Oncology Research, Vol. 25, pp. 389–395
Printed in the USA.

0965-0407/17 $90.00 + .00
DOI: https://doi.org/10.3727/096504016X14743324568129
E-ISSN 1555-3906
www.cognizantcommunication.com

Knockdown of Serine–Arginine Protein Kinase 1 Inhibits the Growth and Migration in Renal Cell Carcinoma Cells

Xingtao Han,*† Jinjian Yang,* Zhankui Jia,* Pengtao Wei,† Han Zhang,† Wenwei Lv,† Jiantao Sun,† and Qingxiang Huo†

*Department of Urology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, P.R. China
†Department of Urology, Luoyang Central Hospital affiliated to Zhengzhou University, Luoyang, P.R. China

The pre-mRNA splicing regulator serine–arginine protein kinase 1 (SRPK1), a member of the SR kinase family, plays an essential role in cancer development and various pathophysiological processes. However, its expression pattern and functions in renal cell carcinoma (RCC) remain unknown. Therefore, the aim of this study was to assess the role of SRPK1 in RCC. Our data showed that SRPK1 was significantly upregulated in human RCC tissues and cell lines. SRPK1 interference significantly inhibited the proliferation of RCC cells and inhibited tumor growth in vivo. In addition, SRPK1 interference also suppressed migration and invasion in RCC cells. A mechanistic study showed that SRPK1 interference inhibited the phosphorylation of PI3K and Akt in RCC cells. In conclusion, our findings suggest that SRPK1 interference inhibits the growth and invasion of RCC cells through suppressing the PI3K/Akt signaling pathway. Thus, SRPK1 might be a therapeutic target for the treatment of RCC.

Key words: Serine–arginine protein kinase 1 (SRPK1); Renal cell carcinoma (RCC); Invasion; PI3K/Akt pathway

INTRODUCTION

Renal cell carcinoma (RCC) is the most common type of malignant tumor of the kidney in adults worldwide[1]. In recent years, the morbidity and mortality of RCC have increased rapidly. Although various treatments for RCC, such as chemotherapy and radiation, have been improved recently, the clinical outcome of patients remains unsatisfactory[2–4]. The prognosis of RCC is highly associated with the progression of localized primary tumors to advanced stages, which ultimately metastasize to multiple organs[5]. Therefore, the identification of novel diagnostic and prognostic biomarkers and therapeutic targets of RCC is of crucial importance for the treatment of RCC.

Serine–arginine protein kinase 1 (SRPK1) is a protein kinase that specifically phosphorylates proteins containing serine–arginine-rich domains, which play an important role in normal cell development and multiple human diseases[6–9]. SRPK1 was shown to be upregulated in several types of cancer. It was found that knockdown of SRPK1 greatly upregulated the levels of antiangiogenic splicing isoform VEGF165b in colon adenocarcinoma cells and inhibited tumor growth in a xenograft model[10]. Another study reported that targeting SRPK1 using small interfering RNA (siRNA) significantly reduced the proliferation of pancreatic tumor cells and altered expression of key apoptotic proteins, and enhanced apoptosis and responsiveness to cytotoxic agents[6]. However, its expression pattern and functions in RCC remain unknown. Therefore, the aim of this study was to assess the role of SRPK1 in RCC. Our data showed that SRPK1 is highly expressed in human RCC cell lines, and knockdown of SRPK1 inhibits the growth and migration in RCC cells.

MATERIALS AND METHODS

Tissue Collection

Human RCC tissues were obtained from patients with RCC who underwent tumor resection between 2013 and 2015 at the Department of Urology, The First Affiliated Hospital of Zhengzhou University (P.R. China). The samples were immediately stored in liquid nitrogen in preparation for use. The study was approved by the medical ethics committee of The First Affiliated Hospital of Zhengzhou University. Informed consent, according to the Declaration of Helsinki, was obtained from each patient.

Cell Culture

Human RCC cell lines (786-O, Caki-1, and UMRC-3) and immortalized proximal tubule epithelial cell line

Address correspondence to Jinjian Yang, Department of Urology, The First Affiliated Hospital of Zhengzhou University, No. 1 Jianshe Road, Erqi District, Zhengzhou 450052, P.R. China. Tel: +86-0371-67967221; Fax: +86-0371-67967221; E-mail: yangjin_jian@126.com

(HK-2) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). They were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 1% (v/v) penicillin–streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in a humidified atmosphere containing 5% CO_2.

RNA Interference and Cell Transfection

Small hairpin RNA (shRNA) for SRPK1 and nontarget shRNA were purchased from GenePharma (Shanghai, P.R. China). RCC cells were seeded at a density of 1×10^5 cells/well. After 24 h, SRPK1-shRNA or nontarget shRNA was transfected into the cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The transfection efficiency of SRPK1 was confirmed by quantitative real-time polymerase chain reaction (qRT-PCR) and Western blotting.

RNA Extraction and Quantitative Real-Time (qRT)-PCR

Total RNA was extracted from human RCC tissues and cells using TRIzol reagent (Invitrogen) and reversely transcribed into cDNA using SuperScript II Reverse Transcriptase (Invitrogen). The sequences of primers are listed as follows: SRPK1, 5′-CACGGCATGCATGGC CTTTGA-3′ (forward) and 5′-CGGCGGCAGTGGCTC TCTTC-3′ (reverse); β-actin, 5′-CCGTGAAAAGATGA CCCAGATC-3′ (forward) and 5′-CACAGCCTGGAT GGCTACGT-3′ (reverse). The steps of RT-PCR were performed as follows: 94°C for 5 min for initial denaturation; 94°C for 30 s, 58°C for 30 s, and 72°C for 45 s; 2 s for plate reading for 40 cycles; and melt curve from 65°C to 95°C. The relative SRPK1 mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ comparative method.

Western Blot Analysis

Total protein was isolated from human RCC tissues and cells using RIPA lysis buffer and then quantified by the BCA assay kit (Bio-Rad, Hercules, CA, USA). Total protein (50 μg) from each sample was separated by 10% SDS polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (GE Healthcare, Milano, Italy) by electroblotting. The membranes were first blocked and then incubated with primary antibodies overnight at 4°C. The antibodies used are as follows: anti-SRPK1, anti-p-PI3K, anti-PI3K, anti-p-Akt, anti-Akt, and anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Following three washes with TBST buffer, horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology) were introduced, and proteins were visualized using the ECL detection system (Thermo Fisher Scientific, Rockford, IL, USA).

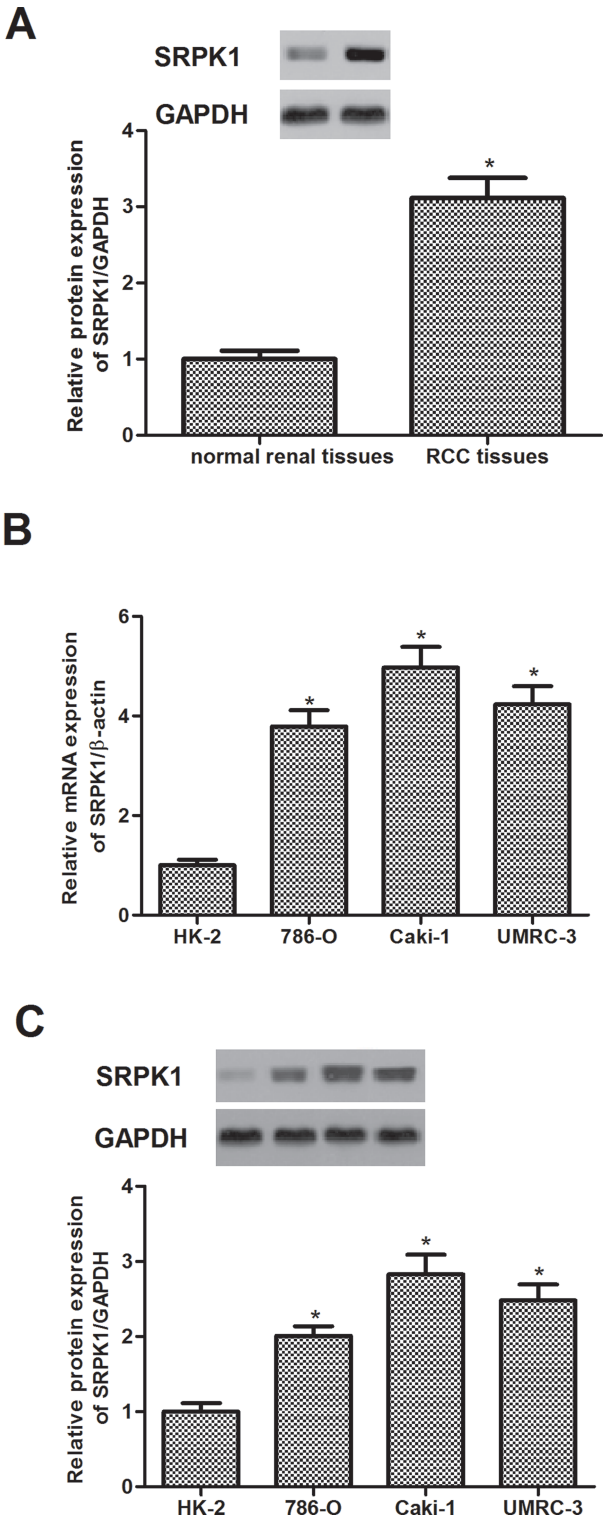

Figure 1. SRPK1 is upregulated in RCC tissues and cell lines. (A) Western blot analysis of SRPK1 expression in human RCC tissues and normal renal tissues. (B) qRT-PCR analysis of SRPK1 mRNA expression in RCC cell lines. (C) Representative Western image of SRPK1 protein in RCC cell lines. Data are expressed as mean ± SD. Experiments were performed in triplicate. *$p<0.05$ compared with the control group.

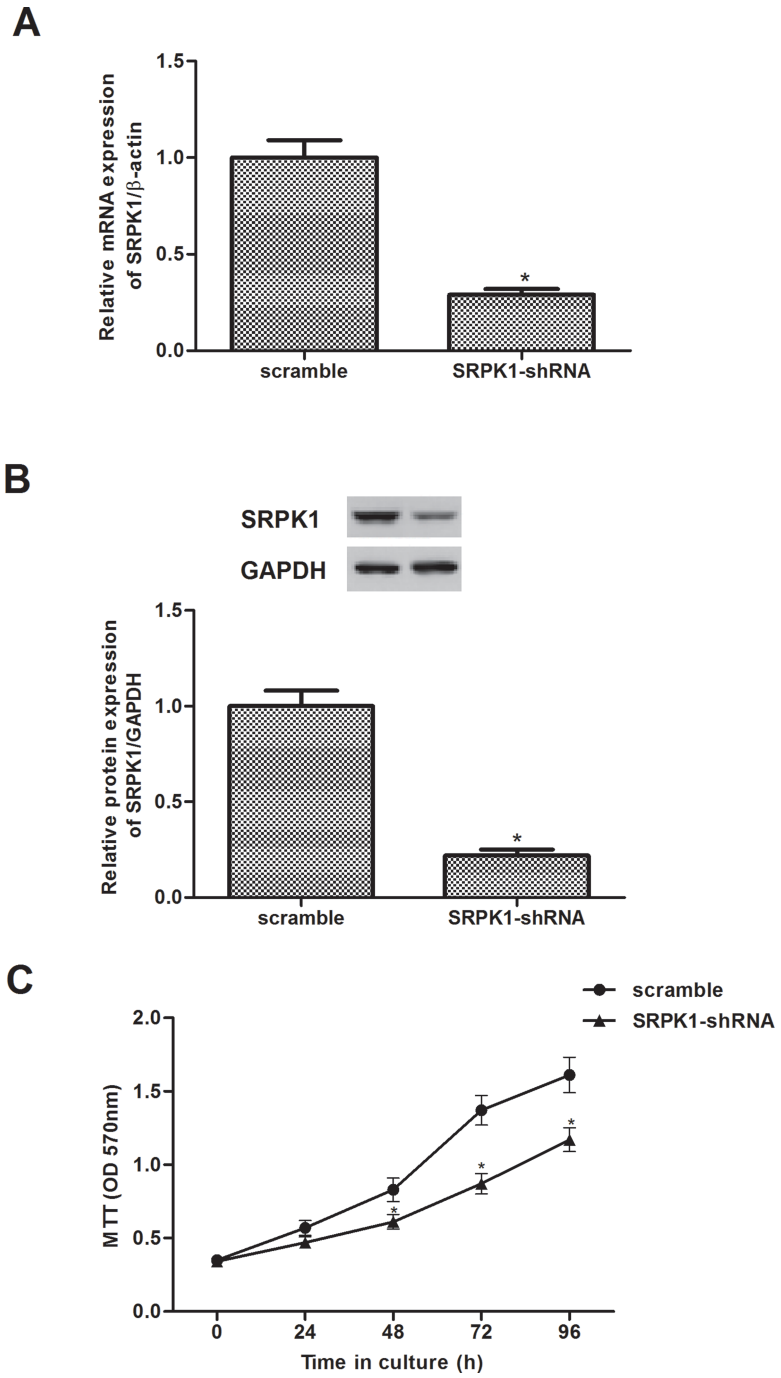

Figure 2. Knockdown of SRPK1 inhibits RCC cell proliferation. Caki-1 cells were transfected with SRPK1-shRNA or nontarget shRNA for 24 h. The expression of SRPK1 at both mRNA (A) and protein (B) levels was detected by qRT-PCR and Western blot. (C) Cell proliferation was evaluated by the MTT assay. Data are expressed as mean±SD. Experiments were performed in triplicate. *$p<0.05$ versus scramble group.

Cell Proliferation Assay

Cell proliferation was evaluated by 3-(4,5-methylthiozol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, Caki-1 cells (1×10^5 cells/well) treated with SRPK1-shRNA or nontarget shRNA were seeded onto 96-well plates and then cultured at 24-h intervals for 4 days. Then the initial culture medium was replaced with fresh medium containing MTT (5 mg/ml; Sigma-Aldrich) and incubated for an additional 4 h. The culture medium was removed, and 150 µl dimethyl sulfoxide

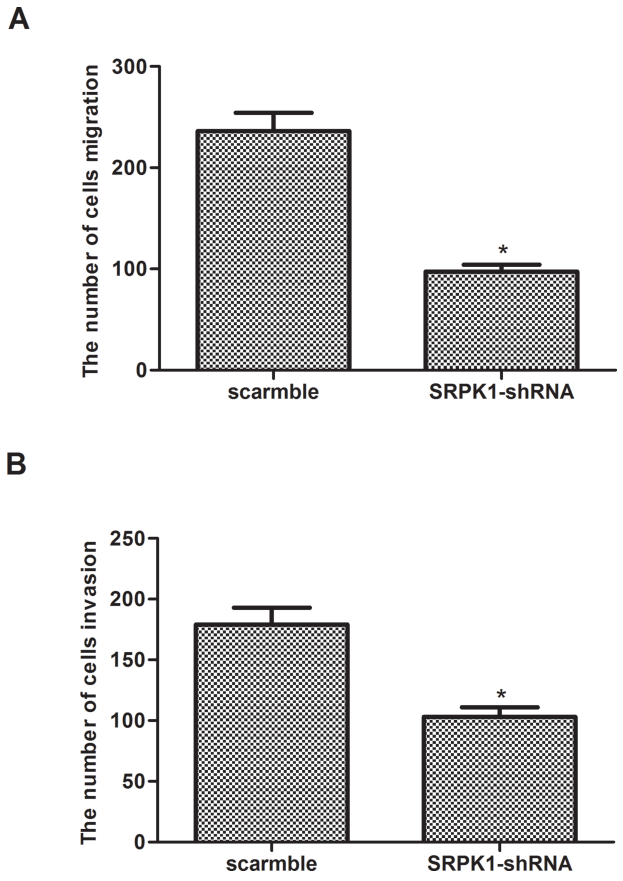

Figure 3. Knockdown of SRPK1 inhibits RCC cell migration and invasion. Caki-1 cells were transfected with SRPK1-shRNA or nontarget shRNA for 24 h. (A) The migration of Caki-1 cells was evaluated by a Transwell assay; cells that have passed through Matrigel for 24 h were counted in five representative microscopic fields. (B) The invasiveness of Caki-1 cells was evaluated by a Matrigel-coated Transwell assay, and cells that have passed through Matrigel for 24 h were counted in five representative microscopic fields. Data are expressed as mean ± SD. Experiments were performed in triplicate. *$p < 0.05$ versus scramble group.

(DMSO; Sigma-Aldrich) was added to each well. The absorbance at 570 nm was measured using a microplate reader (Takara Biotechnology, Dalian, P.R. China).

Migration and Invasion Assays

Cell migration was evaluated using Transwell migration chambers (8-μm pore size; BD Biosciences, Eugene, OR, USA). Briefly, Caki-1 cells (1×10^5 cells/well) transfected with SRPK1-shRNA or nontarget shRNA were added to the upper chamber, and the lower chambers were filled with DMEM containing 10% FBS. After incubation for 24 h at 37°C, nonmigrating cells on the upper side of the filter were wiped off. Membranes were fixed with 4% formaldehyde in PBS and were stained with 1% crystal violet in 20% methanol for 15 min. The number of cells that migrated to the bottom of the filter was counted in five random fields per well under a light microscope (magnification: 100×).

Cell invasion was conducted using Transwell chambers in the presence of Matrigel (BD Biosciences). Then the assay was performed as the migratory assay.

Tumorigenicity in Nude Mice

Male BALB/c nude mice were obtained (Shanghai Slac Laboratory Animal Co. Ltd., P.R. China) and bred under specific pathogen-free conditions. Caki-1 cells (1×10^6) transfected with SRPK1-shRNA or nontarget shRNA were injected subcutaneously in male nude mice. Tumors were measured with a caliper every 7 days, and tumor volume was calculated according to the formula: [(length + width)/2] × length × width. After 5 weeks, the mice were killed, and the tumor volume and weight were measured. All of the animal experiments were performed with the approval of the Animal Care and Use Committee of The First Affiliated Hospital of Zhengzhou University.

Statistical Analysis

Statistical analyses were done using SPSS 13.0 for windows (SPSS, Chicago, IL, USA). The statistical significance of the difference was analyzed by ANOVA and post hoc Dunnett's test. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

SRPK1 Is Upregulated in RCC Tissues and Cell Lines

To examine the protein level of SRPK1 in human RCC tissues, Western blotting was performed. The results demonstrated that the protein expression level of SRPK1 was significantly higher in human RCC tissues than that in normal renal tissues (Fig. 1A). Moreover, SRPK1 expression was assessed in several human RCC cell lines by qRT-PCR and Western blotting. The expression levels of SRPK1 at both mRNA and protein were higher in human RCC cell lines when compared to HK-2 (Fig. 1B and C).

Knockdown of SRPK1 Inhibits RCC Cell Proliferation

To investigate the role of SRPK1 in RCC, we generated a stable knockdown of SRPK1. Caki-1 cells were transduced with lentivirus containing shRNAi to SRPK1 or scrambled shRNAi, and the extent of knockdown was confirmed by both qRT-PCR and Western blotting (Fig. 2A and B). Next we performed an MTT assay to examine the effects of shRNA-SRPK1 on proliferation. We found that knockdown of SRPK1 dramatically suppressed the proliferation of Caki-1 cells, compared with the shRNA scramble group (Fig. 2C).

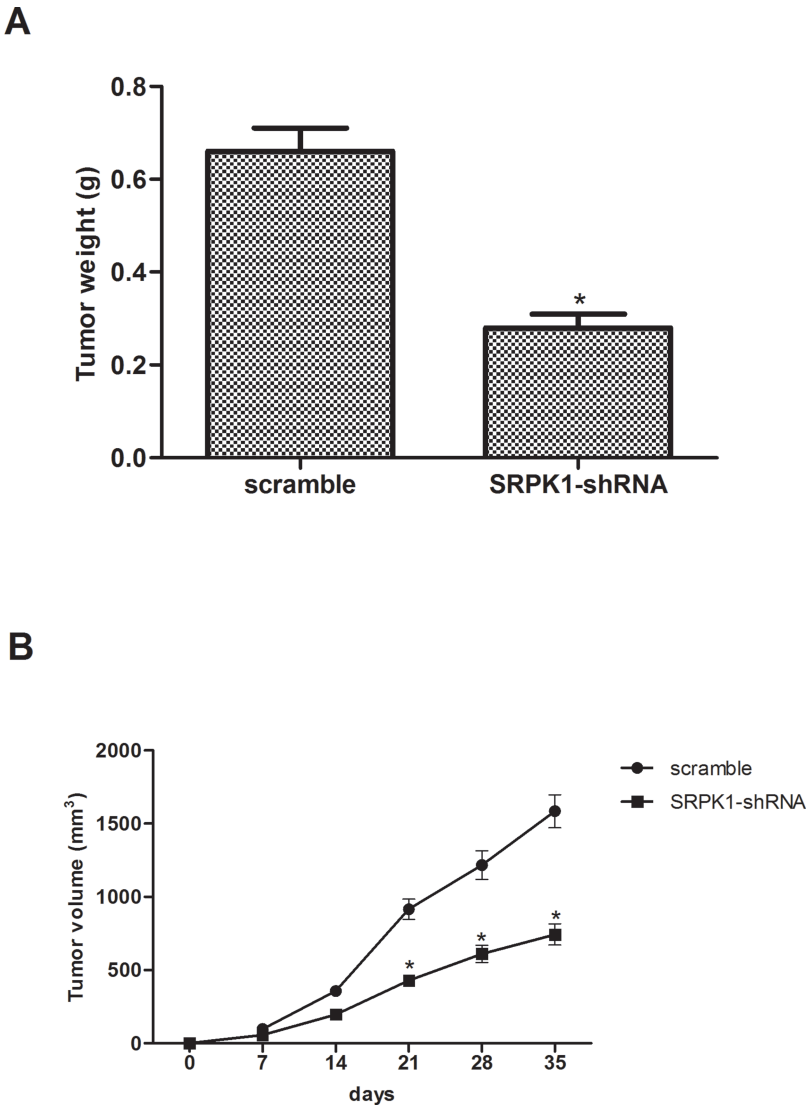

Figure 4. Knockdown of SRPK1 tumorigenesis in vivo. SRPK1-shRNA-treated Caki-1 cells were subcutaneously implanted into nude mice. (A) The tumor weights were calculated at day 35. (B) The tumor volumes were calculated in each group every 7 days from day 0 to day 35. Data are expressed as mean ± SD. Experiments were performed in triplicate. *$p < 0.05$ versus scramble group.

Knockdown of SRPK1 Inhibits RCC Cell Migration and Invasion

Next we examined the effect of SRPK1 on the migration and invasion of RCC cells. SRPK1 interference significantly inhibited migration of Caki-1 cells as assessed by Transwell migration assay when compared with the scramble group (Fig. 3A). Similarly, SRPK1 interference also significantly suppressed Caki-1 cells from invading through Matrigel-coated polycarbonate filter in the Transwell chamber (Fig. 3B).

Knockdown of SRPK1 Tumorigenesis In Vivo

To explore the effects of SRPK1 on tumorigenesis in vivo, SRPK1-shRNA-treated Caki-1 cells were subcutaneously implanted into nude mice. The results indicated that the average weight and tumor size of SRPK1 knockdown tumors were dramatically lower than those of the control group: tumor weight at day 35 was 0.28 ± 0.02 g versus 0.66 ± 0.05 g, respectively (Fig. 4A), while tumor size was 743 ± 61 mm^3 versus 1,585 ± 114 mm^3, respectively (Fig. 4B).

Knockdown of SRPK1 Inhibits the Activation of PI3K/Akt Signaling Pathway in RCC Cells

To elucidate the mechanism by which SRPK1 regulates the proliferation and invasion of RCC cells, we investigated the effect of SRPK1 on the expression of the phosphorylation levels of PI3K and Akt in Caki-1 cells.

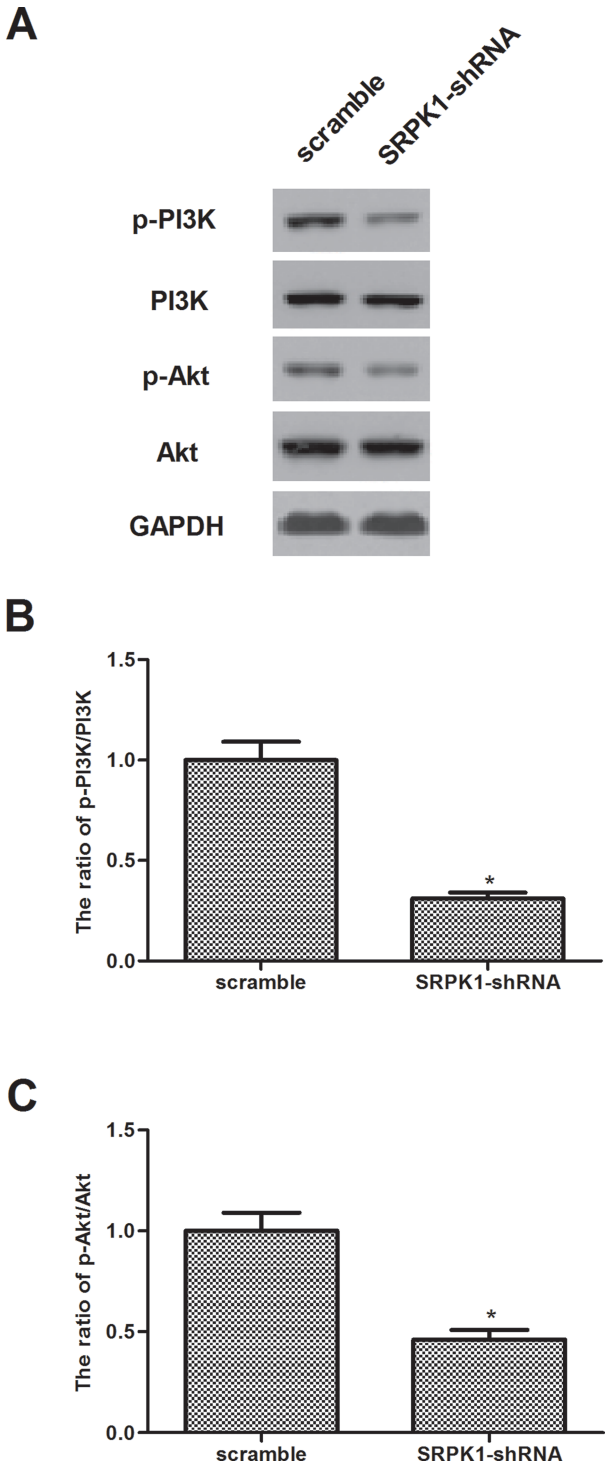

Figure 5. Knockdown of SRPK1 inhibits the activation of the PI3K/Akt signaling pathway in RCC cells. (A) Caki-1 cells were transfected with SRPK1-shRNA or nontarget shRNA, and the levels of phosphorylated PI3K (Tyr607), total PI3K, phosphorylated Akt (Ser473), and total Akt were detected by Western blot analysis. (B, C) Quantification analysis was performed using the Image-Pro Plus 6.0 software and normalized to GAPDH. Data are expressed as mean ± SD. Experiments were performed in triplicate. *$p < 0.05$ versus scramble group.

Data from the Western blotting analysis showed that SRPK1 interference greatly decreased the phosphorylation of PI3K and Akt in Caki-1 cells, compared with the scramble group (Fig. 5).

DISCUSSION

In this study, we found that SRPK1 was significantly upregulated in human RCC tissues and cell lines. SRPK1 interference significantly inhibited the proliferation of RCC cells and inhibited tumor growth in vivo. In addition, SRPK1 interference suppressed migration and invasion in RCC cells. A mechanistic study showed that SRPK1 interference inhibited the phosphorylation of PI3K and Akt in RCC cells.

Previous studies have shown that SRPK1 is highly expressed in many tumor tissues[11–13]. A study from Liu et al. indicated that both the mRNA and the protein levels of SRPK1 were upregulated in non-small cell lung cancer (NSCLC) tissues[13]. In addition, SRPK1 expression was significantly higher in prostate cancer tissues compared with benign tissues[14]. Consistent with these results, in the current study we found that SRPK1 was significantly upregulated in human RCC cell lines. These results suggest that SRPK1 may behave as an oncogene to block the growth of RCC.

Very recently, several studies demonstrated that SRPK1 is involved in the proliferation of cancer cells and tumor growth in vivo[15,16]. Our data showed that SRPK1 interference significantly inhibited the proliferation of RCC cells and inhibited tumor growth in vivo. These results suggest that SRPK1 may play an important role in the development and progression of RCC.

Metastasis is the main cause of cancer-related mortality, and it involves a series of steps, including migration and invasion[17–19]. A recent study showed that transfection of SRPK1 siRNA strongly reduced cell invasion and migration by regulating the expression of MMP2 and MMP9 in glioma cells[20]. Similarly, in the present study we observed that SRPK1 interference suppressed migration and invasion in RCC cells, suggesting that SRPK1 plays an important role in promoting RCC metastasis.

Accumulating evidence has indicated that the PI3K/Akt signaling pathway plays an important role in the development and progression of tumors. Akt, a serine/threonine kinase, is a critical mediator of the PI3K pathway, and activation of Akt phosphorylates a whole network of proteins that regulate cell proliferation, migration, and invasion. The PI3K/Akt pathway is modestly mutated but highly activated in RCC, representing a promising drug target[21–24]. The activation of the PI3K protein was found to be significantly associated with an adverse clinical outcome in RCC[25]. Sun et al. reported that activation of protease-activated receptor 2 (PAR-2)

induced the activation of PI3K and Akt, and the PI3K/Akt inhibitor LY294002 attenuated the invasion and migration of RCC cells stimulated by PAR-2 activation[26]. Most recently, one study showed that knockdown of SRPK1 inhibited glioblastoma cell invasion and the epithelial–mesenchymal transition (EMT) process via suppressing the p-Akt signaling pathway[27]. In this study, we found that SRPK1 interference significantly inhibits the phosphorylation of PI3K and Akt in RCC cells. These data suggest that SRPK1 interference inhibits the growth and invasion of RCC cells through suppressing the PI3K/Akt signaling pathway.

In conclusion, our findings suggest that SRPK1 interference inhibits the growth and invasion of RCC cells through suppressing the PI3K/Akt signaling pathway. Thus, SRPK1 might be a therapeutic target for the treatment of RCC.

ACKNOWLEDGMENT: The authors declare no conflicts of interest.

REFERENCES

1. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. Lancet 2009;373:1119–32.
2. Ko Y, Atkins M. Systemic therapy for renal cell carcinoma. Cancer Chemother Biol Response Modif. 2004; 22:263–72.
3. Chang XLT, Lin Y, Yu J, Liang P. Radiofrequency ablation for renal cell carcinoma. Asian Pac J Surg Oncol. 2016;2:351–60.
4. Kalanovic DJE, Pruthi R. Targeted therapies for treatment of renal cell carcinoma. Asian Pac J Surg Oncol. 2016;2:361–72.
5. Parikh SHS, Thompson RH, Zhang Y. Overall survival and renal function following surgical resection of renal cell carcinoma. Asian Pac J Surg Oncol. 2016;92:373–82.
6. Hayes GM, Carrigan PE, Miller LJ. Serine-arginine protein kinase 1 overexpression is associated with tumorigenic imbalance in mitogen-activated protein kinase pathways in breast, colonic, and pancreatic carcinomas. Cancer Res. 2007;67:2072–80.
7. Ghosh G, Adams JA. Phosphorylation mechanism and structure of serine-arginine protein kinases. FEBS J. 2011;278:587–97.
8. Giannakouros T, Nikolakaki E, Mylonis I, Georgatsou E. Serine-arginine protein kinases: A small protein kinase family with a large cellular presence. FEBS J. 2011;278: 570–86.
9. Mavrou A, Brakspear K, Hamdollah-Zadeh M, Damodaran G, Babaei-Jadidi R, Oxley J, Gillatt D, Ladomery M, Harper SJ, Bates DO. Serine–arginine protein kinase 1 (SRPK1) inhibition as a potential novel targeted therapeutic strategy in prostate cancer. Oncogene 2015;34:4311–9.
10. Gonçalves V, Henriques A, Pereira J, Costa AN, Moyer MP, Moita LF, Gama-Carvalho M, Matos P, Jordan P. Phosphorylation of SRSF1 by SRPK1 regulates alternative splicing of tumor-related Rac1b in colorectal cells. RNA 2014;20:474–82.
11. Lin J-C, Lin C-Y, Tarn W-Y, Li F-Y. Elevated SRPK1 lessens apoptosis in breast cancer cells through RBM4-regulated splicing events. RNA 2014;20:1621–31.
12. Wang F, Zhou J, Xie X, Hu J, Chen L, Hu Q, Guo H, Yu C. Involvement of SRPK1 in cisplatin resistance related to long non-coding RNA UCA1 in human ovarian cancer cells. Neoplasma 2015;62:432–8.
13. Liu H, Hu X, Zhu Y, Jiang G, Chen S. Up-regulation of SRPK1 in non-small cell lung cancer promotes the growth and migration of cancer cells. Tumor Biol. 2015;37:7287–93.
14. Bullock N, Potts J, Simpkin AJ, Koupparis A, Harper SJ, Oxley J, Oltean S. Serine-arginine protein kinase 1 (SRPK1), a determinant of angiogenesis, is upregulated in prostate cancer and correlates with disease stage and invasion. J Clin Pathol. 2016;69:171–5.
15. Wang P, Zhou Z, Hu A, de Albuquerque CP, Zhou Y, Hong L, Sierecki E, Ajiro M. Kruhlak M, Harris C. Both decreased and increased SRPK1 levels promote cancer by interfering with PHLPP-mediated dephosphorylation of Akt. Mol Cell 2014;54:378–91.
16. Zhou B, Li Y, Deng Q, Wang H, Wang Y, Cai B, Han Z-G. SRPK1 contributes to malignancy of hepatocellular carcinoma through a possible mechanism involving PI3K/Akt. Mol Cell Biochem. 2013;379:191–9.
17. Fidler I, Kripke M. Tumor cell antigenicity, host immunity, and cancer metastasis. Cancer Immunol Immunother. 1980;7:201–5.
18. Chambers AF, Groom AC, MacDonald IC. Metastasis: Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2002;2:563–72.
19. Brodland DG, Zitelli JA. Mechanisms of metastasis. J Am Acad Dermatol. 1992;27:1–8.
20. Chang Y, Wu Q, Tian T, Li L, Guo X, Feng Z, Zhou J, Zhang L, Zhou S, Feng G. The influence of SRPK1 on glioma apoptosis, metastasis, and angiogenesis through the PI3K/Akt signaling pathway under normoxia. Tumor Biol. 2015;36:6083–93.
21. Porta C, Figlin RA. Phosphatidylinositol-3-kinase/Akt signaling pathway and kidney cancer, and the therapeutic potential of phosphatidylinositol-3-kinase/Akt inhibitors. J Urol. 2009;182:2569–77.
22. Agouni A, Sourbier C, Danilin S, Rothhut S, Lindner V, Jacqmin D, Helwig J-J, Lang H, Massfelder T. Parathyroid hormone-related protein induces cell survival in human renal cell carcinoma through the PI3K Akt pathway: Evidence for a critical role for integrin-linked kinase and nuclear factor kappa B. Carcinogenesis 2007;28: 1893–901.
23. Banumathy G, Cairns P. Signaling pathways in renal cell carcinoma. Cancer Biol Ther. 2010;10:658–64.
24. Park J-Y, Lin P-Y, Weiss RH. Targeting the PI3K Akt pathway in kidney cancer. Expert Rev Anticancer Ther. 2007;7:863–70.
25. Merseburger AS, Hennenlotter J, Kuehs U, Simon P, Kruck S, Koch E, Stenzl A, Kuczyk MA. Activation of PI3K is associated with reduced survival in renal cell carcinoma. Urol Int. 2008;80:372–7.
26. Sun Z, Cao B, Wu J. Protease-activated receptor 2 enhances renal cell carcinoma cell invasion and migration via PI3K/Akt signaling pathway. Exp Mol Pathol. 2015; 98:382–9.
27. Liao Z, Wu J, Wu M, Yan Y, Wang H, Cheng C, Tang W. The critical role of SRPK1 in EMT of human glioblastoma in the spinal cord. Mol Neurobiol. 2016. [Epub ahead of print].