positive cases, n (%)	χ^2	P value
Age (yrs)	< 60	40	27 (67.5)	0.214	0.652
	\geq 60	50	36 (72.0)		
Sex	Male	59	41 (69.5)	0.021	1.000
	Female	31	22 (71.0)		
Histopathological subtypes	High	3	2 (66.7)	0.146	0.929
	Middle	51	35 (68.6)		
	Low	36	26 (72.2)		
TNM stage	1-II	12	4 (33.3)	8.864	0.006 ^b
	III-IV	78	59 (75.6)		
Tumor size	< 3 cm	60	42 (66.7)	0.952	0.465
	\geq 3 cm	30	23 (76.7)		
Tumor location	Head	37	30 (81.1)	3.674	0.065
	Body/tail	53	33 (62.3)		
Lymph node metastasis	Negative	61	44 (72.1)	0.409	0.624
	Positive	29	19 (65.5)		

^bP < 0.01. TBL1XR1: Transducin (β)-like 1 X-linked receptor 1; PDAC: Pancreatic ductal adenocarcinoma; TNM: Tumor-node-metastasis.

expression of Bax and Bad and reduced the expression of Bcl-2 (Figure 4D). Taken together, these results suggest that TBL1XR1 might regulate PDAC cell apoptosis and cell cycle progression.

TBL1XR1 regulated the proliferation and apoptosis of PDAC cells via the PI3K/AKT pathway

To explore the potential mechanism by which TBL1XR1 influences proliferation and apoptosis in PDAC cells, we examined the expression changes of relevant proteins by western blotting. As shown in Figure 5A, the expression levels of p-AKT and p-PI3K were obviously reduced, while no significant effect on p-STAT3 and p-MEK was observed in the TBL1XR1 downregulation group, which indicated that TBL1XR1 might regulate PDAC cell proliferation and apoptosis *via* the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway. To determine whether TBL1XR1 knockdown-induced inhibition of proliferation was regulated by the PI3K/AKT pathway, we assessed the effect of the presence or absence of LY294002 (a PI3K/Akt inhibitor) on the TBL1XR1-overexpressing cells. Unsurprisingly, overexpression of TBL1XR1 promoted proliferation and suppressed apoptosis in PDAC cells, which was consistent with previous results. LY294002 treatment reversed the TBL1XR1-mediated promotion of proliferation and inhibition of apoptosis (Figure 5B-D). Moreover, LY294002 inhibited the TBL1XR1 overexpression-induced increases in Bcl-2 and decreases in Bad and Bax (Figure 5E). Therefore, we deduced that TBL1XR1 induces PDAC cell proliferation, cell cycle arrest and apoptosis *via* the PI3K/AKT signaling pathway.

DISCUSSION

Although many studies have attempted to elucidate its pathogenesis, PDAC remains a deadly malignancy^[19]. Hence, identification of efficacious early tumor markers for the early diagnosis and treatment of PDAC will have clinical value and significance.

TBL1XR1 (TBLR1) was first identified as a gene transcript in human CD34+CD38-cells; moreover, researchers discovered that TBL1XR1 has an F-box/WD40-repeat

Table 2 Univariate log-rank analysis of overall survival

Parameter	Category	Case number	Median survival time (mo)	P value
			(95%CI)	
Age (yrs)	< 60	40	10.0 (4.8-15.2)	0.413
	≥ 60	50	9.7 (7.41-12.0)	
Sex	Male	59	9.9 (6.7-13.1)	0.983
	Female	31	9.9 (8.1-12.0)	
Histopathological subtypes	High	3	25.0 (-)	0.760
	Middle	51	10.3 (8.1-11.7)	
	Low	36	8.7 (6.0-11.4)	
TNM stage	1-II	12	25.0 (18.1-31.9)	< 0.001 ^c
	III-IV	78	8.0 (5.5-10.5)	
Tumor size	< 3 cm	60	9.8 (8.6-11.0)	0.251
	≥ 3 cm	30	8.3 (4.8-11.8)	
Tumor location	Head	37	9.3 (7.0-11.7)	0.095
	Body/tail	53	11.0 (6.0-16.0)	
Lymph node metastasis	Negative	61	12.0 (7.9-16.1)	0.047 ^a
	Positive	29	7.6 (5.3-9.8)	
TBL1XR1 expression	Low	27	18.9 (10.9-26.9)	< 0.001 ^c
	High	63	7.0 (5.7-8.3)	

^aP < 0.05,^cP < 0.001. TBL1XR1: Transducin (β)-like 1 X-linked receptor 1; CI: Confidence interval; TNM: Tumor-node-metastasis.

sequence^[20]. A growing body of research has revealed that TBL1XR1 can play a vital role in tumorigenesis, invasion, metastasis, and the development of resistance to therapies^[15,21]. Moreover, TBL1XR1 mRNA is highly expressed in many human tissues, such as thyroid, prostate and breast tissues^[22], which indicates that TBL1XR1 may function as an oncogene. TBL1XR1 functions by activating many signal transduction pathways, such as Wnt-β-catenin, NF-κB, and Notch23. Nevertheless, it remains unclear how TBL1XR1 functions in the development of PDAC.

In this research, we discovered that the expression of TBL1XR1 was obviously enhanced in PDAC tissues compared with adjacent tissues. Moreover, we revealed that TBL1XR1 expression, TNM stage and lymph node metastasis were significant independent prognostic factors for patients with PDAC, suggesting that TBL1XR1 expression is a risk factor for PDAC. To further evaluate the function of TBL1XR1 in PDAC, we performed CCK-8 and colony formation assays, and the results showed that the downregulation of TBL1XR1 significantly suppressed PDAC cell proliferation. Moreover, we found that TBL1XR1 knockdown induced PDAC cell cycle arrest in G0/G1 phase and inhibited PDAC cell apoptosis *in vitro*. Additionally, the results of the *in vivo* animal analysis were consistent with the *in vitro* analysis, which indicated that TBL1XR1 might be a potential diagnostic target for PDAC.

The PI3K/AKT signaling pathway has a key impact on multiple cell processes, including cell growth, cell proliferation, angiopoiesis and survival, in both normal and tumor cells^[23]. In our study, we found that TBL1XR1 can induce PDAC cell proliferation and inhibit PDAC cell apoptosis. However, whether the PI3K/AKT signaling pathway is involved in the TBL1XR1-induced promotion of PDAC cell proliferation and inhibition of apoptosis is unknown. Thus, we explored the potential mechanism by which TBL1XR1 influences proliferation and apoptosis in PDAC cells, and we found that inhibition of the PI3K/AKT pathway reversed the TBL1XR1 promotion of proliferation and inhibition of apoptosis, indicating that TBL1XR1 might play an important role in regulating PDAC cell proliferation and apoptosis. In addition, the activity of Akt could be regulated through S-nitrosylation at the kinase cysteine residues, and it was reported to be associated with reduced kinase

Table 3 Multivariate analysis of overall survival

Parameter	Category	Hazard ratio	95%CI	P value
Age (yrs)	< 60	0.139	0.907 (0.544–1.514)	0.709
	≥ 60			
Sex	Male	0.013	1.031 (0.613–1.734)	0.908
	Female			
Histopathological subtypes	High	0.536	1.184 (0.754–1.859)	0.464
	Middle			
	Low			
TNM stage	1-II	5.692	3.046 (1.220–7.604)	0.017 ^a
	III-IV			
Tumor size	< 3 cm	1.297	1.378 (0.794–2.394)	0.255
	≥ 3 cm			
Tumor location	Head	3.450	1.487 (0.978–2.261)	0.063
	Body/tail			
Lymph node metastasis	Negative	7.855	2.231 (1.273–3.909)	0.005 ^b
	Positive			
TBL1XR1 expression	Low	13.951	3.507 (1.815–6.774)	< 0.001 ^c
	High			

^a*P* < 0.05.^b*P* < 0.01.^c*P* < 0.001. TBL1XR1: Transducin (β)-like 1 X-linked receptor 1; CI: Confidence interval; TNM: Tumor-node-metastasis.

activity^[24,25], which provides us with another research direction. Overall, we illuminated the effect and mechanism of TBL1XR1 in human PDAC, and we believe that TBL1XR1 could be a promising diagnostic and therapeutic target for treating patients with advanced PDAC. Targeted therapy is an important aspect of precision therapy. We concluded that TBL1XR1 inhibitors might be promising therapeutic measures for PDAC; however, there are no inhibitors targeting TBL1XR1, which is worth investigating in the future.

In summary, our study showed that TBL1XR1 might regulate PDAC cell proliferation and apoptosis *via* the PI3K/AKT signaling pathway. Therefore, TBL1XR1 may be a promising diagnostic and therapeutic biomarker for patients with advanced PDAC.

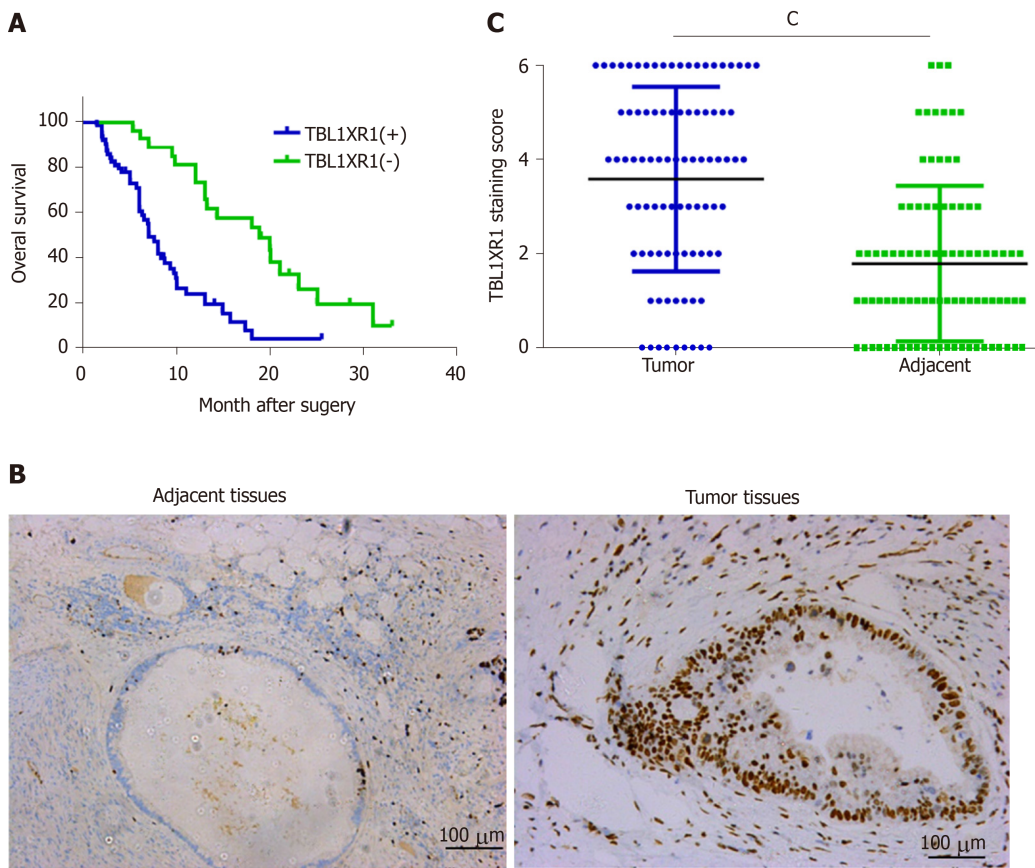


Figure 1 Clinicopathological significance of transducin (β)-like 1 X-linked receptor 1 expression in patients with pancreatic ductal adenocarcinoma. A: Kaplan–Meier plots of overall survival of pancreatic ductal adenocarcinoma (PDAC) patients with positive and negative transducin (β)-like 1 X-linked receptor 1 (TBL1XR1) expression scores; B: Negative and positive staining of TBL1XR1 in PDAC; C: The average staining scores of TBL1XR1 expression in PDAC tissues and adjacent tissues. $^{\circ}P < 0.001$. Scale bar, 100 μ m. TBL1XR1: Transducin (β)-like 1 X-linked receptor 1.

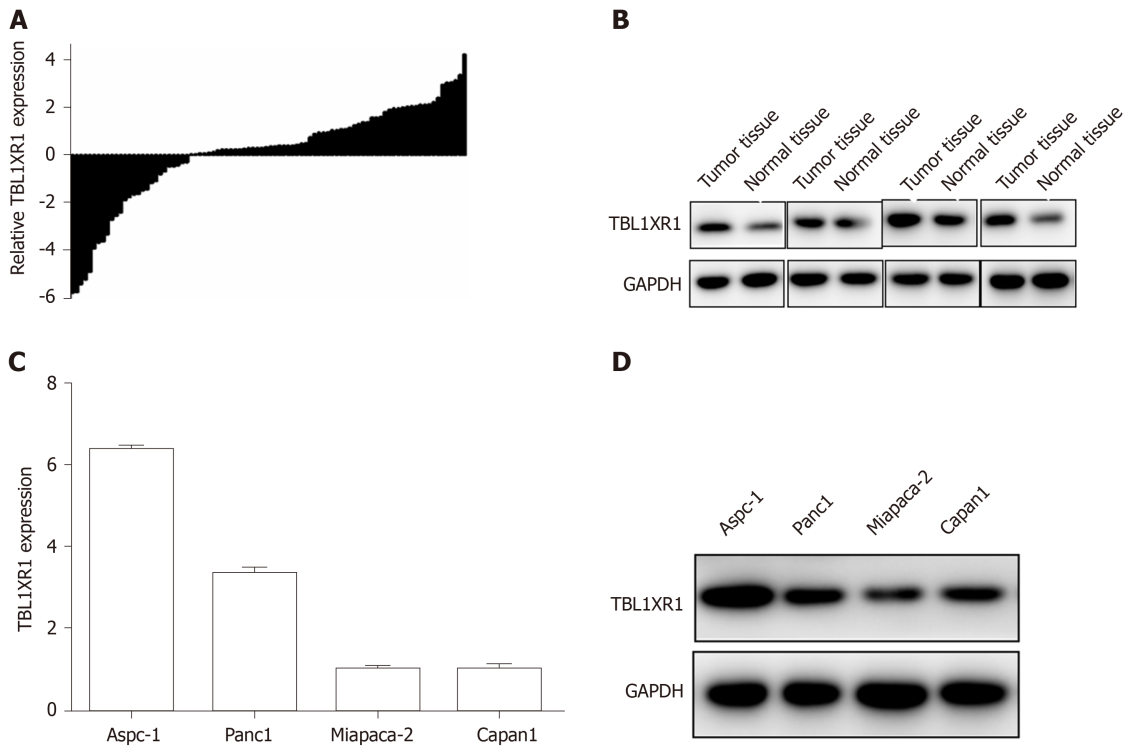


Figure 2 Enhanced expression of transducin (β)-like 1 X-linked receptor 1 in pancreatic ductal adenocarcinoma tissues and cell lines. A and B: The mRNA and protein expression of transducin (β)-like 1 X-linked receptor 1 (TBL1XR1) in pancreatic ductal adenocarcinoma tissues and adjacent nontumor tissues; C and D: The mRNA and protein expression of TBL1XR1 in pancreatic ductal adenocarcinoma cells. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; TBL1XR1: Transducin (β)-like 1 X-linked receptor 1.

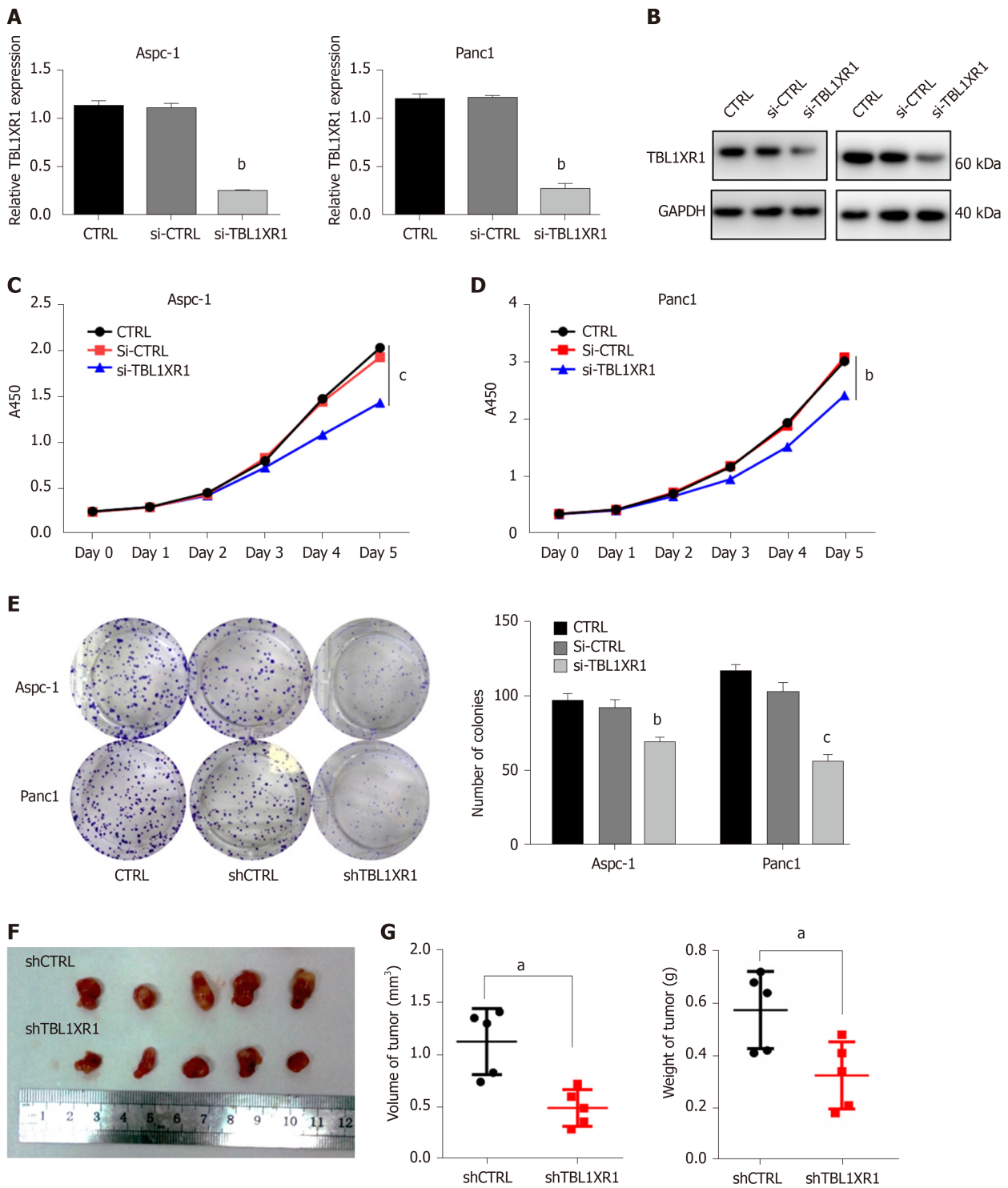
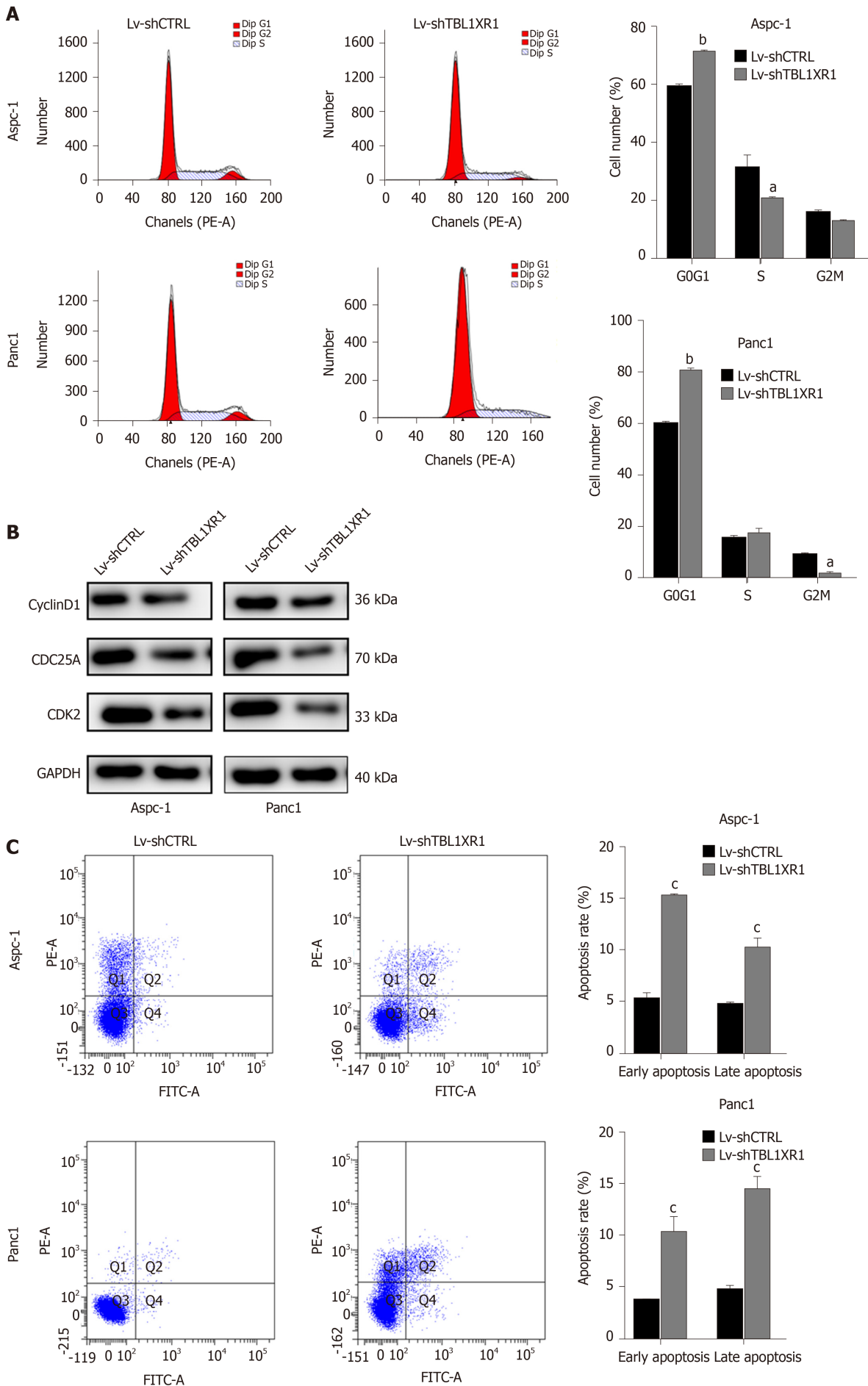


Figure 3 Transducin (β)-like 1 X-linked receptor 1 exhibited oncogenic properties in pancreatic ductal adenocarcinoma cell lines. A: The transfection efficiency of transducin (β)-like 1 X-linked receptor 1 (TBL1XR1) in pancreatic ductal adenocarcinoma (PDAC) cells by quantitative real-time PCR; B: The transfection efficiency of TBL1XR1 in PDAC cells by western blots; C and D: Cellular proliferation of untransfected or transfected Aspc-1 and Panc1 cells was measured using a CCK8 assay daily for 5 d; E: Aspc-1 and Panc1 cells were seeded at 500 cells/well, and the cells were allowed to form colonies. Colony numbers were counted and recorded; F: Mice were treated with Lv-shCTRL and Lv-shTBL1XR1 PDAC cells; G: Tumor volumes and weights were measured. ^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; TBL1XR1: Transducin (β)-like 1 X-linked receptor 1; CTRL: Control; Si-CTRL: CTRL siRNA; Si-TBL1XR1: TBL1XR1 siRNA; ShRNA: Short hairpin RNA; ShCTRL: CTRL shRNA; ShTBL1XR1: TBL1XR1 shRNA.



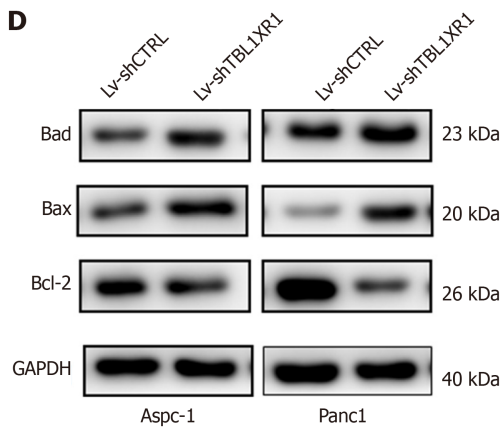
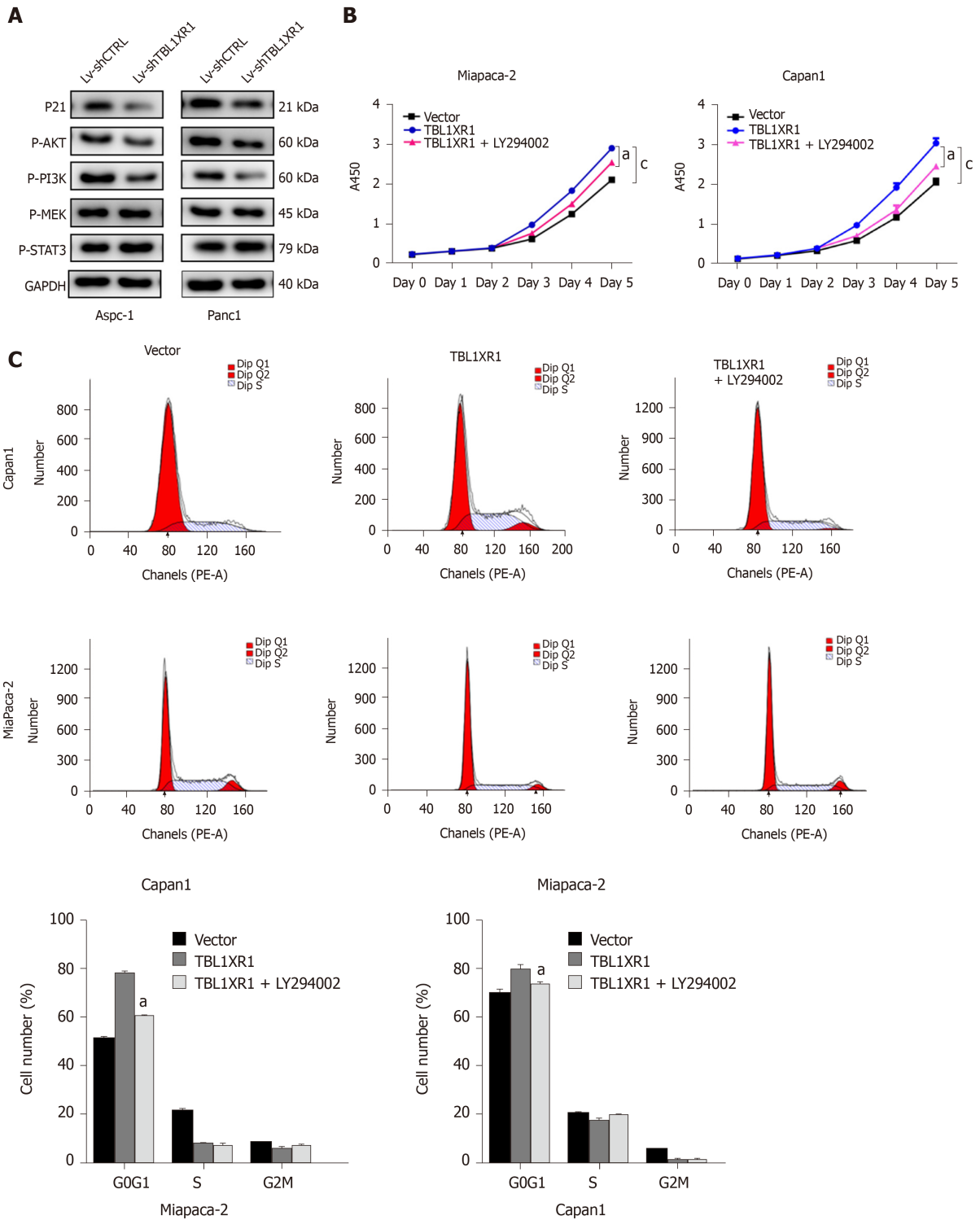


Figure 4 Effects of transducin (β)-like 1 X-linked receptor 1 silencing on cell cycle progression and apoptosis. A: The cell cycle phases of treated cells were evaluated by flow cytometry after transfection for 48 h. The X-axis indicates the different periodic distribution, and the y-axis indicates the number of cells. B: The protein levels of cell cycle regulators, such as CDK2, CDC25A and cyclinD1, were examined by western blotting analysis. C: Untransfected and transfected pancreatic ductal adenocarcinoma cells were analyzed by flow cytometry with annexin V–fluorescein 5-isothiocyanate/propidium iodide (PI) staining. The X-axis indicates annexin V–fluorescein 5-isothiocyanate, and the Y-axis indicates PI staining. Annexin V/PI⁺ represents necrotic cells, annexin V⁺/PI represents early apoptotic cells, and annexin V⁺/PI⁺ represents late apoptotic cells. D: The protein levels of bad, bax and bcl-2 were examined by western blotting analysis. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; TBL1XR1: Transducin (β)-like 1 X-linked receptor 1.



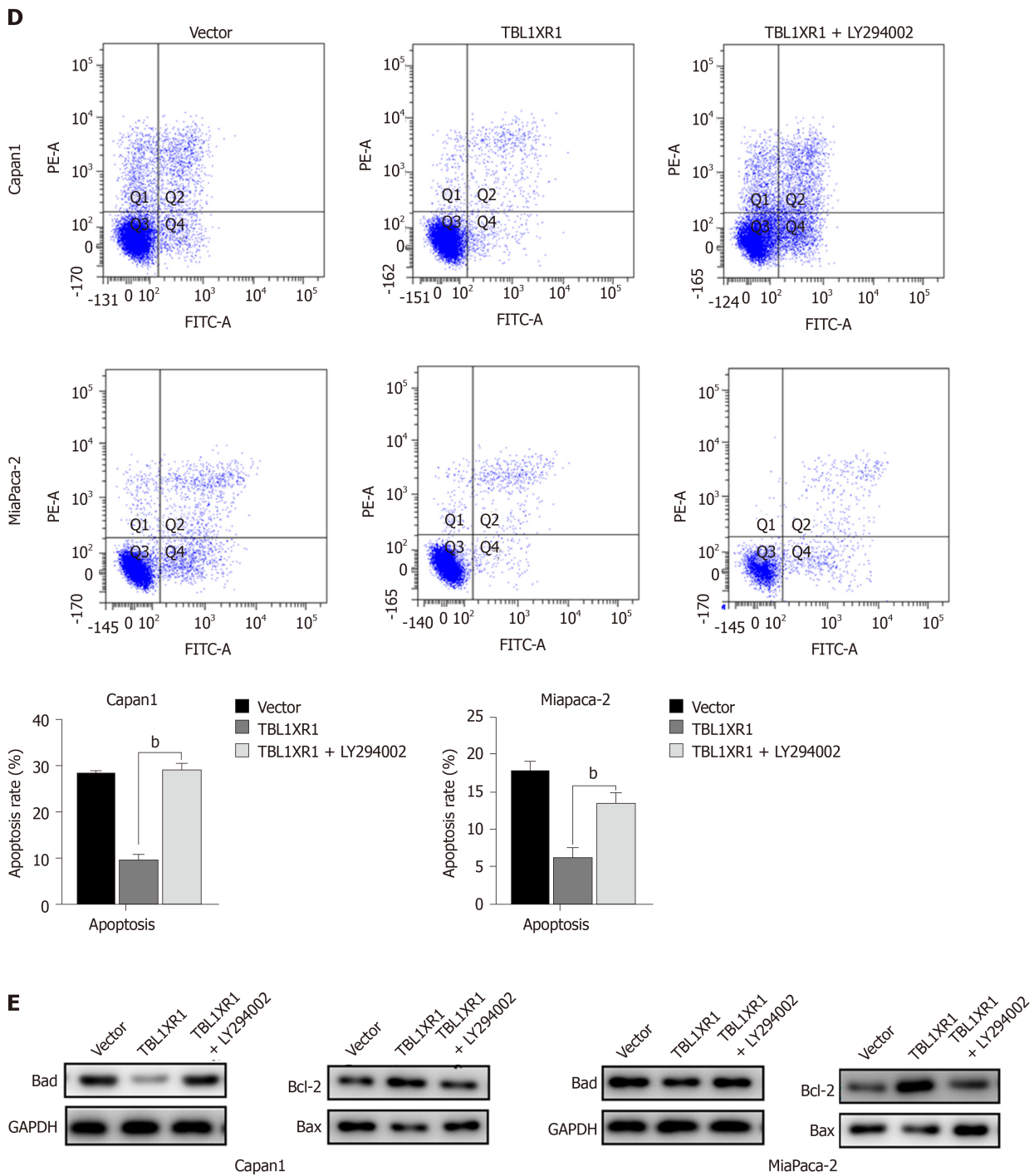


Figure 5 Transducin (β)-like 1 X-linked receptor 1 regulates phosphatidylinositol 3-kinase/protein kinase B signaling in pancreatic ductal adenocarcinoma cells. A: Western blot analysis of phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling-related proteins in both cell lines. GAPDH was used as a loading control; B: Cellular proliferation of pancreatic ductal adenocarcinoma (PDAC) cells treated with or without the PI3K/Akt pathway inhibitor LY294002 was measured using a CCK-8 assay daily for 5 d; C: Cell cycle phase analysis and apoptosis of PDAC cells treated with or without LY294002 were analyzed by flow cytometry. The X-axis indicates the different periodic distributions, and the Y-axis indicates the number of cells. D: Apoptosis analysis of PDAC cells treated with or without LY294002 was analyzed by flow cytometry. The Y-axis indicates V-fluorescein 5-isothiocyanate, and the Y-axis indicates propidium iodide (PI) staining. Annexin V/PI⁺ represents necrotic cells, annexin V⁺/PI represents early apoptotic cells, and annexin V⁺/PI⁺ represents late apoptotic cells. E: Western blot analysis of PI3K/Akt signaling-related proteins in PDAC cells treated with or without LY294002. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; TBL1XR1: Transducin (β)-like 1 X-linked receptor 1.

ARTICLE HIGHLIGHTS

Research background

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest and most aggressive solid tumors and has a poor prognosis. Transducin (β)-like 1 X-linked receptor 1 (TBL1XR1) has been linked to the progression of various human cancers. Nevertheless, the function and role of TBL1XR1 in pancreatic cancers are unclear.

Research motivation

PDAC is one of the deadliest solid tumors. Identification of diagnostic and therapeutic biomarker of PDAC is urgently needed. TBL1XR1 has been linked to the progression of various human cancers. Nevertheless, the function and role of TBL1XR1 are unclear in pancreatic cancers.

Research objectives

To explore the potential effect and mechanism of TBL1XR1 in the development of PDAC.

Research methods

Ninety histologically confirmed PDAC patients were enrolled in this study. PDAC cancer samples and cell lines were used to determine the expression of TBL1XR1. CCK-8 assays and colony formation analysis were performed to assess the viability of PDAC cells. Flow cytometry was carried out to analyze changes in the cell cycle and apoptosis. Related protein expression changes were determined by western blot analysis. Animal analysis was performed to confirm the impact of TBL1XR1 *in vivo*.

Research results

The results showed that patients with TBL1XR1-positive tumors had worse overall survival than those with TBL1XR1-negative tumors. Moreover, we found that TBL1XR1 obviously promoted PDAC cell proliferation and inhibited PDAC cell apoptosis. Moreover, knockdown of TBL1XR1 induced G0/G1 phase arrest. *In vivo* animal studies confirmed that TBL1XR1 accelerated tumor cell growth. The results of western blot analysis showed that TBL1XR1 might play a significant role in regulating PDAC cell proliferation and apoptosis *via* the PI3K/AKT pathway.

Research conclusions

TBL1XR1 promotes PDAC cell progression and might be a probable effective diagnostic and therapeutic marker for pancreatic cancer.

Research perspectives

TBL1XR1 may be an effective diagnosis and therapeutic marker for pancreatic cancer

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