doi:10.4149/neo_2019_190404N295

Silencing of TPD52 inhibits proliferation, migration, invasion but induces apoptosis of pancreatic cancer cells by deactivating Akt pathway

Z. WANG[1,2], Y. LI[1,*], L. FAN[1], Q. ZHAO[1], B. TAN[1], R. LIU[2], F. LI[2]

[1]The Third Department of Surgery, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China; [2]The First Department of General Surgery, Cangzhou Central Hospital, Cangzhou, Hebei, China

**Correspondence: li_yong_hbth@126.com*

Received April 4, 2019 / Accepted July 2, 2019

Pancreatic cancer (PC) is a complex and multifactorial human malignancy with a low survival rate. Tumor protein D52 (TPD52) was abnormally expressed in several cancers and participated in tumorigenesis. However, the oncogenic effect of TPD52 on PC remains unknown. In the present study, after transfecting AsPC-1 and PANC-1 cells with NC or sh-TPD52, the CCK-8 assay, Hoechst staining, western blot, transwell assay, flow cytometry were used to examine the cell proliferation, migration, invasion and apoptosis. qRT-PCR results confirmed that the expression of TPD52 was significantly increased in PC cells, especially AsPC-1 and PANC-1 cells. The present study revealed that silencing of TPD52 significantly suppressed the proliferation, migration and invasion, but induced apoptosis of AsPC-1 and PANC-1 cells in vitro by dephosphorylating Akt at Ser473. Conversely, SC79, an Akt activator, could partially reverse the anti-metastatic effects of sh-TPD52, accompanied by the reactivating of Akt pathway. Additional *in vivo* studies are warranted to elucidate that knockdown of TPD52 could inhibit tumor growth in PC mice models. These findings suggested that TPD52 might be a novel therapeutic target for PC treatment.

Key words: TPD52, pancreatic cancer, proliferation, migration, invasion, apoptosis, Akt

Pancreatic cancer (PC), as a kind of human malignancies, is characterized by aggressive local invasion and metastasis, causing countless deaths worldwide [1]. Due to the secretiveness of pancreatic location and the difficulty of early diagnosis, most patients with pancreatic cancer have been diagnosed as advanced. Given that PC is a complex and multifactorial human malignancy, the traditional radiation or chemotherapy strategies yield disappointing results [2, 3]. Even for PC patients who received treatment, the 5-year survival rate was less than 5% [4, 5]. Thus, it would be important to elucidate the complex mechanism of pancreatic tumorigenesis and to develop more sensitive and specific for early detection and treatment of PC.

Tumor protein D52 (TPD52), also called prostate cancer leucine zipper (PrLZ), acts as an oncogene in many malignant tumors. Previous studies have noted that the TPD52 was abnormally expressed in breast cancer, prostate cancer and ovarian cancer [6–9]. TPD52 participated in tumor progression by regulating proliferation and apoptosis of cancer cells [10]. Murine TPD52 induces cellular transformation, tumorigenesis, and progression to metastasis [11]. However, the precise role of TPD52 in regulating different cell signaling pathways remains unknown. A previous study demonstrated that exogenous overexpression of TPD52 in IMR-32 human neuroblastoma cells induced cell differentiation [12]. Also, TPD52 was caught to express in brain frontal cortex and participated in formation of synaptosomes [13, 14]. TPD52 was identified overexpressed in a variety of malignancies including human breast carcinoma [15–17]. In breast and prostate cancer patients, TPD52 was increased and correlated with poor prognosis [8, 18]. In ovarian carcinoma, increased TPD52 expression acts as a favorable independent prognostic marker [19]. Interestingly, a recent report has identified that TPD52 was associated with migration and invasion in human prostate cancers [20]. TPD52 is highly expressed in pancreatic cancer tissues, suggesting that it may play an important role in PC. Although the oncogenic effect of TPD52 has been well recognized in several cancers, its molecular functions in PC cells are still poorly defined.

This study investigated the function of TPD52 in both PC tissues and cells. The results demonstrated that silencing of TPD52 inhibited the proliferation, migration, invasion, but induced apoptosis in PC cells. These results may provide some clues for exploring new targets and therapies for PC.

Materials and methods

Cell culture and transfection. Human pancreatic cancer cell lines, AsPC-1, PANC-1, SW1990, CFPAC-1 and MIA-PaCa-2, were purchased from Procell (China) and Zhongqiaoxinzhou company (China). AsPC-1 was cultured in RPMI-1640 (Cat no. PM150110, Procell, China) containing 10% Fetal Bovine Serum (FBS) (Cat no. 04-011-1A, BI, Israel). PANC-1 and SW199 were cultured in DEME (Cat no. PM150210, Procell, China) containing 10% FBS. MIA-PaCa-2 was cultured in DMEM (high glucose) (Cat no. ZQ-106, Zhongqiaoxinzhou, China) containing 10% FBS. CFPAC-1 was cultured in IMDM (Cat no. PM150510, Procell, China) containing 10% FBS. All the cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The sh-TPD52 and NC were transfected into AsPC-1 or PANC-1 cells by Lipofectamine 2000 and selected by 300 μg/ml G418 (Cat no. KGY0031, KeyGene Biotech, China) according to the manufacturer's protocol. After transfection, cells were treated with 5 μg/ml SC79 (Cat no. SF2730, Beyotime, China) or equal DMSO for 24 h for further study. Sh-TPD52 sequence (5'–3'): gatccgGGATCACCTACTCTTCAGA-ttcaagagaTCTGAAGAGTAGGTGATCCttttta; agcttaaaaa-GGATCACCTACTCTTCAGAtctcttgaaTCTGAAGAG-TAGGTGATCCcg.

Transwell assay. After transfection or SC79 treatment, cells were digested with 0.25% trypsin and washed with Phosphate Buffer Saline (PBS) before seeding on the top chambers of 24-well transwell culture inserts (Cat no. 3422, Corning, USA). Medium supplemented with 20% FBS was added into the lower chambers. After 24 h, cells were washed with PBS, fixed with 4% formalin for 10 min, and then stained with 0.4% crystal violet for 5 min.

Hematoxylin-eosin (HE) staining. After the rats were sacrificed, tumors were embedded in paraffin and sliced into 0.5 μm sections. Then the sections were first deparaffinized in xylene and dehydrated with gradient concentration of ethanol. Thereafter, the sections were washed by ddH_2O and stained with hematoxylin (Cat no. WLA051a, Wanleibio, China) for 5 min and differentiated in 1% hydrochloric acid ethanol for another 3 s. After 2 min in ddH_2O, eosin dye solution was added to counterstain the tumors for 3 min and then dehydrated with gradient concentration of ethanol for 5 min. Lastly, the tumors sections were detected under a microscope (Cat no. DP73, OLYMPUS, Japan).

Wound healing assay. AsPC-1 and PANC-1 cells were cultured in RPMI-1640 or DEME with 10% FBS. When reaching a confluence of 80–90%, the cells were treated with 1 μg/ml mitomycin C without FBS. Uniform wounds were created by scraping with a sterile pipette tip. The cells were continued to culture in serum-free medium. The wound closure was observed under the microscope and photographed at 0 and 24 h after scratching.

Cell cycle analysis. The cell cycle was assayed by flow cytometry. Briefly, the cells after transfection were harvested. Then the cells were washed with PBS for twice, fixed in 70% ethanol and stored at 4 °C overnight. Next day, the cells were twice washed with PBS, resuspended with 500 μl dye buffer and incubated with propidium iodide/ribonuclease staining solution (Cat no. C1052, Beyotime, China) for 30 min at room temperature, following the manufacturer's instructions. Cell cycle distribution was detected and analyzed using the FACScan instrument and CellQuest program (NovoCyte, ACEABIO, USA).

Hoechst assay. Forty-eight hours after post-transfection, cells were collected and washed with PBS for twice. Then cells were stained by Hoechst Staining Kit (Cat no. C0003, Beyotime, China) and detected by a fluorescent microscope (400×).

Cell counting kit-8 (CCK-8) assay. After transfection, the cells were cultured in 5% CO_2 condition at 37 °C. At 0 h, 24 h, 48 h, and 72 h post-transfection, the CCK-8 assay was used to detect the cell proliferation. Ten μl of CCK-8 reagent (Cat no. 96992, Sigma, China) was added into each well and incubated for 1 h. The optical density of solution in each well at 450 nm (OD_{450}) was detected using a Microplate Reader (ELX-800, BIOTEK, USA).

Annexin V/PI double staining. The cells after transfection for 24 h were collected and washed with PBS for twice. Then the cell apoptosis was measured by Annexin V/PI kit (Cat no. C1062, Beyotime, China) according to the manufacturer's protocol and detected by flow cytometry.

Quantitative real-time PCR (qRT-PCR). Twenty-four hours after post-transfection, total cellular RNA was extracted from AsPC-1 and PANC-1 cells using RNApure total RNA fast isolation kit (Cat no. RP1201, BioTeke, China). Then the extracted RNA was reverse transcribed into cDNA using M-MLV reverse transcriptase (Cat no. 2641A, Takara) following the manufacturer's protocol. Quantitative RT-PCR was performed with Taq HS Perfect Mix (Cat no. R300A, Takara, Japan) by an Exicycler 96 Real-Time Quantitative PCR Detection System (BIONEER, Korea). The primer sequences were as follows:

TPD52-F: 5'-CAGAACATTGCCAAAGGG-3',
TPD52-R: 5'-CTGAGCCAACAGACGAAA-3',
β-actin-F: 5'-GGCACCCAGCACAATGAA-3',
β-actin-R: 5'-CGGACTCGTCATACTCCTGCT-3'.

The cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of 94 °C for 20 s, 60 °C for 30 s and 72 °C for 40 s. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Western blot. Equal protein amount (30 μg) was separated by Sodium Dodecyl Sulfate (SDS) PAGE. Thereafter, the protein bands were transferred onto a polyvinylidene fluoride (PVDF) membrane (Cat no. LC2005, Thermo Fisher Scientific, USA), which was then incubated with TPD52 antibody (1:500 dilution; Cat no. 11795-1-AP, Proteintech, China), pro-caspase-3 antibody (1:500 dilution; Cat no. 19677-1-AP, Proteintech, China), cleaved caspase-3 antibody (1:1000 dilution; Cat no. 9654, CST, USA), Bax

antibody (1:5000 dilution; Cat no. 50599-2-lg, Proteintech, China), Bcl-2 antibody (1:2000 dilution; Cat no.12789-1-AP, Proteintech, China), Akt antibody (1:1000 dilution; Cat no. 4685, CST, USA), p-Akt antibody (1:1000 dilution; Cat no. 4060, CST, USA) or β-actin antibody (1:1000 dilution; Cat no. 60008-1-Ig, Proteintech, China) overnight at 4 °C. The membrane was then incubated with goat anti-rabbit IgG antibody (1:10000 dilution; Cat no. SA00001-2, Proteintech, China) or goat anti-mouse IgG antibody (1:10000 dilution; Cat no. SA00001-1, Proteintech, China) at 37 °C for 40 min. The signal intensity of each protein was measured with ECL reagent (Cat no. E003, Seven Sea biotech, China). β-actin served as a control standard.

Xenograft assay. The animal experiments in this study were approved by the legislation and ethics boards of Cangzhou Central Hospital and obeyed the Guideline for the care and use of laboratory animals. Transfected AsPC-1 cells (2×10^6 cells/mouse) were subcutaneously injected into the right flank of 4-week-old BALB/c male nude mice (HFK Bio-Technology Co., Ltd, Beijing, China). The mice were divided into two groups randomly, 6 mice per group (n=6). Based on the formula $V=0.5\times L\ (\text{length})\times W^2\ (\text{width})$, the tumor volumes were measured every 3 days. Mice were sacrificed 4 weeks after cell inoculation by an overdose of pentobarbital sodium. Solid tumor tissues were removed and weighed.

Statistical analysis. Data analysis was performed using GraphPad Prism 7.0 software (GraphPad Software, USA.). All assays were repeated three times, and the results are presented as the mean ± SD. The statistical significance was determined by One-way ANOVA analysis. A p-value <0.05 was considered to be significant.

Results

Silencing of TPD52 inhibited the proliferation of AsPC-1 and PANC-1 cells but induced cell apoptosis and G1 phase arrest. To explore the function of TPD52 in PC, we first examined the expression of TPD52 in several different PC cells, including PANC-1, AsPC-1, MIA-PaCa-2, SW1990, CFPAC-1 cell by western blotting (Figure 1A). For further study we chose PANC-1 and AsPC-1 cells, which showed the highest expressions of TPD52. Sh-TPD52 or NC was transfected into PANC-1 or AsPC-1 cells, and its effects on the cell proliferation, apoptosis, and cell cycle were investigated. Quantitative RT-PCR was performed to determine the efficacy of transfection of sh-TPD52. As shown in Figure 1B and C, the transfection of sh-TPD52 significantly inhibited both mRNA and protein levels of TPD52 compared with NC in PANC-1 or AsPC-1 cells (***p<0.001). Then, the effect of silencing of TPD52 on the cell proliferation was determined by CCK-8 assay. As shown in Figure 1D, the silencing of TPD52 inhibited the proliferation of PANC-1 or AsPC-1 cells after 72 h incubation (*p<0.05). These results demonstrated that the silencing of TPD52 inhibited the PC cell growth. To investigate the effect of TPD52 on cell apoptosis, the Hoechst staining was performed, and suggested that cell apoptosis was increased after the transfection of sh-TPD52 (Figure 1E). Moreover, the cell apoptosis was measured by Annexin V/PI double staining assay and the results were consistent with the Hoechst staining (Figure 1F). Next, the apoptosis-related protein containing pro-caspase-3, cleaved caspase-3, Bax and Bcl-2 was detected by western blotting (Figure 1G). The results showed that the expressions of cleaved caspase-3 and Bax were remarkably increased by sh-TPD52, the Bcl-2 was decreased and the pro-caspase-3 remained same. Cell cycle assay provided more detailed information by flow cytometry. As shown in Figure 1H, sh-TPD52-treated cells were arrested in G1 phase compared with NC (*p<0.05). Thus, these results revealed that silencing of TPD52 inhibited the proliferation of AsPC-1 and PANC-1 cells but induced cell apoptosis and G1 phase arrest.

Silencing of TPD52 inhibited cell invasion and migration capability in AsPC-1 and PANC-1 cells and alleviated the phosphorylation of Akt (Ser473). To determine the capability of invasion and metastasis, we used wound healing and transwell invasion assay to examine the effect of TPD52 on metastasis in AsPC-1 and PANC-1 cells. The results indicated that sh-TPD52 significantly inhibited cell migration compared with NC, bar = 200 μm (Figure 2A). Transwell invasion assay analysis revealed that there was fewer AsPC-1 or PANC-1 cells which transfected with sh-TPD52 crossed into the transwell membrane compared with NC, bar = 100 μm (Figure 2B). These results demonstrated that silencing of TPD52 could inhibit cell invasion and migration in AsPC-1 and PANC-1 cells. Moreover, the expressions of p-Akt (Ser473) and Akt were assessed by western blotting (Figure 2C). The expression of p-Akt (Ser473) was significantly depressed by transfection with sh-TPD52, with no change in Akt expression. Therefore, these results could conclude that silencing of sh-TPD52 inhibited cell invasion and migration capability in AsPC-1 and PANC-1 cells and alleviated the phosphorylation of Akt (Ser473).

Silencing of TPD52 inhibited cell proliferation, invasion, migration and induced apoptosis by deactivating Akt pathway. The Akt pathway possesses a protective role in tumorigenesis. SC79 was an activator of Akt pathway. To investigate the role and mechanism of TPD52 in cell proliferation, migration, invasion and apoptosis, we treated AsPC-1 and PANC-1 cells with 5 μg/ml SC79, an Akt pathway agonist, or solvent DMSO after transfection. The results showed that SC79 improved the cell proliferation, which was inhibited by sh-TPD52 in AsPC-1 and PANC-1 cells (*p<0.05 vs sh-TPD52+DMSO; Figure 3A). Almost immediately, we found that SC79 inhibited sh-TPD52-induced cell apoptosis by Hoechst staining (Figure 3B). The wound healing assay was used to detect the cell migration, and the result showed that the capability of migration was recovered by SC79, which was significantly decreased by transfected with sh-TPD52 in AsPC-1 and PANC-1 cells (Figure 3C). Although the

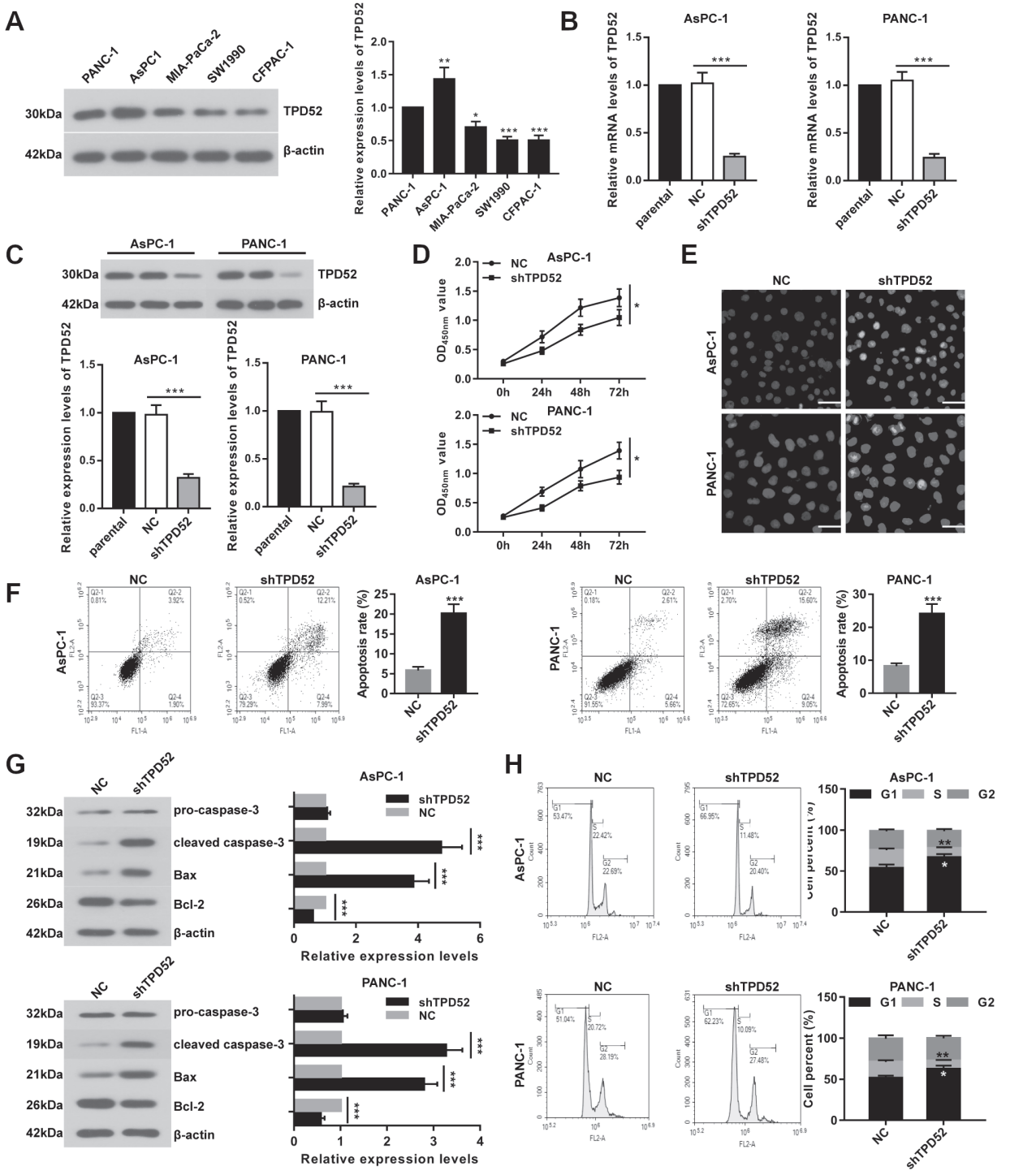

Figure 1. Silencing of TPD52 inhibited the proliferation of AsPC-1 and PANC-1 cells but induced cell apoptosis and G1 phase arrest. A) The expression of TPD52 in PANC-1, AsPC-1, MIA-PaCa-2, SW1990, CFPAC-1 cells was detected by western blotting. *p<0.05, **p<0.01, ***p<0.001, compared with PANC-1. B) The mRNA levels of TPD52 after transfection of sh-TPD52 were determined by RT-PCR in PANC-1 and AsPC-1 cells. ***p<0.001, compared with NC. C) The expression of TPD52 after transfection of sh-TPD52 was determined by western blotting in PANC-1 and AsPC-1 cells. ***p<0.001, compared with NC. D) Silencing of TPD52 inhibited the cell proliferation. Cells were transfected with sh-TPD52 or NC and the cell growth was determined by CCK-8 assay. **p<0.01, compared with NC. E) Silencing of TPD52 induced cell apoptosis. Cells were treated with sh-TPD52 or NC and cell apoptosis was examined by Hoechst assay. F) Cell apoptosis measured by Annexin V/PI staining and detected by flow cytometry. G) The expression of apoptosis-related proteins containing pro-caspase-3, cleaved caspase-3, Bax and Bcl-2 was detected by western blotting. **p<0.01, ***p<0.001, compared with NC. H) Cell cycle assay revealed that silencing of TPD52 induced G1 phase arrest. Bar = 50 μm, *p<0.05, **p<0.01 compared with NC. Each experiment was repeated three times.

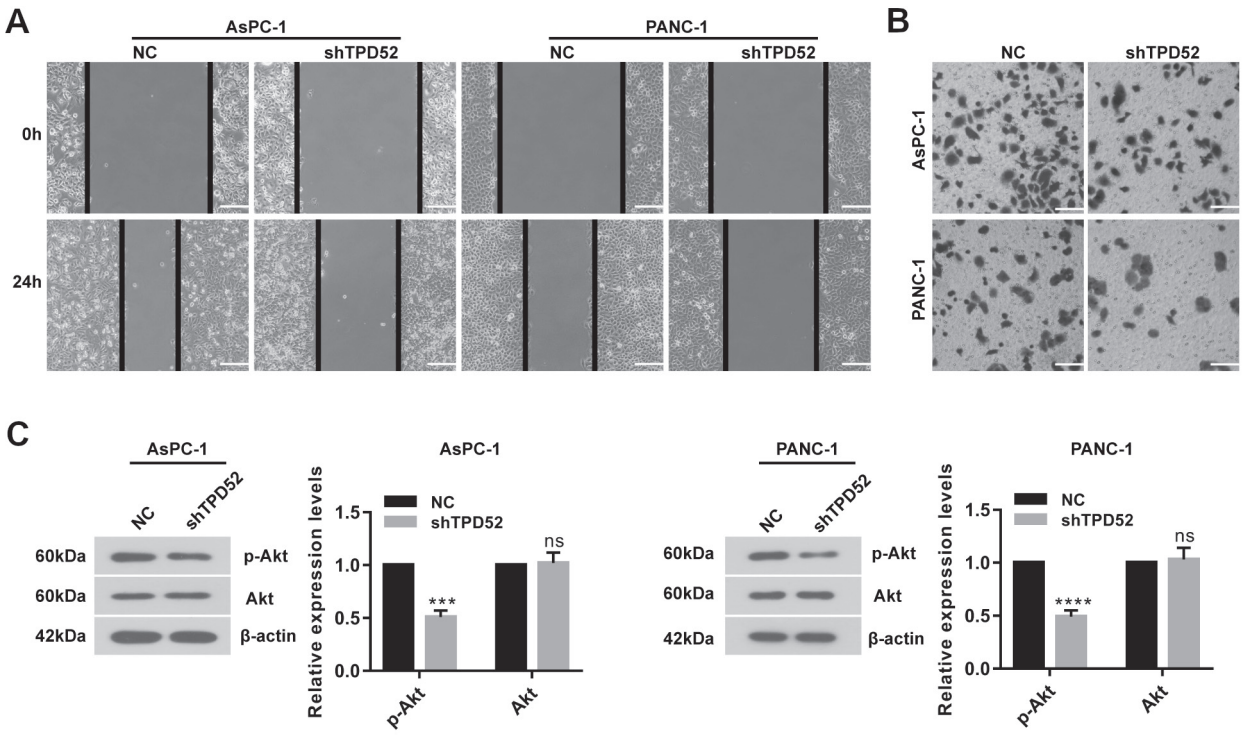

Figure 2. Silencing of TPD52 inhibited cell invasion and migration capability in AsPC-1 and PANC-1 cells and alleviated the phosphorylation of Akt (Ser473). A) Wound healing assay showed that sh-TPD52 inhibited cell migration in AsPC-1 and PANC-1 cells compared with NC, bar = 200 μm. B) Transwell assay revealed that transfection of sh-TPD52 reduced cell invasion through the transwell membrane compared with NC, bar = 100 μm. C) The levels of p-Akt (Ser473) and Akt were detected by western blotting in AsPC-1 and PANC-1 cells. ***p<0.001, ****p<0.0001, compared with NC. Each experiment was repeated three times.

cells invasion ability was decreased by sh-TPD52, transwell invasion assay analysis indicated that SC79 reversed the number of AsPC-1 and PANC-1 cells which crossed into the transwell membrane (Figure 3D). Moreover, the expression of p-Akt (Ser473) was inhibited by sh-TPD52, whereas the consequence was reversed by SC79 treatment (**p<0.01, ***p<0.001 vs sh-TPD52+DMSO; Figure 3E). Thus, all these results could confirm that silencing of TPD52 inhibited cell proliferation, invasion, migration and induced apoptosis by deactivating Akt pathway.

Silencing of TPD52 inhibited tumor growth via deactivating Akt pathway *in vivo*. To investigate the inhibitory effect of TPD52 on human PC cells *in vivo*, AsPC-1 cells were used to establish a nude mouse xenograft model for *in vivo* experiments. The nude mice were randomly divided to two groups (n=6 per group): NC group and sh-TPD52 group. All nude mice were sacrificed 28 days post-treatment, and the xenograft tumors were removed and weighed. The results showed that sh-TPD52 inhibited tumor growth *in vivo* compared with NC mice, including weight and volume (Figures 4A-C). Also, HE staining assay indicated that the sh-TPD52 inhibited the tumor growth and induced the tumor necrosis (Figure 4D). Moreover, the western blotting analysis showed that the expression of p-Akt (Ser473) was decreased, suggesting that the phosphorylation of Akt at Ser473 was significant attenuated (***p<0.001, ****p<0.0001, Figure 4E). Thus, these findings indicated that sh-TPD52 inhibited tumor growth via deactivating Akt pathway *in vivo*.

Discussion

TPD52 was confirmed to participate in several tumor progressions. Previous studies have demonstrated that TPD52 was overexpressed in PC cells and speculated that this overexpression is associated with the progression of PC. However, the effect of TPD52 on PC needs more studies. In the current study, we demonstrated that silencing of TPD52 could inhibit cell proliferation, migration, invasion and induce apoptosis by decreasing the phosphorylation of Akt at Ser473. Nevertheless, these changes were reversed by SC79. Moreover, the *in vivo* experiments also confirmed that knockdown of TPD52 inhibited tumor growth in PC mice models. These results suggest that the roles of TPD52 during PC progression may depend on p-Akt pathway and thus provide a therapeutic target for PC treatment.

Pancreatic cancer has a poor prognosis and leads to death, worldwide. Therefore, the underlying mechanism of PC tumorigenesis urgently needs to be explored. A previous study

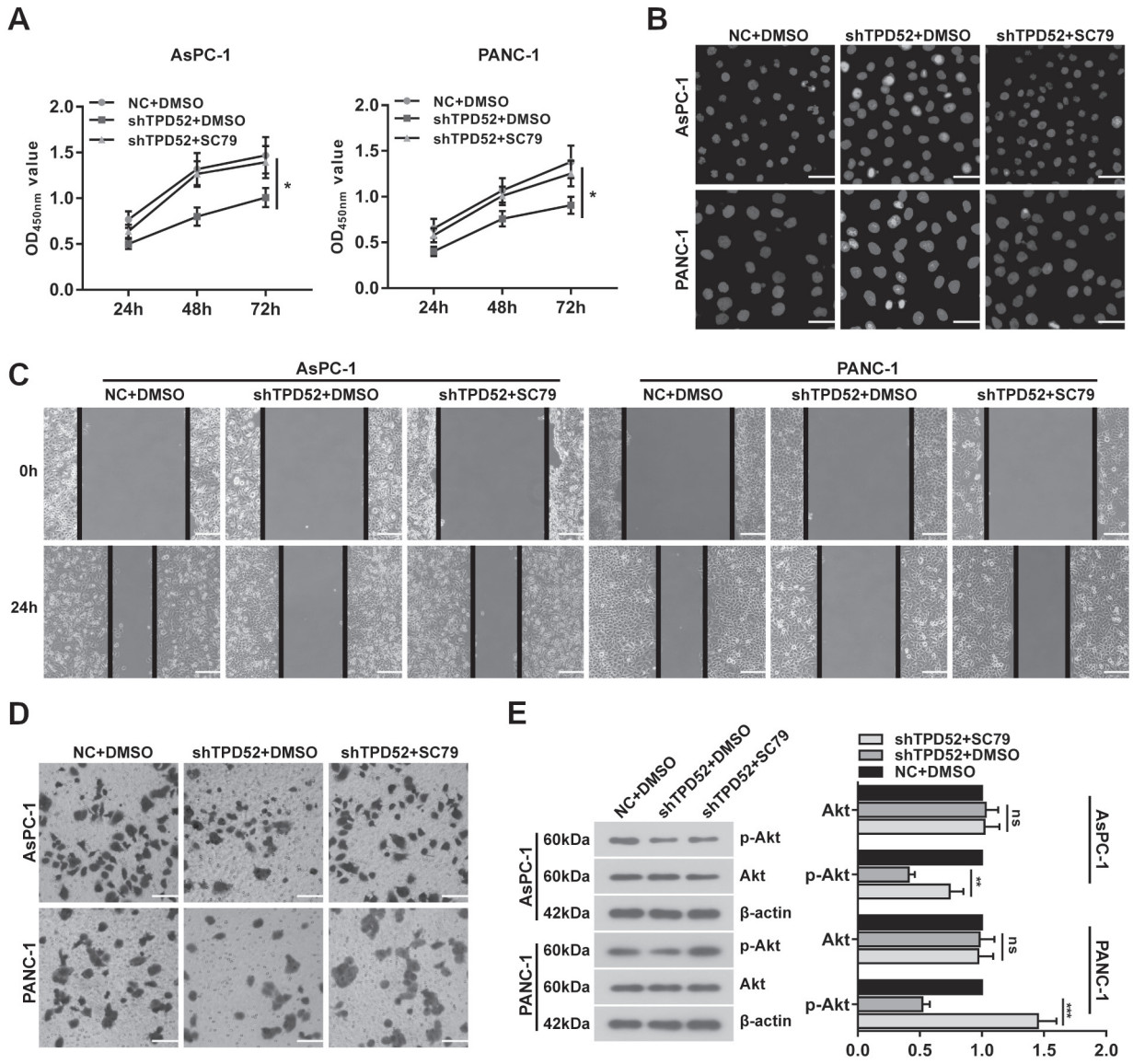

Figure 3. Silencing of TPD52 inhibited cell proliferation, invasion, migration and induced apoptosis by deactivating Akt pathway. After transfection with sh-TPD52, AsPC-1 and PANC-1 cells were treated with 5 μg/ml SC79 or DMSO for 24 h. A) Cell proliferation was determined by CCK-8 assay. B) Cell apoptosis was observed by Hoechst staining, bar = 50 μm. C) Wound healing assay was used to evaluate the cell migration ability changes, bar = 200 μm. D) Transwell assay was used to assess the cell invasion ability changes, bar = 100 μm. E) The levels of p-Akt (Ser473) and Akt were detected by western blotting in AsPC-1 and PANC-1 cells. *p<0.05, **p<0.01, ***p<0.001, compared with sh-TPD52+SC79.

has demonstrated that several molecular events play vital roles in PC progression, containing miRNA, long noncoding RNAs and proteins. MiRNA-143 inhibited migration and invasion but promoted apoptosis of pancreatic cancer cells [21]. LncRNA-BX111887 (BX111), a kind of hypoxia-induced lncRNA, enhanced proliferation and invasion of pancreatic cancer cells [22]. L-type amino acid transporter 2 (LAT2), an oncogenic protein, promoted glycolysis and decreased GEM sensitivity in PC [23]. More importantly, TPD52 is a member of the similarly named gene and protein family. A plenty of studies showed that TPD52 is upregulated in human cancers, along with the association with prostate cancer, colon cancer and lung squamous cell carcinoma (LSCC) metastasis [10, 24–26]. Dysregulation of TPD52 played a role in prostate cancer progression, regulated apoptosis and migration [10, 24]. TPD52 was upregulated in all cases of colorectal cancer investigation and served as a new biomarker in colon cancer [25]. It was reported that TPD52 was overexpressed in LSCC cells and promoted cancer cell aggressiveness. [26]. In the present study, we found that the copy number of TPD52 was increased in PC cells. By transfecting AsPC-1 and PANC-1 cells with sh-TPD52, the cell proliferation, migra-

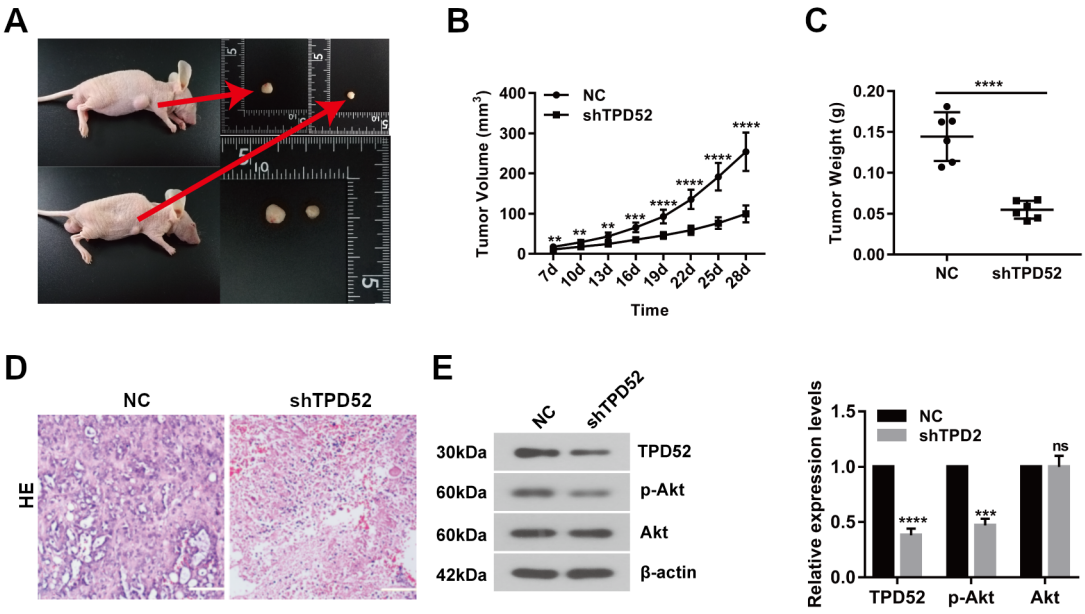

Figure 4. Silencing of TPD52 inhibited tumor growth via deactivating Akt pathway *in vivo*. Total of 12 nude mice were injected with AsPC-1 cells and divided to two groups (n = 6 per group): NC group and sh-TPD52 group. A) Xenograft tumors isolated from mice injected with AsPC-1 cells for 28 days. B) The tumor volume of the xenograft tumors from NC and sh-TPD52 mice. C) The tumor weights of the xenograft tumors from NC and sh-TPD52 mice. D) The tumors were embedded in paraffin and observed by HE staining assay, bar = 100 μm. E) The levels of p-Akt (Ser473) and Akt were detected by western blotting in tumor tissues. **p<0.01, ***p<0.001, ****p<0.0001 vs NC.

tion and invasion were inhibited by sh-TPD52. Also, the cell apoptosis was induced by sh-TPD52. More interestingly, the tumor growth was inhibited by knockdown of TPD52. These findings were consistent with previous studies, which might lay a foundation for researches of PC treatment.

Akt is an important cellular kinase involved in cell proliferation, apoptosis invasion and migration [27]. Several studies concluded that increased Akt activation could enhance the migratory or invasive activity of many cells [28]. Akt pathway is activated by site-specific phosphorylation at Ser473 or Thr308 [29]. Interestingly, we further examined the expression of Akt and p-Akt (Ser473), and the results indicated that the level of Akt caused no change, but the phosphorylation of Akt at Ser473 was significantly decreased *in vivo* and *in vitro*. For the maximum activation of Akt, Ser473 is phosphorylated possibly by autophosphorylation or mTOR signaling [30]. Jin et al. revealed that AKT/mTOR pathway was regulated by multiple genes, including IGF1R, mTOR and AKT1, and contributed to wound healing [31]. SC79, an activator of Akt pathway, could protect hepatocytes from apoptosis by activating Akt pathway *in vivo* and *in vitro* [32]. In the present study, after treatment with SC79, the sh-TPD52-induced changes of AsPC-1 and PANC-1 in cell proliferation, migration, invasion and apoptosis were reversed. Thus, we speculated that TPD52 could induce the phosphorylation of Akt at Ser473 and increase IGF1R, mTOR, and AKT1 and contribute to migration and invasion. Combined treatment of targeting AKT and SRC resulted in a synergistic efficacy against human pancreatic cancer growth and metastasis [32]. Thus, TPD52 might be a vital factor in PC treatment. However, the potential mechanism of TPD52 participating in Akt pathway and influences the cell proliferation, migration, invasion and apoptosis remain still unclear. There are still more investigations for this section in the future.

In conclusion, TPD52 was strongly expressed in PC cells and its expression was found to be associated with tumor metastasis. *In vitro* assays indicated that silencing of TPD52 inhibited cell proliferation, migration and invasion and induced cell apoptosis by dephosphorylating Akt at Ser473. Moreover, these changes were reversed by SC79 – a kind of APK pathway activator. Additional *in vivo* studies are necessary to elucidate that knockdown of TPD52 could inhibit PC tumor growth in mice models. These findings suggested that TPD52 might be a novel therapeutic target for PC treatment.

References

[1] JOOSTE V, DEJARDIN O, BOUVIER V, ARVEUX P, MAYNADIE M et al. Pancreatic cancer: Wait times from presentation to treatment and survival in a population-based study. Int J Cancer 2016; 139: 1073–1080. https://doi.org/10.1002/ijc.30166

[2] CAMPBELL PJ, YACHIDA S, MUDIE LJ, STEPHENS PJ, PLEASANCE ED et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature 2010; 467: 1109–1113. https://doi.org/10.1038/nature09460

[3] MAITRA A, HRUBAN RH. Pancreatic cancer. Annu Rev Pathol 2008; 3: 157–188. https://doi.org/10.1146/annurev.pathmechdis.3.121806.154305

[4] RAHIB L, SMITH BD, AIZENBERG R, ROSENZWEIG AB, FLESHMAN JM et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014; 74: 2913–2921. https://doi.org/10.1158/0008-5472.CAN-14-0155

[5] WEISSLEDER R, PITTET MJ. Imaging in the era of molecular oncology. Nature 2008; 452: 580–589. https://doi.org/10.1038/nature06917

[6] BALLEINE RL, FEJZO MS, SATHASIVAM P, BASSET P, CLARKE CL et al. The hD52 (TPD52) gene is a candidate target gene for events resulting in increased 8q21 copy number in human breast carcinoma. Genes Chromosomes Cancer 2000; 29: 48–57. https://doi.org/10.1002/1098-2264(2000)9999:9999<::aid-gcc1005>3.0.co;2-o

[7] POLLACK JR, SORLIE T, PEROU CM, REES CA, JEFFREY SS et al. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. Proc Natl Acad Sci U S A 2002; 99: 12963–12968. https://doi.org/10.1073/pnas.162471999

[8] WANG R, XU J, SARAMAKI O, VISAKORPI T, SUTHERLAND WM et al. PrLZ, a novel prostate-specific and androgen-responsive gene of the TPD52 family, amplified in chromosome 8q21.1 and overexpressed in human prostate cancer. Cancer Res 2004; 64: 1589–1594. https://doi.org/10.1158/0008-5472.can-03-3331

[9] BYRNE JA, BALLEINE RL, SCHOENBERG FEJZO M, MERCIECA J, CHIEW YE et al. Tumor protein D52 (TPD52) is overexpressed and a gene amplification target in ovarian cancer. Int J Cancer 2005; 117: 1049–1054. https://doi.org/10.1002/ijc.21250

[10] UMMANNI R, TELLER S, JUNKER H, ZIMMERMANN U, VENZ S et al. Altered expression of tumor protein D52 regulates apoptosis and migration of prostate cancer cells. FEBS J 2008; 275: 5703–5713. https://doi.org/10.1111/j.1742-4658.2008.06697.x

[11] LEWIS JD, PAYTON LA, WHITFORD JG, BYRNE JA, SMITH DI et al. Induction of tumorigenesis and metastasis by the murine orthologue of tumor protein D52. Mol Cancer Res 2007; 5: 133–144. https://doi.org/10.1158/1541-7786.MCR-06-0245

[12] KOTAPALLI SS, DASARI C, DUSCHARLA D, KAMI REDDY KR, KASULA M et al. All-Trans-Retinoic Acid Stimulates Overexpression of Tumor Protein D52 (TPD52, Isoform 3) and Neuronal Differentiation of IMR-32 Cells. J Cell Biochem 2017; 118: 4358–4369. https://doi.org/10.1002/jcb.26090

[13] TEE AE, MARSHALL GM, LIU PY, XU N, HABER M et al. Opposing effects of two tissue transglutaminase protein isoforms in neuroblastoma cell differentiation. J Biol Chem 2010; 285: 3561–3567. https://doi.org/10.1074/jbc.M109.053041

[14] BIESEMANN C, GRONBORG M, LUQUET E, WICHERT SP, BERNARD V et al. Proteomic screening of glutamatergic mouse brain synaptosomes isolated by fluorescence activated sorting. EMBO J 2014; 33: 157–170. https://doi.org/10.1002/embj.201386120

[15] BYRNE JA, FROST S, CHEN Y, BRIGHT RK. Tumor protein D52 (TPD52) and cancer-oncogene understudy or understudied oncogene? Tumour Biol 2014; 35: 7369–7382. https://doi.org/10.1007/s13277-014-2006-x

[16] CHEN Y, KAMILI A, HARDY JR, GROBLEWSKI GE, KHANNA KK et al. Tumor protein D52 represents a negative regulator of ATM protein levels. Cell Cycle 2013; 12: 3083–3097. https://doi.org/10.4161/cc.26146

[17] TENNSTEDT P, BOLCH C, STROBEL G, MINNER S, BURKHARDT L et al. Patterns of TPD52 overexpression in multiple human solid tumor types analyzed by quantitative PCR. Int J Oncol 2014; 44: 609–615. https://doi.org/10.3892/ijo.2013.2200

[18] SHEHATA M, BIECHE I, BOUTROS R, WEIDENHOFER J, FANAYAN S et al. Nonredundant functions for tumor protein D52-like proteins support specific targeting of TPD52. Clin Cancer Res 2008; 14: 5050–5060. https://doi.org/10.1158/1078-0432.CCR-07-4994

[19] BYRNE JA, MALEKI S, HARDY JR, GLOSS BS, MURALI R et al. MAL2 and tumor protein D52 (TPD52) are frequently overexpressed in ovarian carcinoma, but differentially associated with histological subtype and patient outcome. BMC Cancer 2010; 10: 497. https://doi.org/10.1186/1471-2407-10-497

[20] GOTO Y, NISHIKAWA R, KOJIMA S, CHIYOMARU T, ENOKIDA H et al. Tumour-suppressive microRNA-224 inhibits cancer cell migration and invasion via targeting oncogenic TPD52 in prostate cancer. FEBS Lett 2014; 588: 1973–1982. https://doi.org/10.1016/j.febslet.2014.04.020

[21] XU B, LIU J, XIANG X, LIU S, ZHONG P et al. Expression of miRNA-143 in Pancreatic Cancer and Its Clinical Significance. Cancer Biother Radiopharm 2018; 33: 373–379. https://doi.org/10.1089/cbr.2018.2500

[22] DENG SJ, CHEN HY, YE Z, DENG SC, ZHU S et al. Hypoxia-induced LncRNA-BX111 promotes metastasis and progression of pancreatic cancer through regulating ZEB1 transcription. Oncogene 2018; 37: 5811–5828. https://doi.org/10.1038/s41388-018-0382-1

[23] FENG M, XIONG G, CAO Z, YANG G, ZHENG S et al. LAT2 regulates glutamine-dependent mTOR activation to promote glycolysis and chemoresistance in pancreatic cancer. J Exp Clin Cancer Res 2018; 37: 274. https://doi.org/10.1186/s13046-018-0947-4

[24] RUBIN MA, VARAMBALLY S, BEROUKHIM R, TOMLINS SA, RHODES DR et al. Overexpression, amplification, and androgen regulation of TPD52 in prostate cancer. Cancer Res 2004; 64: 3814–3822. https://doi.org/10.1158/0008-5472.CAN-03-3881

[25] PETROVA DT, ASIF AR, ARMSTRONG VW, DIMOVA I, TOSHEV S et al. Expression of chloride intracellular channel protein 1 (CLIC1) and tumor protein D52 (TPD52) as potential biomarkers for colorectal cancer. Clin Biochem 2008; 41: 1224–1236. https://doi.org/10.1016/j.clinbiochem.2008.07.012

[26] KUMAMOTO T, SEKI N, MATAKI H, MIZUNO K, KAMIKAWAJI K et al. Regulation of TPD52 by antitumor microRNA-218 suppresses cancer cell migration and invasion in lung squamous cell carcinoma. Int J Oncol 2016; 49: 1870–1880. https://doi.org/10.3892/ijo.2016.3690

[27] MARTINI M, DE SANTIS MC, BRACCINI L, GULLUNI F, HIRSCH E. PI3K/AKT signaling pathway and cancer: an updated review. Ann Med 2014; 46: 372–383. https://doi.org/10.3109/07853890.2014.912836

[28] ADYA R, TAN BK, PUNN A, CHEN J, RANDEVA HS. Visfatin induces human endothelial VEGF and MMP-2/9 production via MAPK and PI3K/Akt signalling pathways: novel insights into visfatin-induced angiogenesis. Cardiovasc Res 2008; 78: 356–365. https://doi.org/10.1093/cvr/cvm111

[29] BELLACOSA A, CHAN TO, AHMED NN, DATTA K, MALSTROM S et al. Akt activation by growth factors is a multiple-step process: the role of the PH domain. Oncogene 1998; 17: 313–325. https://doi.org/10.1038/sj.onc.1201947

[30] TOKER A, NEWTON AC. Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. J Biol Chem 2000; 275: 8271–8274. https://doi.org/10.1074/jbc.275.12.8271

[31] JIN Y, TYMEN SD, CHEN D, FANG ZJ, ZHAO Y et al. MicroRNA-99 family targets AKT/mTOR signaling pathway in dermal wound healing. PLoS One 2013; 8: e64434. https://doi.org/10.1371/journal.pone.0064434

[32] JING ZT, LIU W, XUE CR, WU SX, CHEN WN et al. AKT activator SC79 protects hepatocytes from TNF-alpha-mediated apoptosis and alleviates d-Gal/LPS-induced liver injury. Am J Physiol Gastrointest Liver Physiol 2019; 316: G387–G396. https://doi.org/10.1152/ajpgi.00350.2018