Oncology Research, Vol. 25, pp. 1253–1259
Printed in the USA.

0965-0407/17 $90.00 + .00
DOI: https://doi.org/10.3727/096504017X14854310794561
E-ISSN 1555-3906
www.cognizantcommunication.com

Knockdown of TRIM44 Inhibits the Proliferation and Invasion in Prostate Cancer Cells

Yuying Tan,* Hanxin Yao,† Jinghai Hu,‡ and Lingyun Liu§

*Department of Echocardiography, First Hospital of Jilin University, Changchun, Jilin, P.R. China
†Department of Clinical Laboratory, First Hospital of Jilin University, Changchun, Jilin, P.R. China
‡Department of Urology, First Hospital of Jilin University, Changchun, Jilin, P.R. China
§Department of Andrology, First Hospital of Jilin University, Changchun, Jilin, P.R. China

Tripartite motif 44 (TRIM44), a member of the TRIM protein family, has been shown to play a role in tumor development and progression. However, the potential involvement of TRIM44 in prostate cancer has not been fully explored. Therefore, in the present study, we analyzed the expression of TRIM44 in prostate cancer and assessed the role of TRIM44 in the progression of prostate cancer. Our results showed that the expression of TRIM44 was significantly upregulated in human prostate cancer cell lines. In addition, knockdown of TRIM44 significantly inhibited the proliferation, migration, and invasion of prostate cancer cells in vitro, as well as attenuated the tumor growth in vivo. Mechanistic studies showed that knockdown of TRIM44 significantly reduced the levels of phosphorylated PI3K and Akt in PC-3 cells. In conclusion, this study provided evidence that knockdown of TRIM44 inhibited proliferation and invasion in prostate cancer cells, at least in part, through the inactivation of the PI3K/Akt signaling pathway. These results suggest that TRIM44 may be a potential therapeutic target for the treatment of prostate cancer.

Key words: Tripartite motif 44 (TRIM44); Prostate cancer; Invasion; PI3K/Akt pathway

INTRODUCTION

Prostate cancer is the most common malignancy and the second leading cause of cancer-related deaths in men[1]. Despite the considerable progress that has been made in treatment strategies, including surgical resection, radiation, and chemotherapy, the 5-year survival rate of patients with prostate cancer is very poor[2–4]. This high mortality is attributed to the aggressive migration and invasion of malignant prostate cancer cells. Thus, a good understanding of the molecular mechanisms underlying prostate cancer invasion and metastasis is imperative.

The tripartite motif (TRIM) family of proteins is characterized by three zinc-binding domains, a RING, a B-box type 1, and a B-box type 2, followed by a coiled-coil region[5]. Increasing evidence has reported that TRIM protein family members play a diverse physiological and pathological role in regulating cell cycle, growth, autophagy, and innate immunity[6–8]. Tripartite motif 44 (TRIM44), a member of the TRIM protein family, has been shown to play a role in tumor development and progression[9,10]. Recently, one study reported that the expression of TRIM44 was significantly increased in non-small cell lung cancer tissues compared with normal lung tissues[11]. However, the potential involvement of TRIM44 in prostate cancer has not been fully explored. Therefore, in the present study, we analyzed the expression of TRIM44 in prostate cancer and assessed the role of TRIM44 in the progression of prostate cancer. Our results showed that TRIM44 expression was significantly upregulated in prostate cancer cell lines. Knockdown of TRIM44 suppressed the proliferation and migration of prostate cancer cells, at least in part, through the inactivation of the PI3K/Akt signaling pathway.

MATERIALS AND METHODS

Cell Culture

Human prostate cancer cell lines (LNCaP, DU-145, and PC-3) and the immortalized normal prostate epithelial cell line (RWPE-1) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were maintained in DMEM (Invitrogen, Carlsbad, CA, USA), which was supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 μg/ml penicillin, and 100 μg/ml streptomycin (Sigma-Aldrich,

Address correspondence to Lingyun Liu, Department of Andrology, First Hospital of Jilin University, No. 71 Xinmin Street, Changchun, 130021 Jilin, P.R. China. E-mail: lingyun_liuly@126.com

St. Louis, MO, USA) at 37°C in an incubator (Life Technologies, Baltimore, MD, USA).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The total RNA of prostate cancer cells was extracted and purified using the RNeasy mini kit according to manufacturer's protocol (Qiagen, Santa Clarita, CA, USA). About 3 μg of total RNA for each sample was reverse transcribed into first-strand cDNA using M-MLV reverse transcriptase (Clontech, Palo Alto, CA, USA) for qRT-PCR analysis. The following primer pairs were used: TRIM44, 5′-GTGGACATCCAAGAGGCAAT-3′ (forward) and 5′-AGCAAGCCTTCATGTGTCCT-3′ (reverse); β-actin, 5′-GATCATTGCTCCTCCTGAGC-3′ (forward) and 5′-ACTCCTGCTTGCTGATCCAC-3′ (reverse). Amplification cycles were as follows: 95°C for 3 min, then 35 cycles at 95°C for 30 s, 58°C for 1 min, 72°C for 1.5 min, followed by 72°C for 15 min. The transcript abundance was calculated using the $2^{-\Delta\Delta}$Ct method.

Western Blot

Prostate cancer cells were homogenized and lysed with RIPA lysis buffer (Invitrogen). Total protein (40 μg) from each sample was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a PVDF membrane (Millipore, Billerica, MA, USA). Then nonspecific binding was blocked by incubating with 5% nonfat milk in Tris-buffered-saline with Tween at room temperature for 1 h and then incubated with primary antibodies (anti-TRIM44, anti-E-cadherin, anti-vimentin, anti-N-cadherin, anti-PI3K, anti-p-PI3K, anti-Akt, anti-p-Akt, and anti-GAPDH; Santa Cruz Biotechnology, Santa Cruz, CA, USA). After being washed three times, the membranes were incubated with HRP-labeled conjugated second antibody for 1 h at room temperature. Finally, the target protein was visualized using an enhanced chemiluminescence (ECL) detection system (Amersham, Little Chalfont, UK).

shRNA Transfection

The short hairpin RNA (shRNA) against TRIM44 (shTRIM44) and negative control shRNA (shNC) were purchased from GenePharma Co. (Shanghai, P.R. China). PC-3 cells were seeded into each well of 24-well microplates, grown for 24 h, and then transfected with shTRIM44 or shNC using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Transfection efficiency was assessed 48 h after transfection by qRT-PCR and Western blot.

MTS Proliferation Assay

Cell proliferation was determined via the MTS assay. In brief, PC-3 cells (1×10^4 cells/well) transfected with shTRIM44 or shNC were seeded into 96-well plates and cultured for 24, 48, 72, or 96 h, respectively. Next, 100 μl of MTS solution (Sigma-Aldrich) was added to each well and incubated for 4 h at 37°C. Absorbance was determined at 490 nm using a microplate reader (Invitrogen).

Transwell Migration and Invasion Assays

For the invasion assay, PC-3 cells transfected with shTRIM44 or shNC (1×10^5 cells per well) suspended in 0.1% FBS medium were seeded into the upper compartment. For the invasion assay, PC-3 cells at a density of 1×10^4 cells/well in 100 μl of serum-free DMEM were added into the upper chamber of the insert, with 15% Matrigel tiled on the membrane of the upper chamber. For cell migration and invasion assays, 600 μl of DMEM containing 10% FBS was added to the lower chamber. After incubation for 24 h at 37°C, the cells that invaded/migrated were fixed in methanol for 15 min and stained with 0.05% crystal violet in PBS for 15 min. The number of cells per five high-power fields was counted using a microscope.

In Vivo Tumorigenesis Assay

All of the animal experiments were performed with the approval of The Institutional Animal Care and Use Committee of the First Hospital of Jilin University (P.R. China). Briefly, 1×10^6 PC-3 cells transfected with shTRIM44 and the corresponding control cells were injected subcutaneously into the right flanks of 5-week-old female Balb/c nude mice. Tumor length (L) and width (W) were measured every 7 days, and tumor volume was calculated using the equation: volume $=(W^2\times L)/2$. After 4 weeks, all of the mice were sacrificed, and the tumors were excised and weighed.

Statistical Analysis

All statistical analyses were performed using SPSS 13.0 Software (SPSS Inc., Chicago, IL, USA). Data are expressed as means ± standard deviation (SD). Statistical analysis involved using the Student's t-test for comparison of two groups or one-way ANOVA for multiple comparisons. A value of $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

TRIM44 Was Upregulated in Prostate Cancer Cell Lines

We detected the expression profile of TRIM44 at mRNA in three human prostate cancer cell lines (LNCaP, DU-145, and PC-3) and the immortalized normal prostate epithelial cell line (RWPE-1). The results of the qRT-PCR analysis showed that the mRNA expression levels of TRIM44 in three human prostate cancer cell lines were obviously higher than those in the RWPE-1 cell line (Fig. 1A). Similarly, Western blot analysis

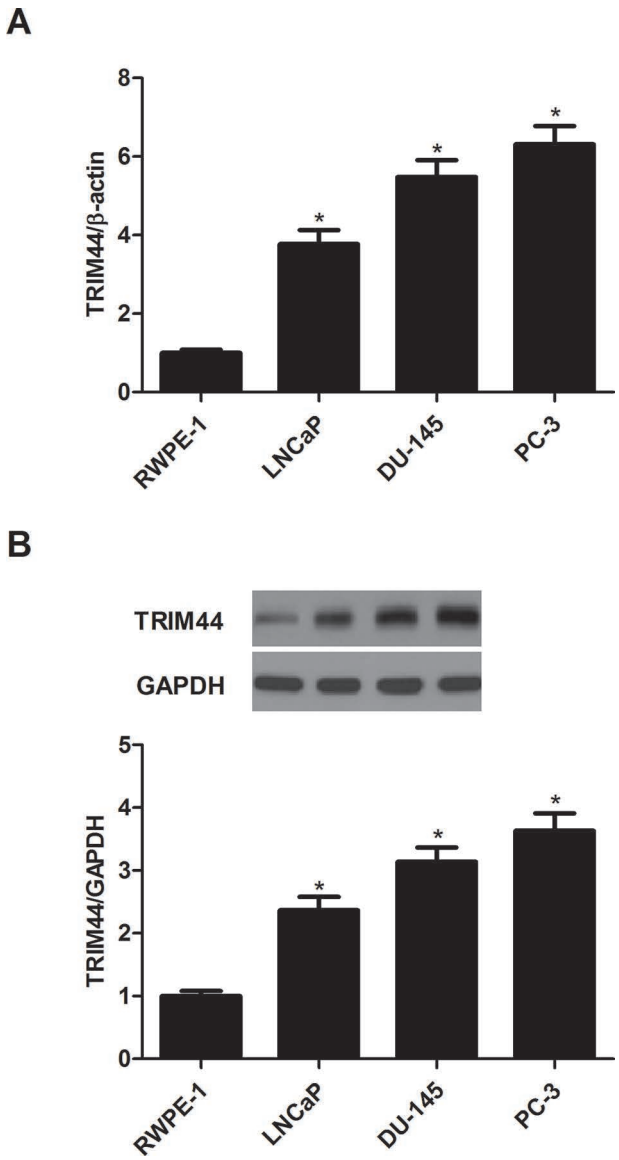

Figure 1. Tripartite motif 44 (TRIM44) was upregulated in prostate cancer cell lines. (A) The expression levels of TRIM44 mRNA were detected in three human prostate cancer cell lines (LNCaP, DU-145, and PC-3) and the immortalized normal prostate epithelial cell line (RWPE-1) using quantitative real-time polymerase chain reaction (qRT-PCR) analysis. (B) TRIM44 protein expression was significantly increased in prostate cancer cell lines. The values shown represent the mean ± standard deviation (SD) and $n = 3$ per group. *$p < 0.05$.

demonstrated that the protein expression of TRIM44 was significantly increased in three human prostate cancer cell lines (Fig. 1B).

Knockdown of TRIM44 Inhibited Prostate Cancer Cell Proliferation In Vitro

To investigate the effect of TRIM44 on prostate cancer tumorigenesis, PC-3 cells were transfected with shTRIM44 or shNC for 48 h. The expression of TRIM44 at both mRNA and protein levels was significantly decreased in PC-3 cells transfected with shTRIM44, compared with the shNC group (Fig. 2A and B). Then we examine the effect of TRIM44 on cell proliferation via the MTS assay. The results indicated that TRIM44 silencing significantly inhibited the proliferation of PC-3 cells, compared with the shNC group (Fig. 2C).

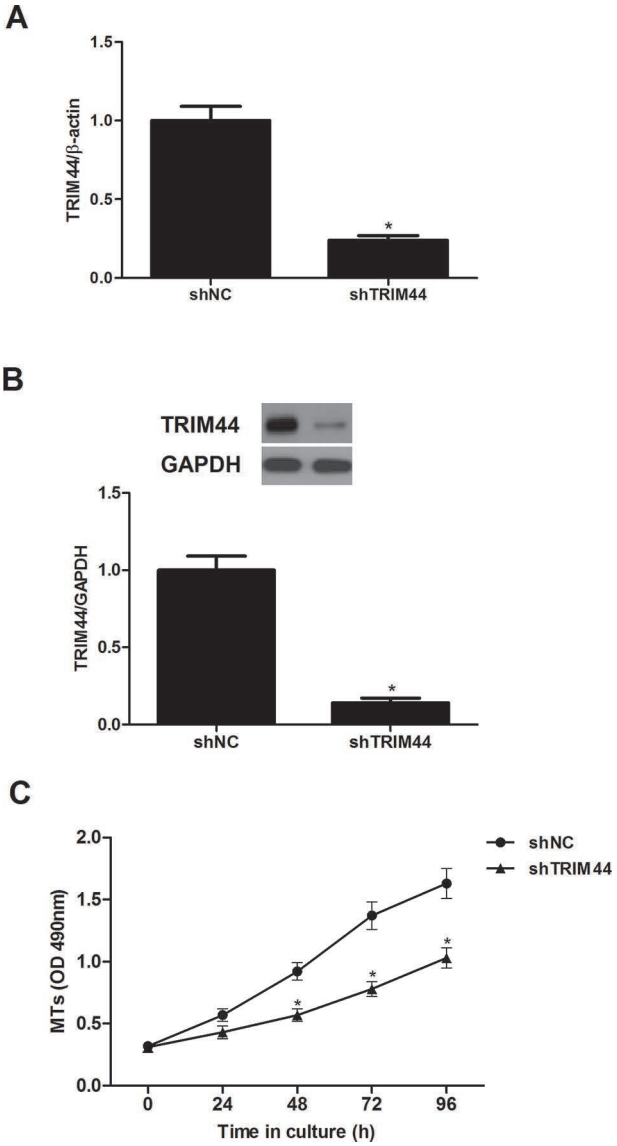

Figure 2. Knockdown of TRIM44 inhibited prostate cancer cell proliferation in vitro. PC-3 cells were transfected with shTRIM44 or shNC for 48 h. (A, B) The transfection efficiency was confirmed by qRT-PCR and Western blot. (C) Cell proliferation was detected via the MTS assay. The values shown represent the mean ± SD and $n = 3$ per group. *$p < 0.05$.

Knockdown of TRIM44 Inhibited Prostate Cancer Cell Migration and Invasion In Vitro

To explore the effect of TRIM44 on prostate cancer cell migration and invasion, we adopted Transwell migration and invasion assays. Compared to the shNC group, downregulation of TRIM44 markedly reduced the number of cells that migrated in PC-3 cells (Fig. 3A). In addition, knockdown of TRIM44 resulted in a significant decrease in the invasive ability in PC-3 cells (Fig. 3B). Furthermore, we examined the effect of TRIM44 on the expression of epithelial–mesenchymal transition (EMT) markers in PC-3 cells. TRIM44 silencing greatly upregulated the protein expression of the epithelial marker (E-cadherin) and reduced the protein expression of mesenchymal markers (vimentin and N-cadherin) in PC-3 cells (Fig. 3C).

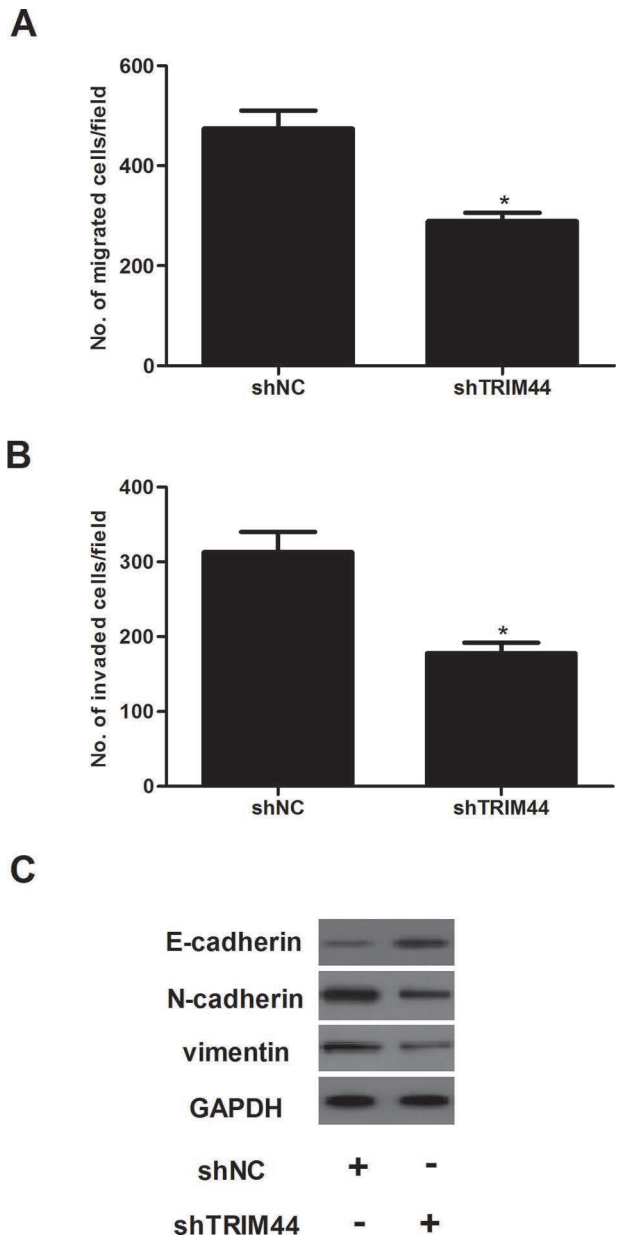

Figure 3. Knockdown of TRIM44 inhibited prostate cancer cell migration and invasion in vitro. PC-3 cells were transfected with shTRIM44 or shNC for 48 h. (A) Cell migration was determined by the Transwell assay. (B) Cell invasion was determined using Matrigel-coated Transwell invasion chamber. (C) The protein expression levels of E-cadherin, N-cadherin, and vimentin were determined by Western blot. The values shown represent the mean ± SD and $n = 3$ per group. *$p < 0.05$.

Knockdown of TRIM44 Attenuated the Tumor Growth In Vitro

To explore the effects of TRIM44 on tumorigenesis in vivo, 1×10^6 PC-3 cells were injected subcutaneously into the right flanks of nude mice. The tumor volume derived

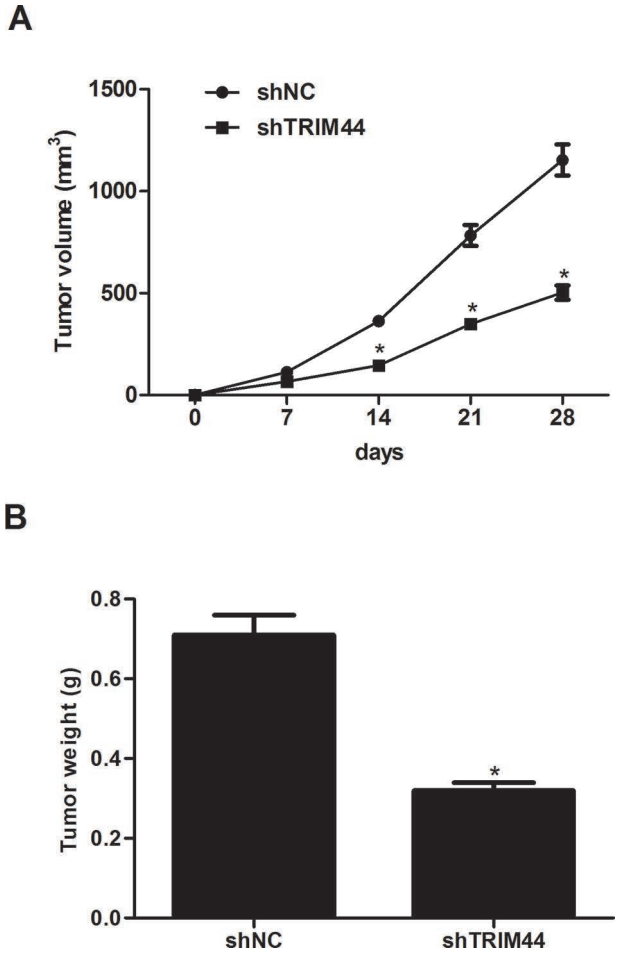

Figure 4. Knockdown of TRIM44 attenuated the tumor growth in vitro. PC-3 cells (1×10^6 cells/0.1 ml) transfected with shTRIM44 or shNC were injected subcutaneously into the flank of nude mice. (A) The tumor volumes were calculated in each group every 7 days from day 0 to day 28. (B) The tumor weights were measured at day 28. The values shown represent the mean ± SD and $n = 3$ per group. *$p < 0.05$.

from shTRIM44-transfected cells was markedly smaller than that of controls (Fig. 4A). In addition, we observed that TRIM44 silencing remarkably reduced the weight of the xenografted tumor (Fig. 4B).

Knockdown of TRIM44 Prevented the Activation of the PI3K/Akt Signaling Pathway in Prostate Cancer Cells

The PI3K/Akt signaling pathway has been shown to play a critical role in promoting metastasis in several types of cancer, including prostate cancer. Thus, we explored the effect of TRIM44 on the activation of the PI3K/Akt pathway in PC-3 cells. The results of the Western blot analysis demonstrated that TRIM44 silencing greatly suppressed the levels of p-PI3K and p-Akt in PC-3 cells, compared with the shNC group (Fig. 5A). Furthermore, we found that the Akt inhibitor (wortmannin) efficiently enhanced the inhibitory effect of shTRIM44 on PC-3 cell invasion (Fig. 5B).

DISCUSSION

This study is the first to demonstrate the expression and function of TRIM44 in human prostate cancer. Our

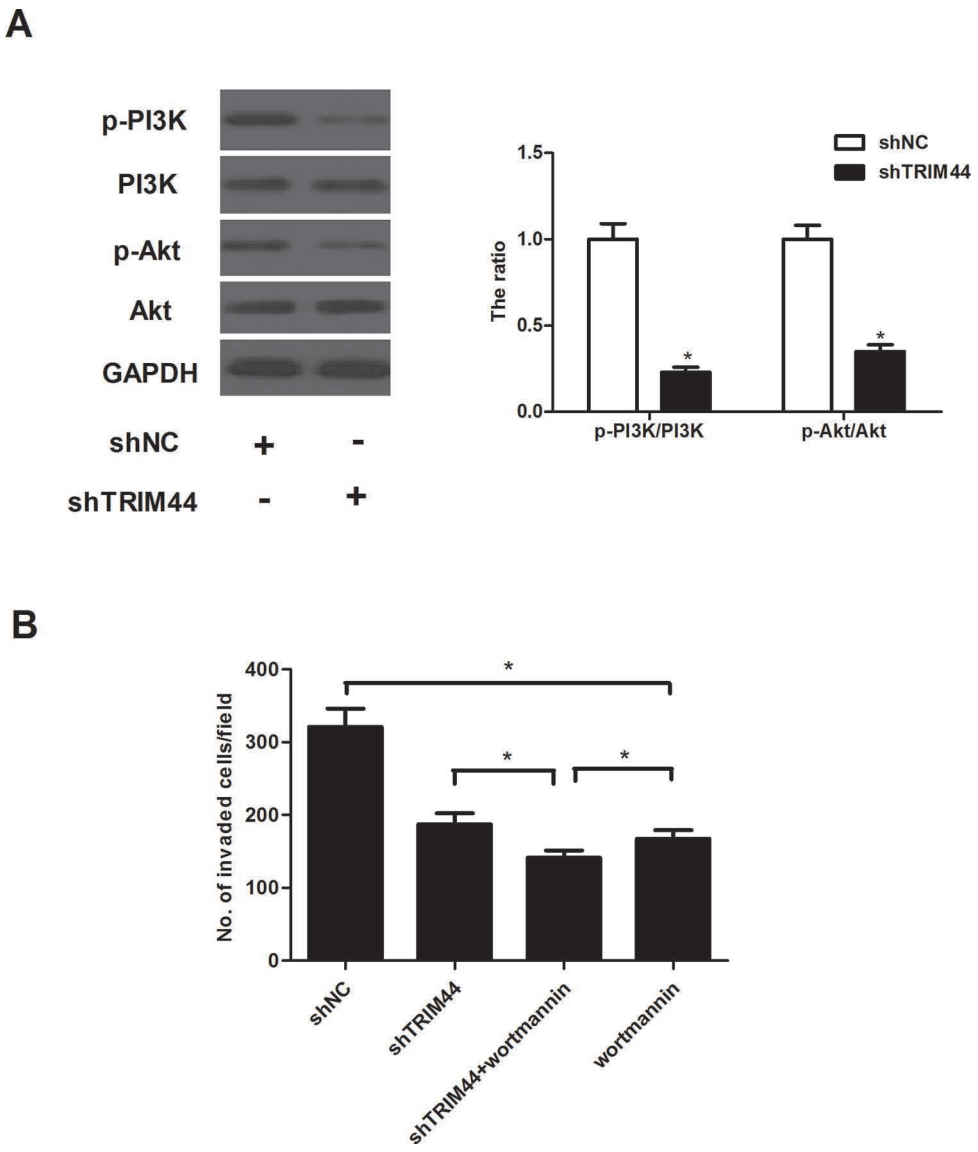

Figure 5. Knockdown of TRIM44 prevented the activation of the PI3K/Akt signaling pathway in prostate cancer cells. (A) PC-3 cells were transfected with shTRIM44 or shNC for 48 h. (A) The protein expression levels of PI3K, p-PI3K, Akt, and p-Akt were determined by Western blot. Quantification analysis was performed using Gel-Pro Analyzer version 4.0 software. (B) PC-3 cells were transfected with shTRIM44 or shNC in the presence or absence of the wortmannin (100 nM) for 24 h. Cell invasion was evaluated by the Matrigel-coated Transwell invasion chamber. The values shown represent the mean ± SD and $n = 3$ per group. *$p < 0.05$.

results showed that the expression of TRIM44 was significantly upregulated in human prostate cancer cell lines. Knockdown of TRIM44 significantly inhibited the proliferation, migration, and invasion of prostate cancer cells in vitro, as well as attenuated the tumor growth in vivo. Mechanistic studies showed that knockdown of TRIM44 significantly reduced the levels of phosphorylated PI3K and Akt in PC-3 cells.

Recent research indicates that TRIM44 may act as an oncogene and is closely associated with poor prognosis in several types of cancer. A study showed that TRIM44 expression was increased in gastric cancer cell lines and primary tumor samples of gastric cancer, and downregulation of TRIM44 expression obviously suppressed the proliferation of gastric cancer cells[10]. Another study reported that TRIM44 mRNA and protein expression were sharply upregulated in hepatocellular carcinoma (HCC) compared with matched normal tissues, and overexpression of TRIM44 efficiently induced cell proliferation via accelerating the G_1/S phase transition in HCC cells[12]. In accordance with previous studies, in our study we found that the expression of TRIM44 was significantly upregulated in human prostate cancer cell lines. Knockdown of TRIM44 significantly inhibited the proliferation of prostate cancer cells in vitro, as well as attenuated the tumor growth in vivo. These data suggest that TRIM44 may be a novel candidate oncogene in the progression of prostate cancer.

The most common cause of death from prostate cancer is metastasis[13]. Accumulating evidence indicates that EMT plays an essential role in the regulation of metastasis and diseases progression of prostate cancer[14–16]. It has been reported that the expression of E-cadherin was reduced or absent in high-grade prostate cancer[17]. In this study, we observed that downregulation of TRIM44 markedly suppressed the migration and invasion of prostate cancer cells, which was associated with upregulated protein expression of the epithelial marker (E-cadherin) and reduced protein expression of the mesenchymal markers (vimentin and N-cadherin) in PC-3 cells. These results strongly suggest that TRIM44 silencing suppressed the EMT process in prostate cancer cells, leading to reduced cell migration and invasive capacities.

Increasing evidence has reported that the PI3K/Akt pathway plays an important role in the development and progression of prostate cancer[18–20]. A study by Dai et al. confirmed that the expression of p-Akt and p-mTOR levels was markedly higher in prostate cancer tissues than in benign prostatic hyperplasia tissues[21]. Akt, a serine/threonine protein kinase, was activated through the PI3K pathway, which has been implicated in cancer cell migration, invasion, and EMT phenotype[22,23]. Activated Akt can induce EMT, which would increase cell invasion and metastasis[24]. In the present study, we found that knockdown of TRIM44 significantly reduced the levels of phosphorylated PI3K and Akt in PC-3 cells. In addition, we observed that the Akt inhibitor (wortmannin) efficiently enhanced the inhibitory effect of shTRIM44 on PC-3 cell invasion. These results suggest that knockdown of TRIM44 inhibited the proliferation and invasion of prostate cancer cells, at least in part, through the PI3K/Akt signaling pathway.

In summary, this study provided evidence that knockdown of TRIM44 inhibited the proliferation and invasion of prostate cancer cells, at least in part, through the inactivation of PI3K/Akt signaling pathway. These results suggest that TRIM44 may be a potential therapeutic target for the treatment of prostate cancer.

ACKNOWLEDGMENT: The authors declare no conflicts of interest.

REFERENCES

1. Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. N Engl J Med. 2003;349:366–81.
2. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001;1:34–45.
3. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, de Wit R, Mulders P, Chi KN, Shore ND, Armstrong AJ, Flaig TW, Fléchon A, Mainwaring P, Fleming M, Hainsworth JD, Hirmand M, Selby B, Seely L, de Bono JS, AFFIRM Investigators. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367:1187–97.
4. Tangen CM, Thompson IM, Goodman PJ, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA Jr. The influence of finasteride on the development of prostate cancer. N Engl J Med. 2003; 349:215–24.
5. Reymond A, Meroni G, Fantozzi A, Merla G, Cairo S, Luzi L, Riganelli D, Zanaria E, Messali S, Cainarca S, Guffanti A, Minucci S, Pelicci PG, Ballabio A. The tripartite motif family identifies cell compartments. EMBO J. 2001;20: 2140–51.
6. Kawai T, Akira S. Regulation of innate immune signalling pathways by the tripartite motif (TRIM) family proteins. EMBO Mol Med. 2011;3:513–27.
7. Song X, Fu C, Yang X, Sun D, Zhang X, Zhang J. Tripartite motif-containing 29 as a novel biomarker in non-small cell lung cancer. Oncol Lett. 2015;10:2283–8.
8. Kimura T, Jain A, Choi SW, Mandell MA, Schroder K, Johansen T, Deretic V. TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. J Cell Biol. 2011;210:973–89.
9. Luo Q, Lin H, Ye X, Huang J, Lu S, Xu L. Trim44 facilitates the migration and invasion of human lung cancer cells via the NF-κB signaling pathway. Int J Clin Oncol. 2015;20:1–15.
10. Kashimoto K, Komatsu S, Ichikawa D, Arita T, Konishi H, Nagata H, Takeshita H, Nishimura Y, Hirajima S, Kawaguchi T, Shiozaki A, Fujiwara H, Okamoto K, Tsuda H, Otsuji E. Overexpression of TRIM44 contributes to malignant outcome in gastric carcinoma. Cancer Sci. 2012; 103:2021–6.

11. Xing Y, Meng Q, Chen X, Zhao Y, Liu W, Hu J, Xue F, Wang X, Cai L. TRIM44 promotes proliferation and metastasis in non-small cell lung cancer via mTOR signaling pathway. Oncotarget 2016;7:30479–91.
12. Zhu X, Wu Y, Miao X, Li C, Yin H, Yang S, Lu X, Liu Y, Chen Y, Shen R, Chen X, He S. High expression of TRIM44 is associated with enhanced cell proliferation, migration, invasion, and resistance to doxorubicin in hepatocellular carcinoma. Tumor Biol. 2016;37:1–14.
13. Krishna SN, Bergan RC. Therapeutic modulation of prostate cancer metastasis. Future Med Chem. 2014;6:223–39.
14. Gravdal K, Halvorsen OJ, Haukaas SA, Akslen LA. A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. Clin Cancer Res. 2007;13:7003–11.
15. Zhang X, Zhang T, Yang K, Zhang M, Wang K. miR-486-5p suppresses prostate cancer metastasis by targeting Snail and regulating epithelial–mesenchymal transition. Oncotargets Ther. 2016;9:6909–14.
16. Lu Q, Liu Z, Li Z, Chen J, Liao Z, Wu WR, Li YW. TIPE2 Overexpression suppresses the proliferation, migration, and invasion in prostate cancer cells by inhibiting PI3K/Akt signaling pathway. Oncol Res. 2016;24:305–13.
17. Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HE, Debruyne FM, Isaacs WB. Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. Cancer Res. 1992;52:5104–9.
18. Gao N, Zhang Z, Jiang BH, Shi X. Role of PI3K/AKT/mTOR signaling in the cell cycle progression of human prostate cancer. Biochem Biophys Res Commun. 2003; 310:1124–32.
19. Vo BT, Morton D Jr, Komaragiri S, Millena AC, Leath C, Khan SA. TGF-beta effects on prostate cancer cell migration and invasion are mediated by PGE(2) through activation of PI3K/AKT/mTOR pathway. Endocrinology 2013; 154:1768–79.
20. Yadav SS, Li J, Stockert JA, O'Conor J, Herzog B, Elaiho C, Galsky MD, Tewari AK, Yadav KK. Combination effect of therapies targeting the PI3K- and AR-signaling pathways in prostate cancer. Oncotarget 2016;7:76181–96.
21. Dai B, Kong YY, Ye DW, Ma CG, Zhou X, Yao XD. Activation of the mammalian target of rapamycin signaling pathway in prostate cancer and its association with patient clinicopathological characteristics. BJU Int. 2009;104:1009–16.
22. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metast Rev. 2009;28:15–33.
23. Yoo YA, Kang MH, Kim JS, Sang CO. Invasion in BMP2-induced EMT is mediated through down-regulation of PI-3 kinase/Akt pathway. Cancer Res. 2008;68:3538S.
24. Grille SJ, Bellacosa A, Upson J, Kleinszanto AJ, Roy FV, Leekwon W, Donowitz M, Tsichlis PN, Larue L. The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res. 2003;63:2172–8.