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Original Article

Curcumin inhibits cell growth and invasion and induces apoptosis through down-regulation of Skp2 in pancreatic cancer cells

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Abstract: Natural polyphenol compound curcumin has been found to exhibit its anticancer activity in a variety of human malignancies including pancreatic cancer (PC). However, the underlying mechanism has not been fully understood. Accumulating evidence has demonstrated that Skp2 (S-phase kinase associated protein 2) plays an oncogenic role in the development and progression of human cancers. In this study, we aim to explore the molecular basis of curcumin-induced cell growth inhibition in PC cells. Multiple methods such as CTG assay, Flow cytometry, clonogenic assay, wound healing assay, Transwell invasion assay, Western blotting, and transfection were performed to validate the oncogenic role of curcumin in PC cells. We found that curcumin suppressed cell growth, clonogenic potential, migration and invasion, and induced cell apoptosis and cell cycle arrest. Moreover, we observed that overexpression of Skp2 significantly promoted cell growth, whereas down-regulation of Skp2 with siRNAs inhibited cell growth. The molecular basis of curcumin-mediated cell growth inhibition we identified is that curcumin significantly suppressed Skp2 expression and subsequently induced p21 expression. These findings suggested that targeting Skp2 by curcumin could be a promising therapeutic strategy for the treatment of PC patients.

Keywords: Curcumin, pancreatic cancer, Skp2, invasion, proliferation

Introduction

Pancreatic cancer (PC) is one of the most aggressive and lethal forms of human malignancies worldwide [1]. PC is the fourth leading cause of cancer-related deaths with an approximately annual incidence of 46,420 and mortality of 39,590 in the United States in 2014 [2]. In China, PC is the seventh deadliest disease with an overall 5-year survival rate of 4.1% and a median survival time of 3.9 months [3]. The PC patients are always diagnosed at an advanced stage and effective targeted therapy is extremely limited. It has been well known that the majority of the PC patients become intractable to standard chemotherapeutic drugs such as gemcitabine and 5-FU (5-fluorouracil) or their combinations [4]. Thus, there is an urgent need to explore the underlying molecular mechanisms of PC pathogenesis and to develop efficient pharmacological agents for the treatment.

Skp2 (S-phase kinase associated protein 2) is a crucial component of the SCF (Skp1-Cullin1-F-box) type of E3 ubiquitin-ligase complexes involved in cell cycle progression through degradation of its ubiquitination targets [5]. Noteworthy, accumulated evidence has demonstrated that Skp2 plays a critical role in the development and progression of human cancers including PC [6]. Skp2 is a *bona fide* proto-oncoprotein and exerts its oncogenic activity by targeting and degrading its ubiquitination targets such as p21 [7], p27 [8], p57 [9], E-cadherin [10], and FOXO1 [11]. Consistent with this notion, Skp2 plays a key role in regulating cell growth, apoptosis, differentiation, cell cycle progression and metastasis [12]. One study has shown that acetylated by p300, Skp2 is localized in cytoplasm and subsequently enhances cell migration via degradation of E-cadherin [10, 13]. Lin et al. reported that Akt directly phosphorylates Skp2, leading to promotion of cell proliferation

and tumorigenesis [14]. They also proved that inactivation of Skp2 suppresses tumorigenesis [15]. Moreover, Skp2 is over-expressed and correlated with poor prognosis in a variety of human cancers, including PC [12, 16], prostate cancer [12], breast cancer [17, 18], nasopharyngeal carcinoma [19], and glioma [20]. Remarkably, over-expression of Skp2 is associated with the extent of lymph node metastasis, higher histological grade, and poorer patient outcome in PC patients [16]. Schuler et al. further demonstrated that Skp2 confers resistance of PC cells towards TRAIL (tumor necrosis factor-related apoptosis-inducing ligand)-induced apoptosis [21]. Notably Skp2 activates Akt ubiquitination, glycolysis, herceptin sensitivity and tumorigenesis [22]. Strikingly, pharmacological inactivation of Skp2 ubiquitin ligase restricts cancer stem cell traits and cancer progression [23] and tumorigenesis [24]. Altogether, these findings indicated that inactivation of Skp2 could be a promising approach for better management of human cancer patients.

Curcumin is a natural polyphenol compound derived from turmeric (*Curcuma longa*). A growing body of evidence implicates that curcumin exhibits multiple activities such as antioxidant, anti-inflammatory, anti-diabetic, antiviral, antifungal, antibacterial, wound-healing and neuroprotective properties [25]. Over the last two decades, a number of studies have proved that curcumin has anticancer effects against a variety of tumors both *in vitro* and *in vivo* [26]. More importantly, in contrast with conventional cytotoxic drugs, curcumin has minimal toxicity and is safety at high dose by human clinical trials [27, 28]. Curcumin exerts anticancer effects, both alone and in combination with other anticancer drugs (e.g. gemcitabine, 5-FU, and oxaliplatin), by modulating a variety of molecular targets. To date, more than 30 molecular targets have been identified, including NF-κB (nuclear factor-κB), Akt, Notch, mTOR (mammalian target of rapamycin), and Hedgehog [26, 29, 30]. Although numerous studies have indicated curcumin's anticancer effects, the underlying mechanism has not been fully understood.

Therefore, in the current study, we explored whether high-level Skp2 was responsible for cell growth, clonogenic ability, migration, invasion, apoptosis and cell cycle arrest. We also determined whether curcumin exhibited its anticancer activity against PC cell lines via inactivation of Skp2. We found that Skp2 was critically involved in PC tumorigenesis. A significantly down-regulation of Skp2 after curcumin treatment was observed, resulting in up-regulation of p21, which could lead to restraint of tumorigenesis. These findings suggest that inhibition of Skp2 by curcumin could be an imperative approach for the treatment of PC.

Materials and methods

Cell culture and reagents

Human PC cell lines Patu8988 and Panc-1 were obtained from ATCC and maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin in a 5% CO_2 atmosphere at 37°C. Primary antibodies against Skp2, β-actin and the secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-p21 and p27 antibodies were purchased from Cell Signaling Technology. Lipofectamine 2000 was purchased from Invitrogen. Curcumin (CAS number 458-37-7, 99.5% purity) was obtained from Sigma-Aldrich (St. Louis, MO). Curcumin was dissolved in DMSO to make a 30 mM stock solution and was added directly to the medium at different concentrations. Cells were treated with 0.1% DMSO as the control group. CellTiter-Glo Luminescent Cell Viability Assay (CTG, Promega) was carried out by following the manufacture's instruction.

Cell viability assay

The Patu8988 and Panc-1 cells (4×10^3) were seeded in a 96-well plate. After an overnight culture, cells were treated with different concentrations of curcumin for 48 h and 72 h. At the end of treatment period, 20 μL of reagent CTG was added to each well. Mix contents for 2 minutes on an orbital shaker to induce cell lysis. Allow the plate to incubate for 10 minutes at room temperature and then detect the omitted luminescence using a plate reading luminometer.

Clonogenic assay

In order to examine the survival of cells treated with curcumin, Patu8988 and Panc-1 cells were plated (3×10^5 per well) in a 6-well plate and incubated overnight. After 72 h exposure to different concentrations of curcumin, the viable

cells were counted and seeded into 60 mm dishes in a range of 1,000 cells per plate. The cells were then incubated for 21 days at 37°C in a humidified 5% CO_2 atmosphere. All the colonies were stained with 2% crystal violet.

Wound healing assay

Patu8988 and Panc-1 cells were seeded in a 6-well plate at the concentration of 2×10^6 cells per well. After cells converged almost 100%, absorbed the supernatant carefully and scratched the cells with a yellow pipette tips. The wound was generated and then washed the cells with PBS. Added medium containing curcumin to the cells and incubated for 20 h. The scratched area was photographed with an Olympus microscope at 0 h and 20 h, respectively.

Cell apoptosis analysis

The apoptotic cells were detected with Annexin V-FITC/PI apoptosis detection kit (Biouniqure, China). Briefly, PC cells were incubated in 6-well plate overnight and treated with various concentrations of curcumin for 48 h. Cells were harvested by centrifugation, washed with PBS, and then resuspended in 500 μl of binding buffer with 5 μl Propidium iodide (PI) and 5 μl FITC-conjugated anti-Annexin V antibody. All the samples were kept in the dark for 15 min at room temperature. Apoptosis was analyzed using a flow cytometer (BD, USA).

Cell cycle analysis

Exponentially growing PC cells were seeded in the 6-well plate and incubated overnight. The cells were then treated with curcumin and cultured for 48 h. At the end of treatment period, cells were collected and fixed with ice-cold 70% (v/v) ethanol and kept at 4°C overnight. The cells were collected and washed with ice-cold PBS. Then, the cell pellets were re-suspended at 1×10^6 cells/ml in PBS and incubated with 0.1 mg/ml RNase I and 50 mg/ml Propidium iodide (PI) at 37°C for 30 min. DNA contents were determined with a flow cytometer (BD, USA).

Transwell invasion assay

Cell invasive capacity of Patu8988 and Panc-1 was performed using Transwell Filter (8 μm pore size, Corning) with Matrigel (BD Biosciences). Briefly, PC cells treated with curcumin or Skp2 transfection or combination were tr ansferred in each upper chamber in 200 μL of serum-free medium. And 500 μL of complete medium was added into each bottom chamber with the same concentration of curcumin. After incubation for 24 h, the cells in the upper chamber were removed, and the invaded cells in the membrane were stained with Wright's-Giemsa. The stained cells were photographed and counted under a light microscope in at least six randomly-selected fields.

Transfection

PC cells were seeded into 6-well plates and transfected with Skp2 cDNA or Skp2 siRNA or empty vector using lipofectamine 2000 by following the manufacture's instruction. Skp2 siRNA oligonucleotides were purchased from GenePharma (Shanghai, China): sense 5'-GGA GUG ACA AAG ACU UUG UTT-3'; antisense 5'-ACA AAG UCU UUG UCA CUC CTT-3'. After the indicated periods of incubation, the cells were subjected to further analysis as described under the results sections.

Western blotting analysis

The harvested PC cells were washed by PBS and lysed with cell lysis buffer (Cell Signaling, Danvers, MA). The protein concentrations were tested by BCA Protein Assay kit (Thermo Scientific, MA). Equal amount of protein samples were prepared and fractionated by electrophoresis in Sodium Dodecyl Sulfonate (SDS)-polyacrylamide gel and then transferred onto a Polyvinylidene Fluoride (PVDF) membrane. Appropriate primary antibodies were added and then incubated at 4°C overnight. The membranes were washed 3 times with TBST and then incubated with second antibody at room temperature for 1 h. The protein bands were subsequently detected by electrochemiluminescence (ECL) assay.

Statistical analysis

All data analyses were conducted using GraphPad Prism 4.0 (Graph Pad Software, La Jolla, CA). Statistical comparisons were performed using the Student *t* test. Results are expressed as means ± SD. P values < 0.05 were considered statistically significant.

Results

Curcumin suppressed cell proliferation

In order to determine whether curcumin treatment suppresses cell growth in PC cells, CTG

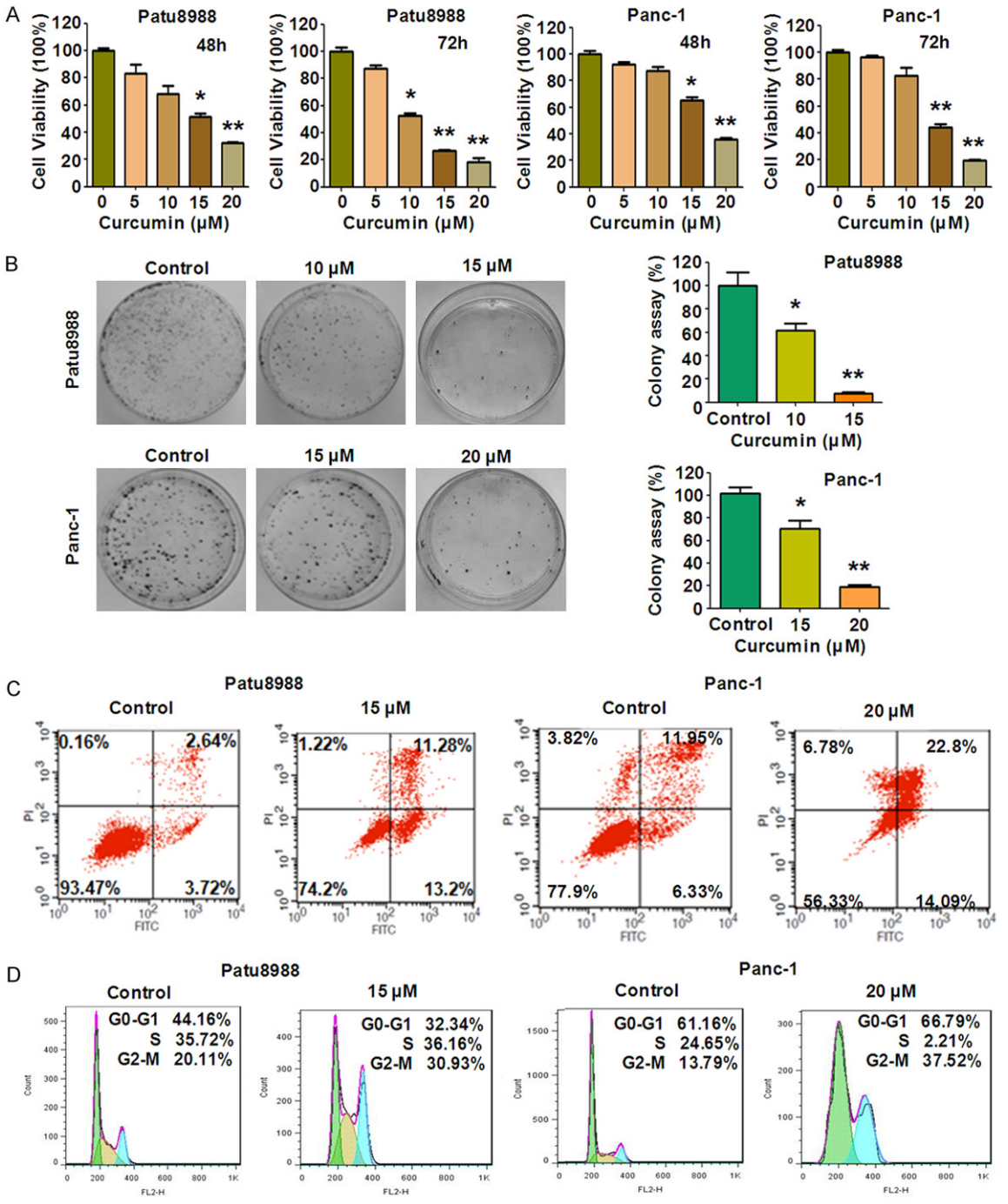
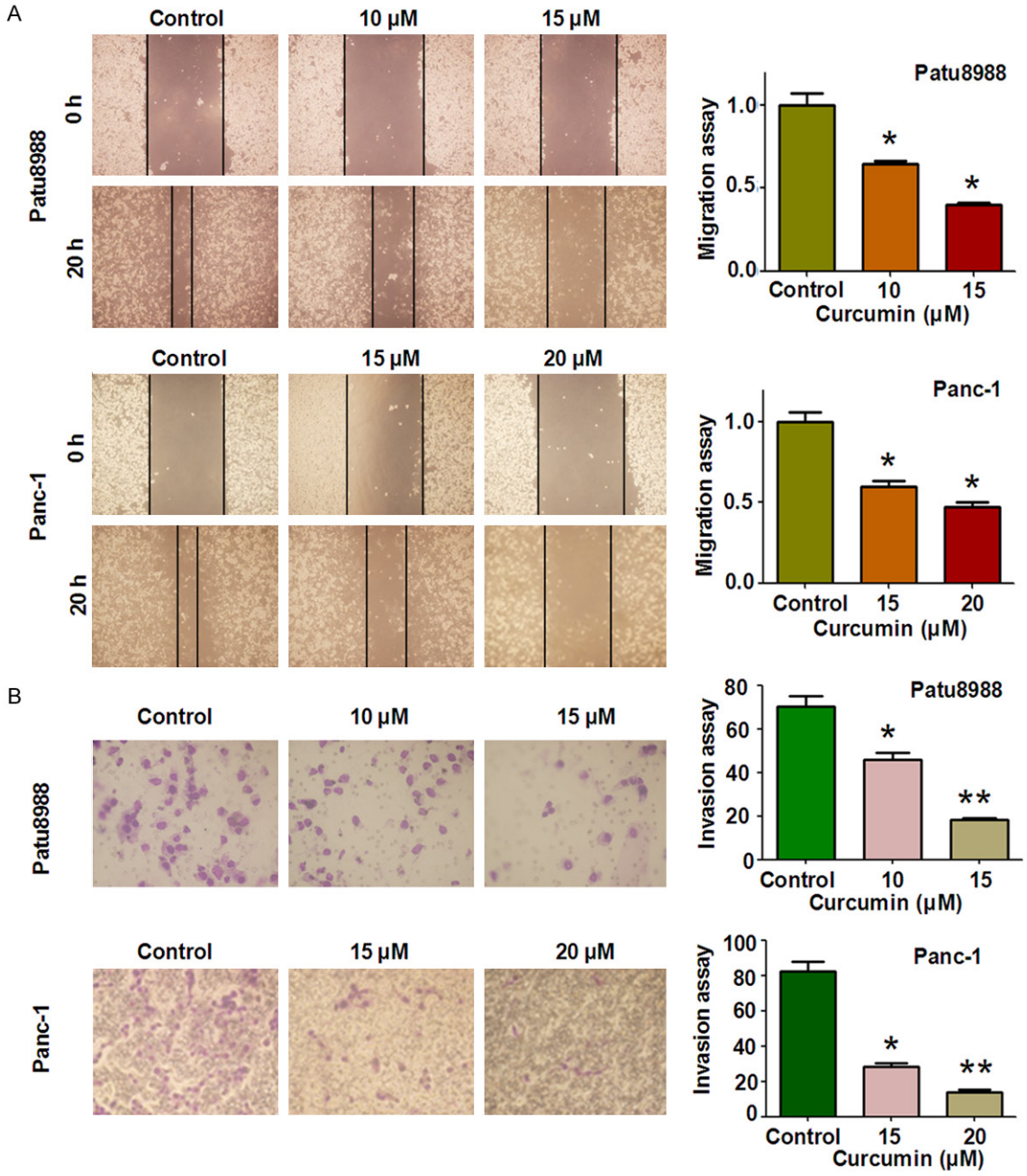


Figure 1. Effect of curcumin on PC cell growth, apoptosis, and cell cycle. A. Effect of curcumin on PC cells growth was detected by CTG assay after treatment with curcumin for 48 and 72 h. *P < 0.05, **P < 0.01, compared to the control groups (DMSO treatment). B. Left panel, Colony formation viability of PC cells treated with curcumin was evaluated by clonogenic assay. Right panel, quantitative results are illustrated for left panel. *P < 0.05, **P < 0.01 vs control. X axis: concentrations of curcumin. Y axis: percentage of colony numbers in control groups. C. Cell apoptosis in PC cells treated with curcumin was accessed by Flow cytometry. D. Curcumin induced PC cell cycle arrest at G2-M Phase.

measurement was carried out to test the growth viability in Patu8988 and Panc-1 cells treated with various concentrations of curcumin for 48 hours and 72 hours, respectively. We found that curcumin significantly suppressed cell growth in a time- and dose-dependent


A
Control
10 μM
15 μM
Patu8988
0 h
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Migration assay
Patu8988
1.0
0.5
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Curcumin (μM)
Control
15 μM
20 μM
Panc-1
Panc-1
Control 15 20
Curcumin (μM)
B
Invasion assay
Patu8988
80
60
40
20
0
Control 10 15
Curcumin (μM)
Panc-1
100
80
60
40
20
0
Control 15 20
Curcumin (μM)
C
Patu8988
Panc-1
Control
10 μM
15 μM
20 μM
Skp2
p21
β-actin
Tubulin

Figure 2. Curcumin inhibited cell migration and invasion in PC cells. A. Left panel, the inhibitory effect of curcumin on PC cell migration was detected using wound healing assay in Patu8988 cells and Panc-1 cells. Right panel, quantitative results are illustrated for left panels. *P < 0.05, vs control (DMSO treatment). B. The inhibitory effect of curcumin on PC cell invasion was detected by Transwell chambers assay in Patu8988 cells and Panc-1 cells. Right panel, quantitative results are illustrated for left panel. *P < 0.05, **P < 0.01 vs control. C. The expression levels of Skp2 and p21 were determined by Western blotting analysis in Patu8988 and Panc-1 cells after curcumin treatment.

manner in both Patu8988 and Panc-1 cells (**Figure 1A**). Moreover, the 50% inhibitive concentration (IC_{50}) of curcumin was examined. After a 72 h treatment, the IC_{50} values of Patu8988 and Panc-1 cells were found to around 10 μM and 15 μM, respectively (**Figure 1A**). Therefore, we selected 10 μM and 15 μM of curcumin for Patu8988 cells treatment and 15 μM and 20 μM for Panc-1 cells in the following studies.

Curcumin inhibited colony formation

We further confirmed the effects of curcumin on cell growth by clonogenic assay. Compared with control, curcumin treatment caused a significant inhibition of colony formation in both PC cells in a dose-dependent manner (**Figure 1B**). In accordance with CTG assay (**Figure 1A**), the results from clonogenic assay indicate that curcumin inhibited cell survival in both Patu8988 and Panc-1 cells.

Curcumin induced apoptosis

Next, we measured apoptotic cells using Annexin V-FITC/PI apoptosis detection kit. Patu8988 cells were treated with 15 μM curcumin for 48 hours and Panc-1 cells were treated with 20 μM curcumin. As shown in flow cytometer data, the percentage of apoptotic cells increased from 6.36% in the control to 24.48% and from 18.28% in control cells to 36.89% in curcumin-treated Patu8988 and Panc-1 cells (**Figure 1C**), respectively. These results indicated that curcumin treatment caused a statistically evident increase of apoptotic cells, leading to notable cell growth inhibition in both PC cell lines.

Curcumin induced cell cycle arrest

To detect whether curcumin abolished cell cycle progression, PI staining and flow cytometry assay were performed in both PC cells treated with curcumin for 48 hours. As a result, a typical G2/M arrest pattern was identified. The G2/M phase fraction increased from 20.11% in control cells to 30.93% in curcumin-treated Patu8988 cells (**Figure 1D**). Similar G2/M arrest was found in curcumin-treated Panc-1 cells (**Figure 1D**). These findings revealed that curcumin treatment could induce obvious G2/M phase arrest in PC cells.

Curcumin inhibited cell migration and invasion

Wound healing assay and Transwell assay were conducted to examine whether curcumin inhibited the motility of the PC cells. Compared with control group, our wound healing assay showed that curcumin significantly inhibited the migration of Patu8988 and Panc-1 cells (**Figure 2A**). Transwell assay further demonstrated that curcumin treatment inhibited the invasion of PC cells transit from the matrigel-coated membrane. Moreover, we found that curcumin inhibited cell migration and invasion of both PC cells in a dose-dependent manner (**Figure 2B**). These results clearly suggest that curcumin possesses an anti-invasive function in PC cells.

Curcumin down-regulated Skp2 expression

Recently, it has been reported that Skp2 plays its oncogenic roles in tumorigenesis [31]. To further investigate the underlying molecular mechanism of curcumin-mediated anticancer activities, alterations in Skp2 were examined using Western blotting analysis in PC cells treated with curcumin. As shown in **Figure 2C**, Skp2 was markedly down-regulated in both patu8988 and Panc-1 cells after curcument treatment. Consistent with the note that p21 was one of Skp2 downstream targets, we further confirm whether curcumn-mediated inactivation of Skp2 could impact the expression of p21 in PC cells. As a result, we found that curcumin treatment significantly enhanced the accumulation of tumor suppressor p21 in both PC cell lines (**Figure 2C**). Our findings suggest that curcumin exerts its anticancer activities at least partly through inactivation of Skp2 and subsequent up-regulation of its target in PC cells.

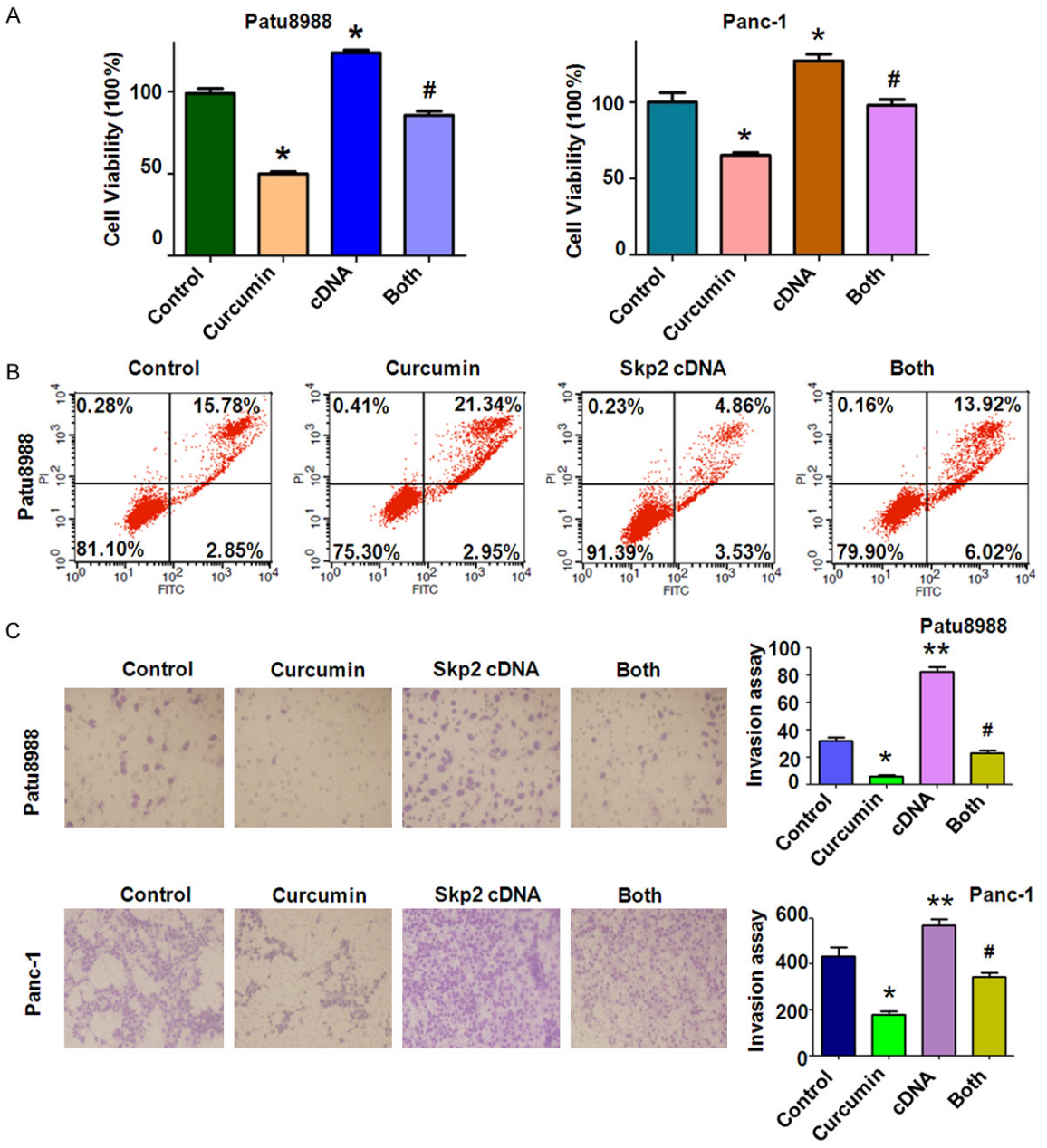


Figure 3. Overexpression of Skp2 promoted cell proliferation and inhibited apoptosis in PC cells. A: The effect of Skp2 overexpression in combination with curcumin treatment on PC cell growth was detected by CTG assay. Control: control cDNA (pcDNA 3.1); cDNA: Skp2 cDNA; Both: Skp2 cDNA+Curcumin. B: Cell apoptosis was accessed by Flow cytometry. *P < 0.05, **P < 0.01, compared with control; #P < 0.05 compared with curcumin treatment or Skp2 cDNA transfection. C. Left panel, the PC cells invasion was detected after Skp2 cDNA transfection and curcumin treatment. Right panel, Quantitative results are illustrated for left panel. *P < 0.05, **P < 0.01, compared with control; #P < 0.05 compared with curcumin treatment or Skp2 cDNA transfection.

Over-expression of Skp2 rescued curcumin-induced cell growth inhibition and apoptosis

In order to detect whether curcumin exerts its anticancer effects through inhibition of Skp2 in PC cells, Patu8988 and Panc-1 cells were transfected with Skp2 cDNA or empty vector as control. We found that re-expression of Skp2 promoted cell growth (**Figure 3A**). Moreover, over-expression of Skp2 partly abrogated curcumin-induced cell growth inhibition in both PC cells (**Figure 3A**). Next, we measured whether

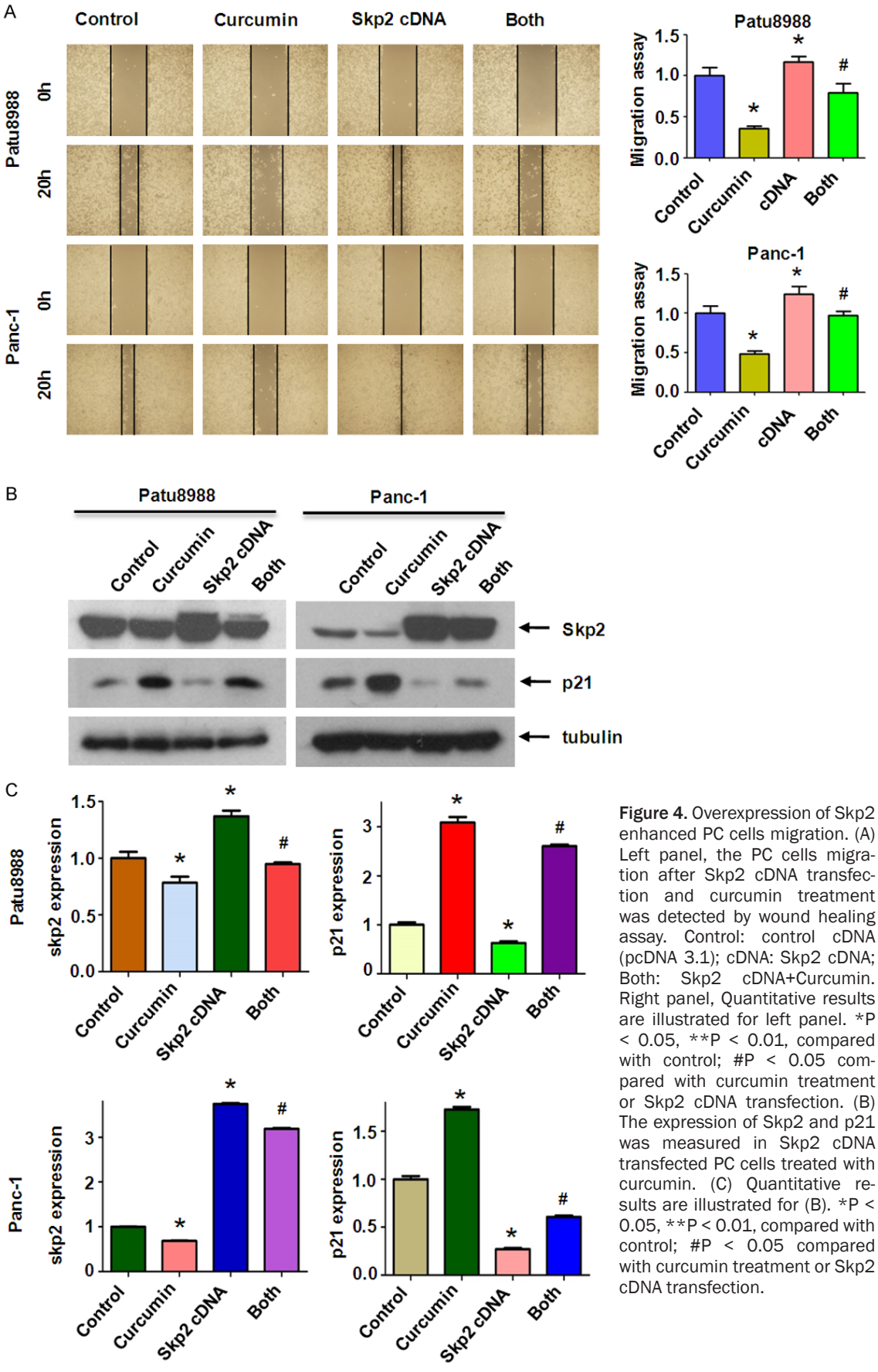


Figure 4. Overexpression of Skp2 enhanced PC cells migration. (A) Left panel, the PC cells migration after Skp2 cDNA transfection and curcumin treatment was detected by wound healing assay. Control: control cDNA (pcDNA 3.1); cDNA: Skp2 cDNA; Both: Skp2 cDNA+Curcumin. Right panel, Quantitative results are illustrated for left panel. *P < 0.05, **P < 0.01, compared with control; #P < 0.05 compared with curcumin treatment or Skp2 cDNA transfection. (B) The expression of Skp2 and p21 was measured in Skp2 cDNA transfected PC cells treated with curcumin. (C) Quantitative results are illustrated for (B). *P < 0.05, **P < 0.01, compared with control; #P < 0.05 compared with curcumin treatment or Skp2 cDNA transfection.

over-expression of Skp2 could reverse curcumin-induced apoptosis. Indeed, we found that over-expression of Skp2 significantly reduced percentage of apoptotic cells in Patu8988 cells (**Figure 3B**). Moreover, Skp2 cDNA transfection decreased curcumin-induced apoptosis in both PC cells (**Figure 3B**). These results suggest that curcumin-induced apoptosis could be partly due to down-regulation of Skp2 in PC cells.

Over-expression of Skp2 enhanced cell motility in PC cells

In order to verify the contribution of Skp2 to cell motility and invasiveness in PC cell lines, we performed wound healing assay and Transwell assay to examine the migration and invasion potential of Skp2 cDNA tranfected cells. We found that Skp2 cDNA tranfection triggered the migration and invasion abilities in Patu8988 cells (**Figures 3C**, **4A**). Notably, over-expression of Skp2 abrogated the inhibitory effects of curcumin on cell migration and invasion in PC cells. We further identified that over-expression of Skp2 counteracted activation of p21 induced by curcumin to a certain degree (**Figure 4B**, **4C**).

Depletion of Skp2 promoted curcumin-induced anti-tumor activities

To further verify the role of Skp2 in PC cells, we depleted Skp2 expression by transient transfection of Skp2 siRNA oligonucleotides. We found that depletion of Skp2 markedly inhibited cell growth (**Figure 5A**). Depletion of Skp2 in combined with curcumin treatment promoted cell growth inhibition to a greater degree compared with curcumin alone or siRNA transfection alone (**Figure 5A**). Next, we found that Skp2 siRNAs significantly promoted apoptosis of both PC cells (**Figure 5B**). Depletion of Skp2 enhanced curcumin-triggered apoptosis in PC cells. We further identified that down-regulation of Skp2 inhibited migration and invasion in both PC cells (**Figures 5C**, **6A**). Notably, depletion of Skp2 combined with curcumin suppressed cell migration and invasion to maximum effects compared with siRNA treatment alone or curcumin alone (**Figures 5C**, **6A**). We also observed that Skp2 siRNA transfection led to elevated p21 level (**Figure 6B**, **6C**). Taken together, these results suggested that curcumin exerts its anticancer activity through down-regulation of Skp2 signaling pathway.

Discussion

A growing body of evidence has demonstrated the anti-cancer effects of curcumin against PC cells [32]. Moreover, curcumin exhibits its anti-cancer effects through modulating the activity of various molecules that play important roles in cancer progression [38]. For instance, down-regulation of NF-κB by curcumin was associated with the suppression of proliferation and the induction of apoptosis in human PC cells [32]. One study has demonstrated that curcumin induced apoptosis in PC cells through the induction of forkhead box O1 and inhibition of the PI3K/Akt pathway [33]. Moreover, curcumin induced PC cells death via reduction of the inhibitors of apoptosis [34]. Consistent with these findings, we found that curcumin inhibited cell growth and induced apoptosis in Patu8988 and Panc-1 cells. Notably, in our present study, we also observed that curcumin suppressed cell migration and invasion. These findings suggest that curcumin could be an effective agent for the treatment of PC patients.

Recent studies have revealed the essential oncogenic function of Skp2 in pancreatic tumorigenesis [21]. Previous study has shown that down-regulation of Skp2 by ATO was associated with the cell growth inhibition and apoptosis in PC cells [31]. Therefore, inactivation of Skp2 could bring us considerable therapeutic benefits in treating PC patients. In fact, several specific small molecular inhibitors of Skp2 have already been developed using in silico screens [35]. These small molecules promoted p27 accumulation in a Skp2-dependent manner and induced cell-type specific blocks in the G1 or G2/M phases [35]. Specifically, compound CpdA inhibited Skp2 E3 ligase activity, and subsequently induced p27 accumulation via preventing it from recruitment to Skp2 ligase complex [36]. Additionally, SMIP0004 has been found through a high-throughput screening, and was report to restore p27 due to its ability to reduce Skp2 abundance, leading to anti-proliferative activity in prostate cancer cells [37]. Compound 25, also known as SZL-P1-41, has been developed to selectively suppress Skp2 E3 ligase activity and to restrict cancer progression both *in vitro* and *in vivo* [23]. However, these inhibitors could have unexpected effects in clinical trials. Actually, inactivating Skp2 by nature agents, such as curcumin, quercetin, lycopene, silibinin, epigallocatechin-3-gallate, and Vitamin D3, could be a safer approach for

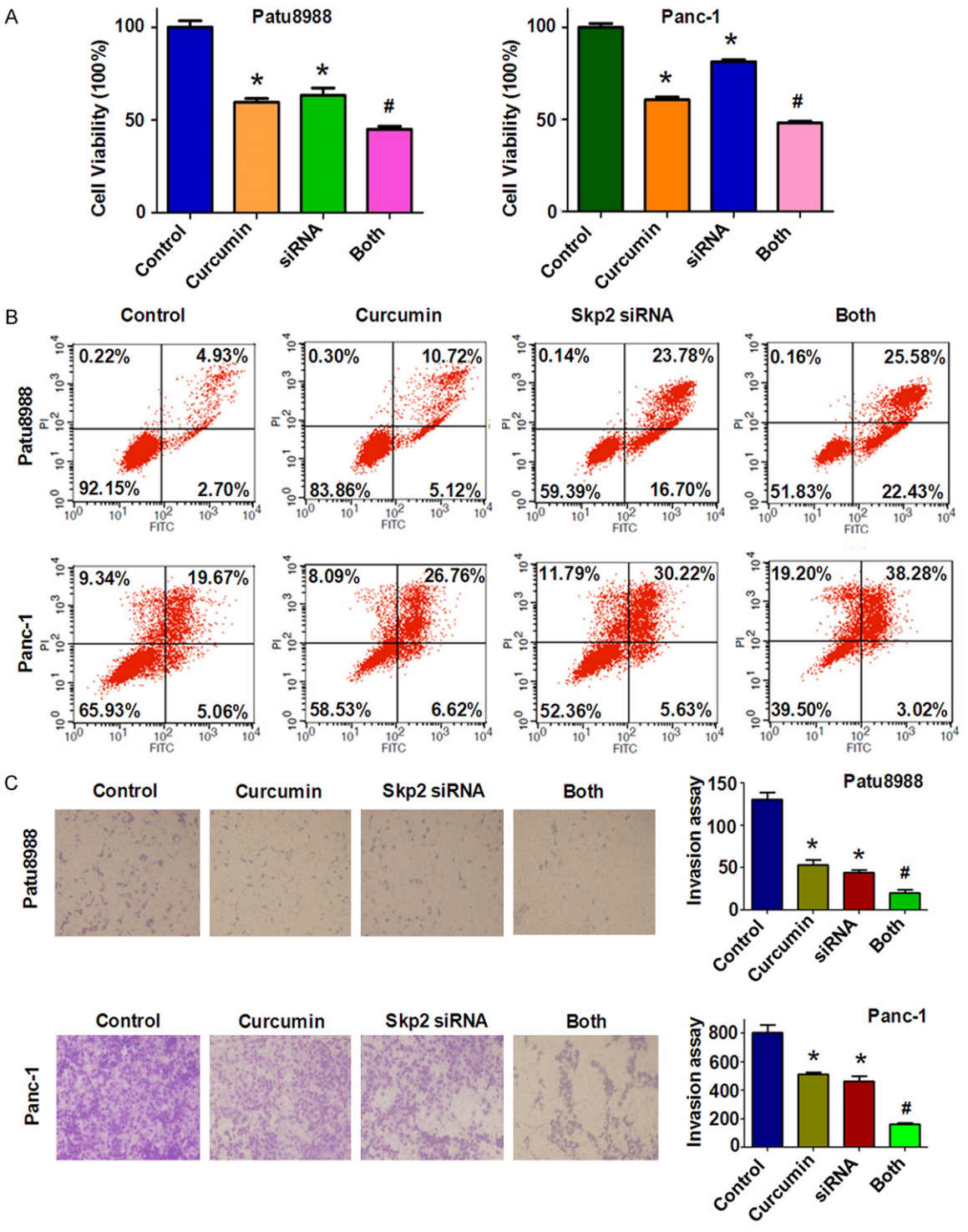
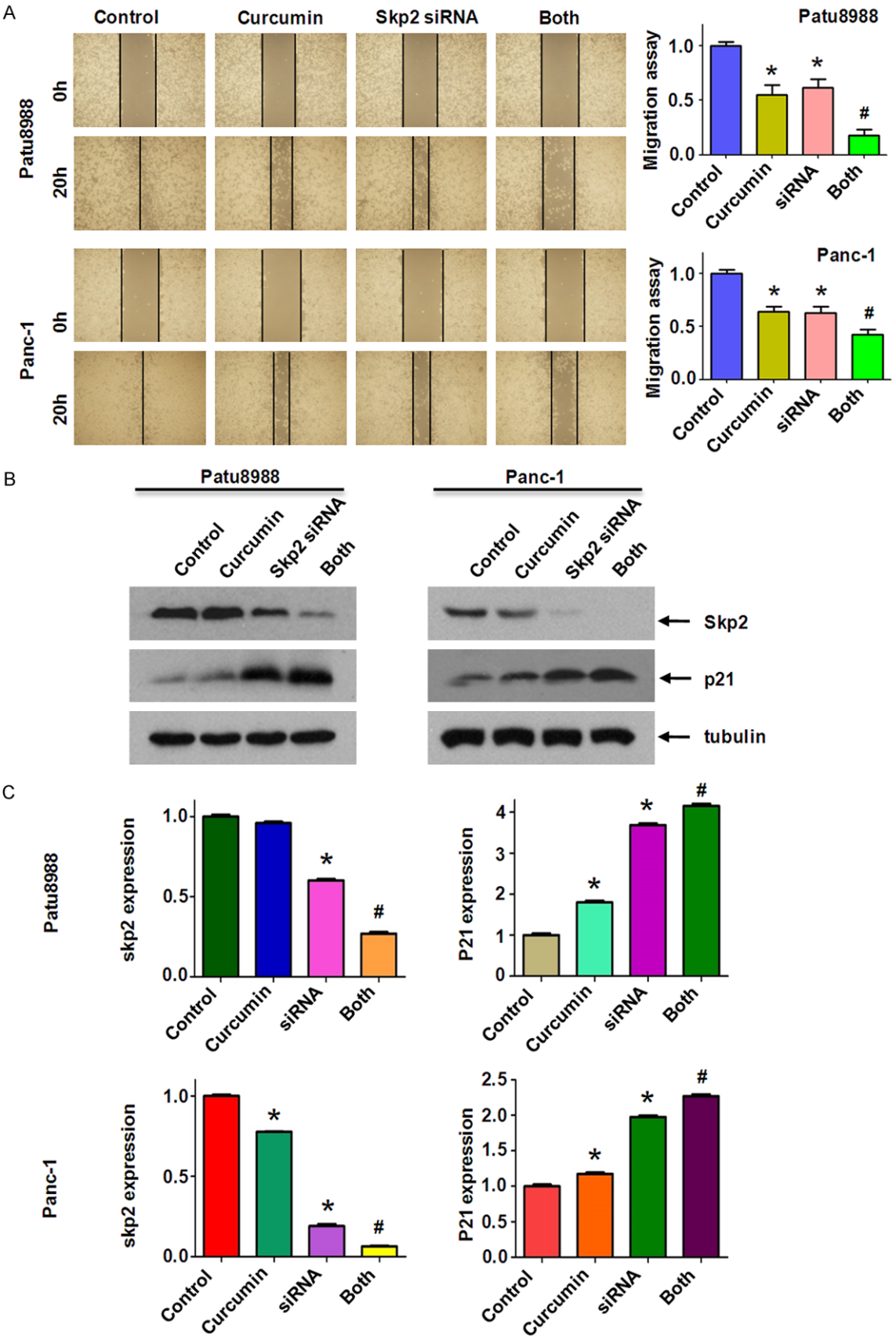


Figure 5. Knockdown of Skp2 inhibited cell proliferation and invasion and facilitated cell apoptosis. A: The effect of down-regulated Skp2 in combination with curcumin treatment on PC cell growth was detected by CTG assay. Control: control siRNA; siRNA: Skp2 siRNA; Both: Skp2 siRNA+Curcumin. *P < 0.05, **P < 0.01, compared with control; #P < 0.05 compared with curcumin treatment or Skp2 siRNA transfection. B: Cell apoptosis was accessed by Flow cytometry in PC cells treated with Skp2 siRNA and curcumin. C. Left panel, PC cells invasion was detected after Skp2 siRNA transfection and curcumin treatment. Right panel, Quantitative results are illustrated for left panel. *P < 0.05, **P < 0.01, compared with control; #P < 0.05 compared with curcumin treatment or Skp2 siRNA transfection.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Curcumin
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Figure 6. Knockdown of Skp2 inhibited PC cells migration. (A) Left panel, the PC cells migration after Skp2 siRNA transfection and curcumin treatment was detected by wound healing assay. Control: control siRNA; siRNA: Skp2 siRNA; Both: Skp2 siRNA+Curcumin. Right panel, Quantitative results are illustrated for left panel. (B) The expression of Skp2 and p21 was measured in Skp2 siRNA transfected PC cells treated with curcumin. (C) Quantitative results are illustrated for (B). *P < 0.05, **P < 0.01, compared with control; #P < 0.05 compared with curcumin treatment or Skp2 siRNA transfection.

treating PC patients [38-41]. Nevertheless, further *in vitro* cell culture as well as *in vivo* mouse modeling studies should be further pursued to validate the anti-tumor effects of these above mentioned Skp2 inhibitors.

It was reported that curcumin promoted the expression of P53 through a PPARγ activation-dependent mechanism and induced hepatic stellate cell senescence by elevating the expression of senescence markers p16, p21 and Hmga1 [42]. Zhao et al. have confirmed that curcumin significantly decreased PC cell proliferation, which was associated with increased expression of the p21/CIP1, p27/KIP1 and FOXO1 by inhibition of the PI3K/Akt pathway [33]. In the present study, we validated that curcumin exerts its anticancer activity through down-regulation of Skp2 signaling pathway. Although these findings demonstrate a promising anticancer potential of curcumin, it is worth noting that the therapeutic use of curcumin is limited. Rapid metabolism and poor absorption of curcumin should account for this case. Therefore, it is necessary to aggrandize the bioavailable efficiency and/or improve delivery methods of curcumin to overcome the blood-brain barrier. In the present study, we revealed the anticancer activity of curcumin through suppression of Skp2 and subsequently induction of p21 in PC cells. Undoubtedly, it is necessary to determine whether curcumin exhibits its anticancer effects via inhibiting Skp2 expression in PC mouse models *in vivo*. In conclusion, our findings demonstrated that curcumin-mediated cell growth inhibition, apoptosis, cell cycle arrest, invasion and migration suppression in PC cells could be partly due to the down-regulation of Skp2. These results suggest that inhibiting Skp2 by curcumin could bring benefits for the treatment of PC patients.

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Disclosure of conflict of interest

None.

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