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Knockdown of TACC3 Inhibits the Proliferation and Invasion of Human Renal Cell Carcinoma Cells

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Transforming acidic coiled-coil protein 3 (TACC3) is a member of the TACC family and plays an important role in regulating cell mitosis, transcription, and tumorigenesis. However, the expression pattern and roles of TACC3 in renal cell carcinoma (RCC) remain unclear. The aim of this study was to investigate the role of TACC3 in RCC. We demonstrated overexpression of TACC3 in human RCC cell lines at both RNA and protein levels. Moreover, knockdown of TACC3 repressed RCC cell proliferation, migration, and invasion in vitro. In addition, knockdown of TACC3 inactivated PI3K/Akt signaling in RCC cells. Furthermore, knockdown of TACC3 significantly reduced tumor growth in xenograft tumor-bearing mice. Taken together, our findings showed that TACC3 was increased in human RCC cell lines, and knockdown of TACC3 inhibited the ability of cell proliferation, migration, invasion, and tumorigenesis in vivo. Therefore, TACC3 may act as a therapeutic target for the treatment of human RCC.

Key words: Transforming acidic coiled-coil protein 3 (TACC3); Renal cell carcinoma (RCC); Proliferation; Invasion; PI3K/Akt pathway

INTRODUCTION

Renal cell carcinoma (RCC) is the most lethal malignant tumor of the urologic system and accounts for approximately 3% of all adult malignancies[1]. The incidence of RCC has increased rapidly in recent years. Despite recent advances in surgery and adjuvant therapy[2–4], about 30% of patients with RCC experience local recurrence or distant metastasis after traditional partial or radical nephrectomy[5]. Thus, exploring the molecular mechanism of RCC invasion and metastasis is important for developing novel therapeutic strategies.

Transforming acidic coiled-coil protein (TACC) family members are characterized by a conserved C-terminal coiled-coil domain and comprise centrosome- and microtubule-associated proteins[6,7]. TACC3 is a member of the TACC family and plays an important role in regulating cell mitosis and transcription[8]. TACC3 has been involved in both centrosome- and chromatin-driven major microtubule assembly pathways during mitotic spindle assembly[9]. Increasing evidence suggests that TACC3 is associated with various types of human cancer[10,11]. It was reported that TACC3 expression was significantly upregulated in colorectal cancer tissues and cells; its expression was closely correlated with clinical stage and overall survival[12]. However, the expression pattern and roles of TACC3 in RCC remain unclear. The aim of this study was to investigate the role of TACC3 in RCC. In the present study, we demonstrated that TACC3 was highly expressed in human RCC cell lines. We also provided evidence that knockdown of TACC3 inhibited the proliferation and invasion of RCC cells, at least partly, through suppressing the PI3K/Akt signaling pathway.

MATERIALS AND METHODS

Cell Culture

The RCC cell lines (Caki-1, 786-O, and UMRC-3) and immortalized proximal tubule epithelial cell line (HK-2) were obtained from the American Type Culture Collection (Manassas, VA, USA). All cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) at 37°C under 5% CO_2 and 95% humidified air.

Quantitative Real-Time PCR Analysis

Total RNA was isolated from RCC cells using RNeasy Mini kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's protocol. First-strand cDNA was synthesized from 5 μg of total RNA using Superscript II reverse transcriptase (Invitrogen). Real-time (RT)-PCR

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was conducted using an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) and performed with SYBR Green PCR Master Mix (Toyobo, Osaka, Japan). The primer sequences used were as follows: for TACC3, 5′-CCTCTTCAAGCGTTTTG AGAAAC-3′ and 5′-GCCCTCCTGGGTGATCCTT-3′; for β-actin, 5′-GGTGGCTTTTAGGATGGCAAG-3′ and 5′-ACTGGAACGGTGAAGGTGACAG-3′. The PCR products were resolved on a 1.5% agarose gel and stained with ethidium bromide. Finally, the relative quantification of the gene of interest was determined using the $2^{-\Delta\Delta}$CT method[13].

Western Blot

Cells were washed twice with ice-cold PBS and lysed on ice with RIPA buffer. After detecting the protein concentration (Bicinchoninic Acid Protein Assay Kit; Pierce, Rockford, IL, USA), aliquots of 30 μg of protein from each sample were separated by 12% SDS-polyacrylamide gel and transferred onto PVDF membranes (Invitrogen). Subsequently, the membranes were blocked with 5% nonfat milk and incubated with anti-TACC3, anti-E-cadherin, anti-vimentin, anti-p-PI3K, anti-PI3K, anti-p-Akt, anti-Akt, or anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight, followed by incubation with HRP-conjugated secondary antibodies for 1 h at room temperature. Finally, the blots were visualized using an enhanced chemiluminescence detection kit (Pierce).

Short Hairpin RNA and Cell Transfection

Short hairpin RNA (shRNA) for TACC3 (shRNA-TACC3) and nontargeting shRNA (shRNA-Scr) were purchased from GenePharma (Shanghai, P.R. China). Caki-1 cells (1×10^5 cells/well) were transfected with shRNA-TACC3 or shRNA-Scr using Lipofectamine 2000 (Invitrogen). The transfection efficiency of TACC3 was confirmed by qRT-PCR and Western blot.

Proliferation Assay

Cell counting kit-8 (CCK-8) was employed to detect cell proliferation. In brief, Caki-1 cells at a density of 1×10^5 cells/well were seeded into 96-well culture plates and allowed to adhere. At different time points, 10 μl of CCK-8 solution was added into each well of the plate, and the plates were incubated for another 4 h at 37°C. The absorbance at 490 nm was read on a DIAS Microplate Reader (Dynex Technologies, Chantilly, VA, USA).

Transwell Migration and Invasion Assays

Cell migration was detected using Transwell inserts (8-μm pore size; Corning Costar, Lowell, MA, USA) assay. Caki-1 cells were trypsinized and resuspended in serum-free medium, and 1×10^5 cells/0.1 ml were added to the upper chamber, while 500 μl of DMEM was added into the lower compartment as a chemoattractant. Following incubation for 24 h, cells were fixed with formalin for 15 min and stained with crystal violet (Sigma-Aldrich, St. Louis, MO, USA). The number of cells that migrated through the membrane was determined by counting six high-power fields (×100) under a light microscope. The invasion assay was performed in six-well Transwell units with 8-μm filters coated with Matrigel (BD Biosciences, San Jose, CA, USA).

Xenograft Models

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Central Hospital of Wuhan (P.R. China). Six-week-old female BALB/c nude mice were purchased from the Laboratory Animal Center of Wuhan University (P.R. China) and were maintained under specific pathogen-free conditions. Infected 1×10^6 Caki-1 cells were injected subcutaneously into the flanks of nude mice ($n=10$/group). Tumor volume was measured using calipers and calculated as follows: $0.5\times\text{length}\times\text{width}^2$. Three weeks after injection, mice were sacrificed, and the tumors were removed and weighed.

Statistical Analysis

All statistical tests were performed with SPSS for Windows, version 13.0 (SPSS, Chicago, IL, USA). Data are presented as means±SD. Statistical differences between groups were evaluated using the Student's paired *t*-test after the normal distribution test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Upregulation of TACC3 Expression in Human RCC Cell Lines

We first assessed TACC3 gene expression in a panel of RCC cell lines by the method of qRT-PCR. The mRNA expression of TACC3 was much higher in human RCC cell lines than in the normal cell line (Fig. 1A). Similarly, Western blot analysis indicated that the protein expression of TACC3 was significantly upregulated in human RCC cell lines (Fig. 1B).

Effects of TACC3 Downregulation on RCC Cell Proliferation

To examine TACC3 loss of function, Caki-1 cells were transfected with shRNA-TACC3 or shRNA-Scr for 24 h, respectively. TACC3 mRNA and protein expression were markedly reduced compared with the shRNA-Scr-transfected cells (Fig. 2A). We then investigated the effect of TACC3 on cellular proliferation in Caki-1 cells using the MTT assay. The results demonstrated that

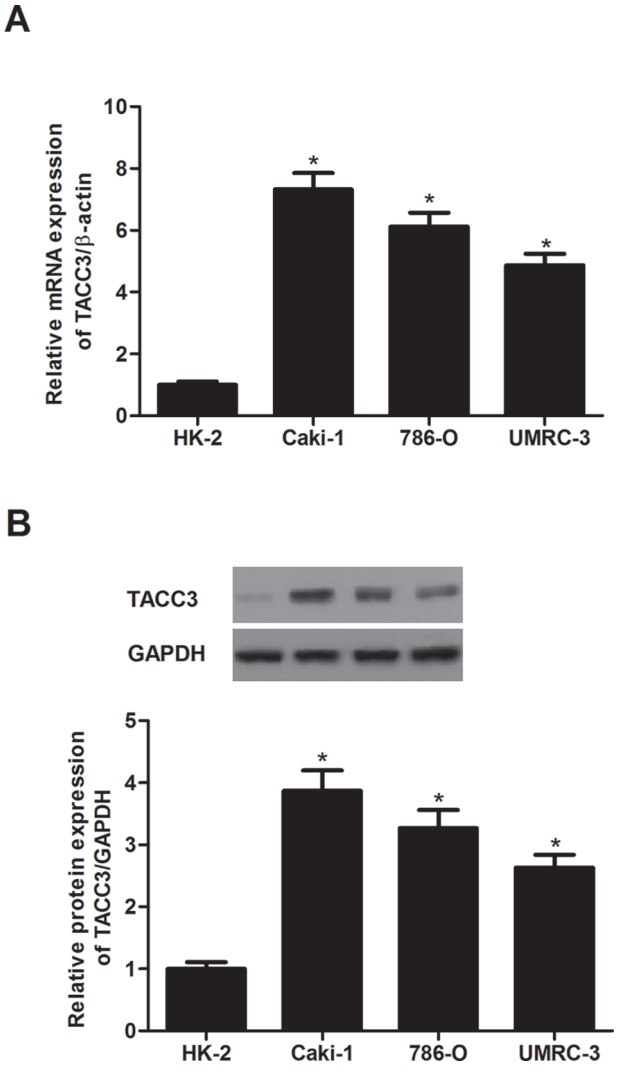

Figure 1. Upregulation of TACC3 expression in human renal cell carcinoma (RCC) cell lines. (A) The mRNA expression of transforming acidic coiled-coil protein 3 (TACC3) in human RCC cell lines was evaluated by quantitative real-time (qRT)-PCR. (B) Protein expression of TACC3 in human RCC cell lines was evaluated by Western blot. *$p < 0.05$ versus HK-2 group.

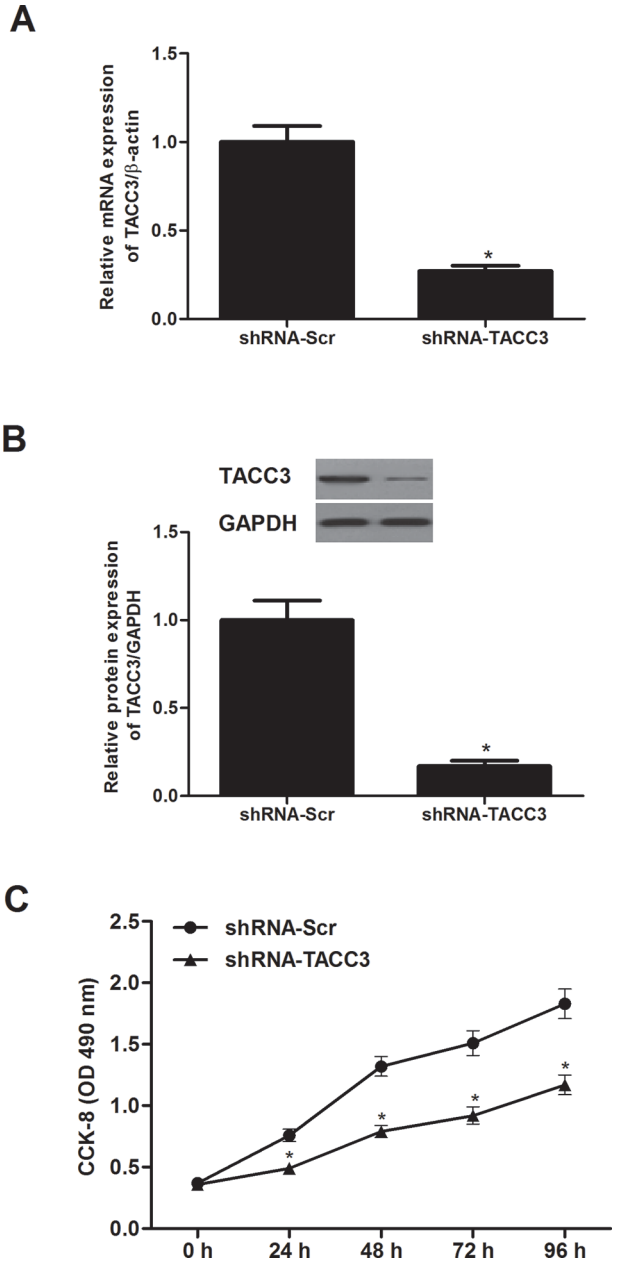

Figure 2. Effects of TACC3 downregulation on RCC cell proliferation. Caki-1 cells were transfected with shRNA-TACC3 or shRNA-Scr for 24 h, respectively. (A) The mRNA expression of TACC3 in Caki-1 cells was evaluated by qRT-PCR. (B) Protein expression of TACC3 in Caki-1 cells was evaluated by Western blot. (C) The effect of TACC3 on cellular proliferation in Caki-1 cells was measured using the MTT assay. *$p < 0.05$ versus shRNA-Scr group.

silencing of TACC3 significantly inhibited the proliferation of Caki-1 cells (Fig. 2C).

Effects of TACC3 Downregulation on RCC Cell Migration and Invasion

To investigate the influence of TACC3 on the migratory and invasive behaviors of Caki-1 cells, the Transwell migration and Matrigel invasion assays were introduced into our study. Knockdown of TACC3 sharply reduced the number of Caki-1 cells that migrated into the lower chamber, when compared with the cells infected with shRNA scramble (Fig. 3A). In addition, silencing of TACC3 efficiently suppressed Caki-1 cells from invading through the Matrigel-coated polycarbonate filter in the Transwell chamber (Fig. 3B). Furthermore, we investigated the role of TACC3 in the EMT process in Caki-1 cells. The results showed that the expression level of E-cadherin was increased, whereas vimentin expression

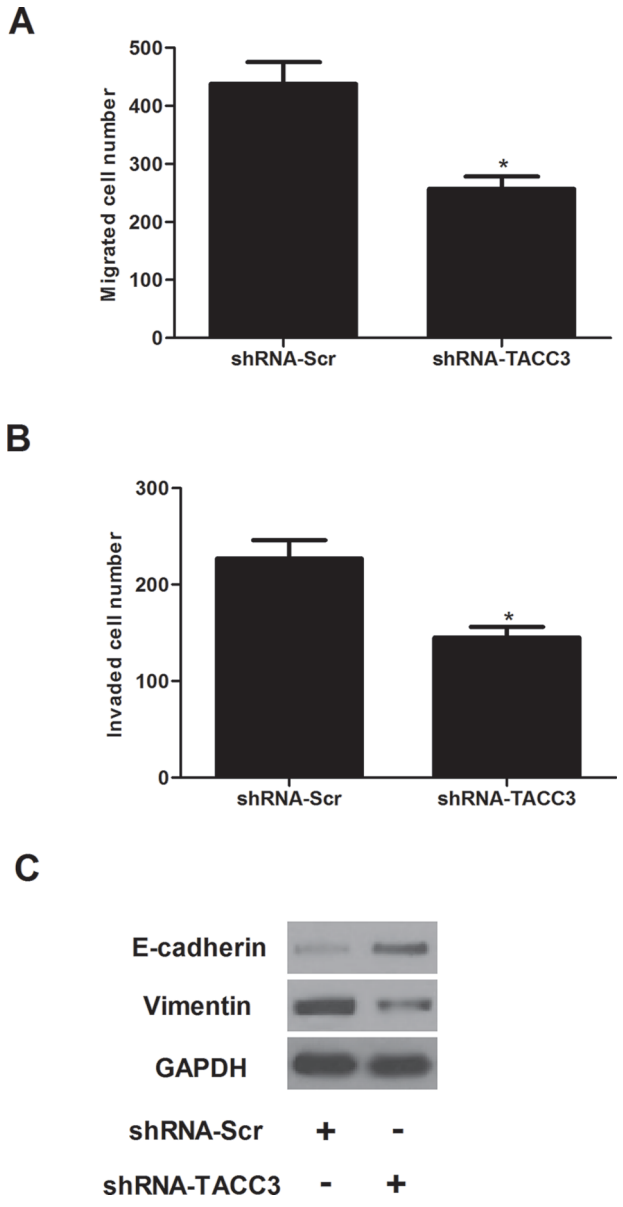

Figure 3. Effects of TACC3 downregulation on RCC cell migration and invasion. Caki-1 cells were transfected with shRNA-TACC3 or shRNA-Scr for 24 h, respectively. (A) The effect of TACC3 on cellular migration in Caki-1 cells was detected using the Transwell migration assay. (B) The effect of TACC3 on cellular invasion in Caki-1 cells was evaluated using the Matrigel invasion assay. (C) The protein expression levels of E-cadherin and vimentin were detected by Western blot. *$p<0.05$ versus shRNA-Scr group.

was decreased in the shRNA-TACC3 group when compared to the shRNA-Scr group (Fig. 3C).

Silencing TACC3 Inhibits the PI3K/Akt Signaling Pathway in RCC Cells

To elucidate the mechanism by which TACC3 regulates the proliferation and invasiveness of RCC cells, we examined whether TACC3 inhibition regulated the activation of the PI3K/Akt signaling pathway. Compared to the shRNA-Scr group, the expression levels of p-PI3K and p-Akt were greatly downregulated in Caki-1 cells transfected with shRNA-TACC3, while the expression of total PI3K and Akt levels remained stable (Fig. 4A). Quantification analysis is shown in Figure 4B. Moreover, we observed that the inhibitory effects of TACC3 downregulation on cell proliferation (Fig. 4C) and invasion (Fig. 4D) were enhanced by Akt inhibitor (MK-2206) exposure.

Effect of TACC3 Expression on In Vivo Caki-1 Tumorigenicity

Next, we examined whether downregulation of TACC3 expression could influence the growth of tumors in vivo. Volume was significantly lower in tumors derived from the shRNA-TACC3 group than that in the control group (Fig. 5A). In addition, we found that tumor weight at 3 weeks after SC injection was lower by 54.7% in mice injected with TACC3 knockdown cells (Fig. 5B).

DISCUSSION

In this study, we demonstrated overexpression of TACC3 in human RCC cell lines at both RNA and protein levels. Moreover, knockdown of TACC3 repressed RCC cell proliferation, migration, and invasion in vitro. In addition, knockdown of TACC3 inactivated PI3K/Akt signaling in RCC cells. Furthermore, knockdown of TACC3 significantly reduced tumor growth in xenograft tumor-bearing mice.

TACC3 has been found to be deregulated in different types of cancer, and it plays an important role in tumor progression[11,14–16]. A study by He et al. reported that TACC3 was highly expressed in cholangiocarcinoma tissues, and downregulation of TACC3 induced G_2/M cycle arrest and inhibited the invasion, metastasis, and proliferation of cholangiocarcinoma cells[17]. In the current study, we consistently showed that the expression of TACC3 at both the mRNA and protein levels was obviously increased in human RCC cell lines. Moreover, knockdown of TACC3 suppressed the proliferation of RCC cells in vitro, as well as attenuated xenografted tumor growth in vivo. These results imply that TACC3 may be an oncogene, which is required for the progression of RCC.

Excessive migration and invasion of tumor cells are the main biological characteristics of malignant tumors[18]. EMT is a critical biological process characterized by epithelial cells' loss of epithelial characteristics and gain of properties typical of mesenchymal cells. Reducing the expression of E-cadherin can trigger EMT, thereby inducing invasiveness and the metastatic potential of tumor cells[19,20]. In the current study, we observed that knockdown of TACC3 sharply inhibited the migration

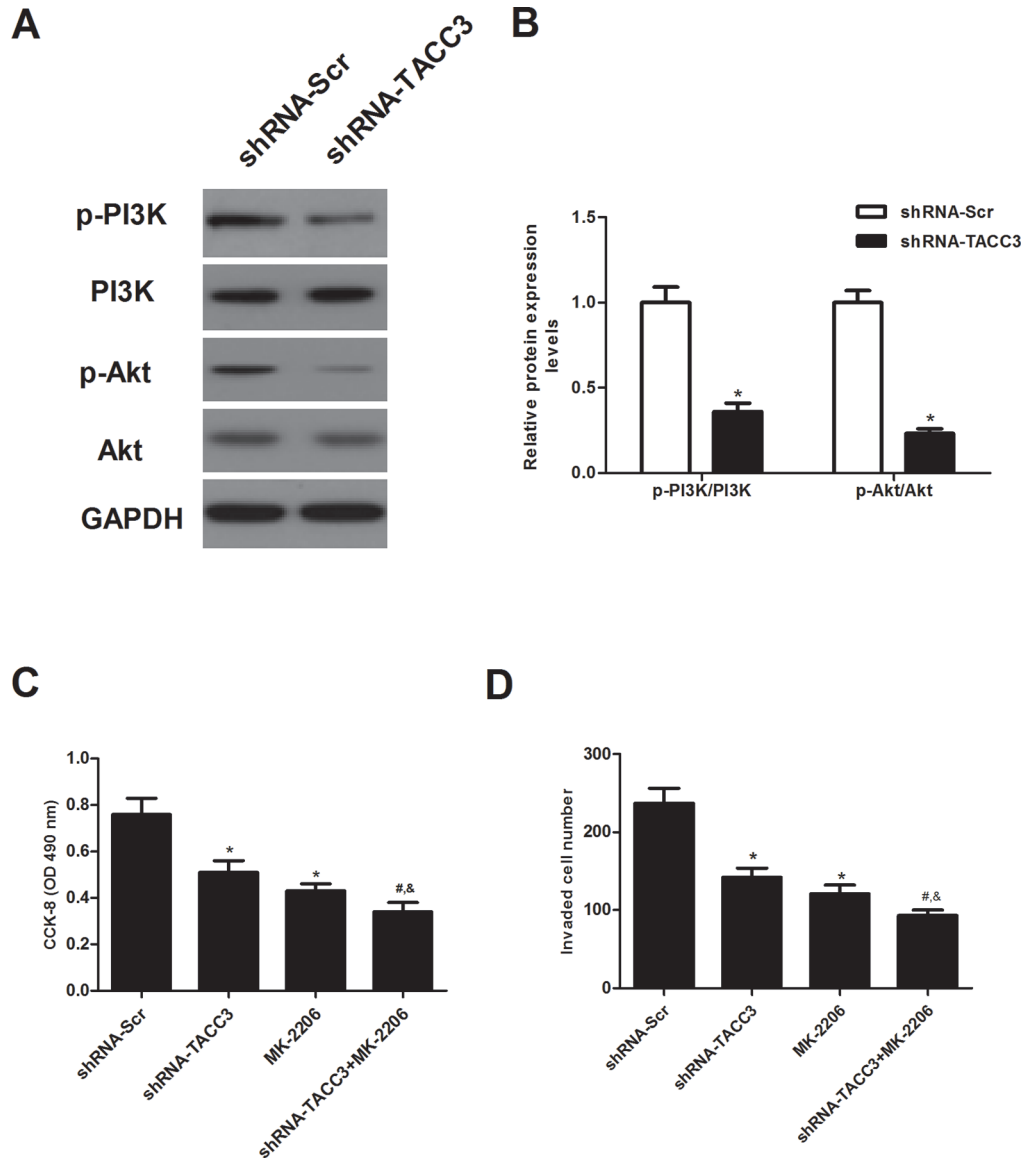

Figure 4. Silencing TACC3 inhibits the PI3K/Akt signaling pathway in RCC cells. Caki-1 cells were transfected with short hairpin RNA (shRNA)-TACC3 or shRNA-Scr for 24 h, respectively. (A) Protein expression levels of p-PI3K and p-Akt were detected by Western blot. (B) Quantification analysis was performed using Gel-Pro Analyzer version 4.0 software. (C, D) Caki-1 cells were infected with shRNA-TACC3 or shRNA-Scr, and migration and invasion assays were performed in the presence of either DMSO or Akt inhibitor (MK-2206, 10 μM). *$p<0.05$ versus shRNA-Scr group, #$p<0.05$ versus shRNA-TACC3 group, &$p<0.05$ versus MK-2206 group.

and invasion of RCC cells. Moreover, the expression of E-cadherin was upregulated, while the expression of vimentin was downregulated in Caki-1 cells transfected with shRNA-TACC3. Part of these results was consistent with the previous study demonstrating that depletion of TACC3 suppressed migratory/invasive behavior as well as the expression of EMT-related markers in cancer cells[21]. These results suggest that TACC3 positively regulates the EMT phenotype, consequently affecting RCC cell migration and invasion in vitro.

Previous studies revealed that the PI3K/Akt signaling pathway plays an important role in regulating a variety of cellular functions including cell survival, growth, angiogenesis, metabolism, tumorigenesis, and metastasis[22–24]. The PI3K/Akt pathway is highly activated in RCC[25]. Thus, suppression of the PI3K/Akt pathway may be a good approach for inhibition of RCC progression[26–28]. More importantly, one study reported that knockdown of TACC3 inhibited the migration and invasion of HTR8/SVneo cells through suppression of the PI3K/Akt

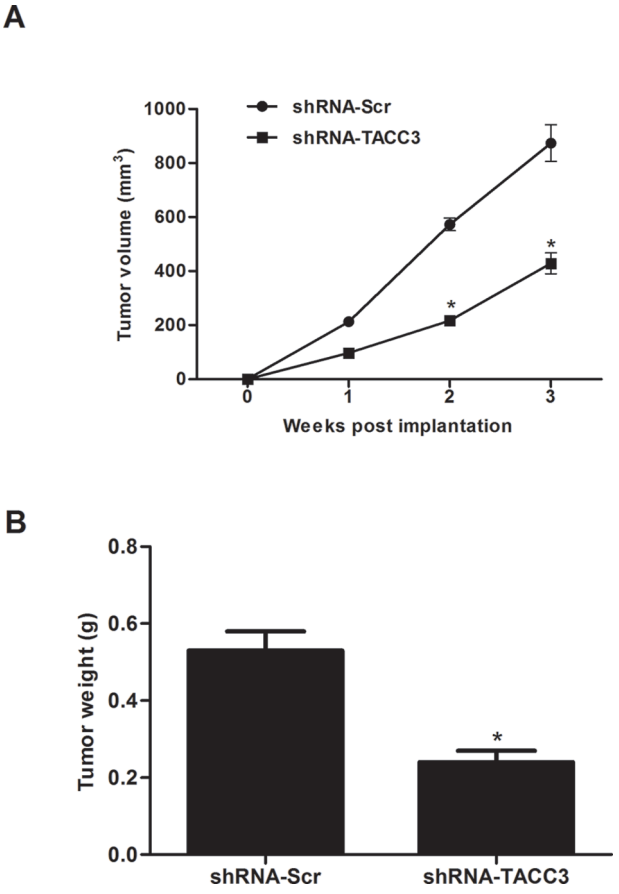

Figure 5. The effect of TACC3 expression on in vivo Caki-1 tumorigenicity. Infected 1×10^6 Caki-1 cells were injected subcutaneously into the flanks of nude mice ($n=10$/group). (A) Tumor volume was measured using calipers. (B) Three weeks after injection, mice were sacrificed, and the tumors were removed and weighed. *$p<0.05$ versus shRNA-Scr group.

signaling pathway[29]. Similarly, in the current study, we found that knockdown of TACC3 repressed the expression levels of p-PI3K and p-Akt in Caki-1 cells. More importantly, we observed that the inhibitory effects of TACC3 downregulation on cell proliferation and invasion were enhanced by AKT inhibitor (MK-2206) exposure. These results suggest that knockdown of TACC3 inhibited the ability for cell proliferation, migration, invasion, and tumorigenesis in vivo, at least in part, through inactivation of the PI3K/Akt signaling pathway.

In conclusion, we shed light on the expression and role of TACC3 in human RCC cells. Our findings showed that TACC3 was increased in human RCC cell lines, and knockdown of TACC3 inhibited the ability of cell proliferation, migration, invasion, and tumorigenesis in vivo. Therefore, TACC3 may act as a therapeutic target for the treatment of human RCC.

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REFERENCES

1. Ljungberg B, Hanbury DC, Kuczyk MA, Merseburger AS, Mulders PF, Patard JJ, Sinescu IC. Renal cell carcinoma guideline. Actas Urol Esp. 2009;33:270–9.
2. Deveson KS. Renal cell carcinoma: Treatment options. Br J Nurs. 2011;20:536, 538–9.
3. Escudier B, Porta C, Schmidinger M, Algaba F, Patard JJ, Khoo V, Eisen T, Horwich A. Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012;23:40–8.
4. Hagan K, Trapp JD, Rhamy RK, Reynolds VH. Treatment of metastatic renal cell carcinoma. Cancer Chemother Pharm. 2009;67:1175–8.
5. Drucker BJ. Renal cell carcinoma: Current status and future prospects. Cancer Treat Rev. 2005;31:536–45.
6. Gergely F, Karlsson C, Still I, Cowell J, Kilmartin J, Raff JW. The TACC domain identifies a family of centrosomal proteins that can interact with microtubules. Proc Natl Acad Sci USA 2001;97:14352–7.
7. Peset I, Seiler J, Sardon T, Bejarano LA, Rybina S, Vernos I. Function and regulation of Maskin, a TACC family protein, in microtubule growth during mitosis. J Cell Biol. 2005;170:1057–66.
8. Hood FE, Royle SJ. Pulling it together: The mitotic function of TACC3. Bioarchitecture 2011;1:105–9.
9. Kinoshita K, Noetzel TL, Pelletier L, Mechtler K, Drechsel DN, Schwager A, Lee M, Raff JW, Hyman AA. Aurora A phosphorylation of TACC3/maskin is required for centrosome-dependent microtubule assembly in mitosis. J Cell Biol. 2005;170:1047–55.
10. Yun M, Rong J, Lin ZR, He YL, Zhang JX, Peng ZW, Tang LQ, Zeng MS, Zhong Q, Ye S. High expression of transforming acidic coiled coil-containing protein 3 strongly correlates with aggressive characteristics and poor prognosis of gastric cancer. Oncol Rep. 2015;34:1397–405.
11. Huang ZL, Lin ZR, Xiao YR, Cao X, Zhu LC, Zeng MS, Zhong Q, Wen ZS. High expression of TACC3 in esophageal squamous cell carcinoma correlates with poor prognosis. Oncotarget 2015;6:6850–61.
12. Du Y, Liu L, Wang C, Kuang B, Yan S, Zhou A, Wen C, Chen J, Wu Y, Yang X. TACC3 promotes colorectal cancer tumourigenesis and correlates with poor prognosis. Oncotarget 2016;7:41885–97.
13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402–8.
14. Stewart JP, Thompson A, Santra M, Barlogie B, Lappin TR, Jr SJ. Correlation of TACC3, FGFR3, MMSET and p21 expression with the t(4;14)(p16.3;q32) in multiple myeloma. Br J Haematol. 2004;126:72–6.
15. Ulisse S, Baldini E, Toller M, Delcros JG, Guého A, Curcio F, De AE, Giacomelli L, Ambesiimpiombato FS, Bocchini S. Transforming acidic coiled-coil 3 and Aurora-A interact in human thyrocytes and their expression is deregulated in thyroid cancer tissues. Endocr Relat Cancer 2007;14:827–37.
16. Singh D, Iavarone A. Transforming fusions of FGFR and TACC genes in human glioblastoma. Science 2012;337:1231–5.

17. He JC, Yao W, Wang JM, Schemmer P, Yang Y, Liu Y, Qian YW, Qi WP, Zhang J, Shen Q. TACC3 overexpression in cholangiocarcinoma correlates with poor prognosis and is a potential anti-cancer molecular drug target for HDAC inhibitors. Oncotarget 2016;12254–65.
18. Valastyan S, Weinberg RA. Tumor metastasis: Molecular insights and evolving paradigms. Cell 2011;147:275–92.
19. Kalluri R. EMT: When epithelial cells decide to become mesenchymal-like cells. J Clin Invest. 2009;119:1417–9.
20. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metast Rev. 2009;28:15–33.
21. Ha GH, Park JS, Breuer EKY. TACC3 promotes epithelial–mesenchymal transition (EMT) through the activation of PI3K/Akt and ERK signaling pathways. Cancer Lett. 2013; 332:63–73.
22. Vara JÁF, Casado E, Castro JD, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signalling pathway and cancer. Cancer Treat Rev. 2004;30:193–204.
23. Martini M, Santis MCD, Braccini L, Gulluni F, Hirsch E. PI3K/AKT signaling pathway and cancer: An updated review. Ann Med. 2014;46:372–83.
24. Carnero A, Blanco-Aparicio C, Renner O, Link W, Leal JF. The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. Curr Cancer Drug Targets 2008; 8:187–98.
25. 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.
26. Lin Y, Yang Z, Xu A, Dong P, Huang Y, Liu H, Li F, Wang H, Xu Q, Wang Y. PIK3R1 negatively regulates the epithelial-mesenchymal transition and stem-like phenotype of renal cancer cells through the AKT/GSK3β/CTNNB1 signaling pathway. Sci Rep. 2015;5:8997.
27. Yao X, Qi L, Chen X, Du J, Zhang Z, Liu S. Expression of CX3CR1 associates with cellular migration, metastasis, and prognosis in human clear cell renal cell carcinoma. Urologic Oncol. 2014;32:162–70.
28. Ribback S, Cigliano A, Kroeger N, Pilo MG, Terracciano L, Burchardt M, Bannasch P, Calvisi DF, Dombrowski F. PI3K/AKT/mTOR pathway plays a major pathogenetic role in glycogen accumulation and tumor development in renal distal tubules of rats and men. Oncotarget 2015; 6:13036–48.
29. Zhu X, Cao Q, Li X, Wang Z. Knockdown of TACC3 inhibits trophoblast cell migration and invasion through the PI3K/Akt signaling pathway. Mol Med Rep. 2016;14:3437–42.