Original Article

Central Asian Journal of Medical Sciences
CAJMS

Cent Asian J Med Sci. 2025 Mar;11(4):27-33 https://doi.org/10.24079/CAJMS.2025.01.004

Association between prostate cancer development and TARBP1

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Submitted date: Feb 2, 2025
Accepted date: Mar 27, 2025

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Objective: Prostate cancer is the most frequent cancer among elderly men worldwide and second cause of cancer-related deaths in man. Prostate cancer characterized a clinically heterogeneous and genetic alterations in cells play a pivotal role in carcinogenesis. A detailed understanding of the genetic alterations in cancer cells are important for developing new therapeutic medicine. Transactivation response RNA-binding protein1 (TARBP1) encodes a protein with RNA-binding, tRNA methyltransferase activity, and RNA interference regulation functions, but its association with prostate cancer remains unclear. The aim of this study was to investigate clinical significance of TARBP1 in prostate cancer. **Method:** Relative messenger ribonucleic acid (mRNA) and protein expression of TARBP1 in prostate cancer cell and tumor tissue was determined by qRT-PCR and Western blot analysis. LNCaP cells with lentivirus vector to silence the expression of TARBP1 were injected into nude mice. After 24 days, the mice were killed and tumor tissues were excised. The size of tumors was measured using a ruler and body weight of mice were weighed using a scale. **Results:** Through qRT-PCR and Western blot analysis, we validated that the expression levels of TARBP1 was decreased in TARBP1-knockdown prostate cancer cell compared with cells transfected with negative control LV-shNC RNA. The tumor growths were significant smaller in the group B (LNCaP-LV-shTARBP1) compared with group A (LNCaP-LV-shNC). Knocking down TARBP1 significantly suppressed the tumor progression of BALB/C nude xenograft. Moreover, body weight of mice in LNCaP-LV-shNC group increased significant slowly compared with LNCaP-LV-shTARBP1 group. The result of western blot analysis for TARBP1 in tumor tissues from xenograft nude mice bearing orthotopic LNCaP tumors shows that the expression of TARBP1 was also significantly decreased in the LNCaP-LV-shTARBP1 group compared with LNCaP-LV-shNC group ($P < 0.05$). **Conclusion:** We revealed that the TARBP1 highly expressed in prostate cancer and its role in promoting tumor growth. TARBP1 could serve as a potential therapeutic target for prostate cancer treatment and it is also a biomarker for tumor diagnosis and prognosis.

Keywords: TARBP1, Prostate cancer, LNCaP, LV-shTARBP1.

Introduction

Prostate cancer is the most frequent cancer among elderly men worldwide and second cause

of cancer-related deaths in man.[1,2] The complexity of prostate cancer pathogeny, the low rate of early-stage diagnosis and the treatment is still not completely resolved. Despite extensive progress in clinical and experimental oncology studies are done, the prognosis of the majority of prostate cancer patients is still poor. Prostate cancer characterized a clinically heterogeneous and genetic analysis revealed that pathogenic alterations in cells play a pivotal role in carcinogenesis. Targeted genomic analysis of prostate cancer could offer opportunity for improving treatment rate of cancer cases.[2-4] Therefore, it is important to discover novel molecular biomarkers related to prostate cancer as therapeutic targets for the diagnosis and treatment of prostate cancer.

Transactivation response RNA-binding protein1 (TARBP1) encodes a protein with RNA-binding, tRNA methyltransferase activity, and RNA interference regulation functions.[5,6] Moreover, TARBP1 is mainly localized in the nucleus, may have a significant function in modulating transcription.[7] TARBP1 is considered a novel oncogene through mediating tRNA modification and promotes the tumorigenesis.[8] Some studies have indicated that TARBP1 expression may be altered in certain cancers, potentially contributing to tumor progression or metastasis, although these findings are not as well-established as for other RNA-binding proteins.[9-11] But its association with prostate cancer remains unclear.

While the role of TARBP1 has been explored in other cancers, such as hepatocellular carcinoma (HCC) and non-small cell lung cancer (NSCLC), its association with prostate cancer has not been thoroughly investigated. Limited studies have suggested that alterations in RNA-binding proteins, including TARBP1, may influence tumor progression in prostate cancer. Previous research has demonstrated that RNA-binding proteins can impact prostate cancer development through mechanisms such as RNA interference and regulation of gene expression. However, direct evidence of TARBP1 expression and its functional role in prostate cancer tissues or cells remains scarce. This study aims to fill this knowledge gap by investigating the expression and impact of TARBP1 in prostate cancer.

Materials and Methods

Cell Culture and Transfection

Human prostate cancer LNCaP cells were purchased from ATCC (Manassas, VA, USA) and cultured in RPMI-1640 medium containing penicillin (100 units/ml), streptomycin (100 μg/ml) and supplemented with 10% fetal bovine serum in an incubator at 37^{0}C and 5% CO^{0}C. When the cell density reached 90%, the cells were washed with PBS. The cells were digested with trypsin, rounded and terminated by adding medium. Cells were collected by centrifugation, counted and inoculated for culture as needed. For cell counting, cells were resuspended after centrifugation, and 10μL of the cell solution was mixed with PBS and trypan blue. LNCaP cells were infected with lentivirus vector (GenePharma, Shanghai, China) carrying to silence the expression of TARBP1 following the manufacturer's instructions. The cells were divided into LNCaP-LV-shNC (infected with LV-shNC, a negative control shRNA) and LNCaP-LV-shTARBP1(infected with LV-shTARBP1).

Real-Time PCR

Total ribonucleic acid (RNA) from LNCaP cells was isolated using TRIpure Total RNA Extraction Reagent (Sigma-Aldrich, Shanghai, China) and transcribed using QuantiTect Reverse Transcription Kit (Qiagen, Venlo, The Netherland) according to the manufacturer's instructions. Quantitative real-time PCR analysis was performed on a LightCycler 350S Real-Time PCR system (Roche Diagnostics, Mannheim, Germany) using g QuantiTect Primer Assay and QuantiTect SYBR Green PCR (Qiagen) according to the manufacturer's instructions. Polymerase chain reaction using the following primers: Forward, 5′- CAGGGAATGCCAAGAAG-3′; and reverse, 5′- GGCGTGGAAGGATGTAA-3′.

Western Blot Analysis

LNCaP cells were lysed using the PMSF-RO according to the manufacturer's instructions (Roche). After centrifugation at 12000 rpm for 10min, the supernatant was removed and the total protein extract stored at -80^{0}C. Protein concentration was determined with a protein assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were separated by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and electrophoretically transferred to polyvinylidene difluoride membrane (Thermo Fisher Scientific, Waltham, MA, USA). The primary antibody of TARBP1 (Mybiosource, San Diego, California, USA) was incubated in PVDF membranes overnight at 4^{0}C. The primary antibody was rinsed with TBST, and the secondary antibody (HRP-conjugated goat anti-rabbit IgG, wanleibio, Shenyang, China) was diluted and incubated at 37^{0}C for 45min. The secondary antibody was rinsed six times by TBST, followed by luminescence with ECL reagent (wanleibio, Shenyang, China) and exposure in a dark room.

Xenograft Tumor Model in Nude Mice

Male BALB/c nude mice (5 weeks old) were purchased from Crea Japan (Tokyo, Japan). All animal procedures were approved by the local animal care and uses ethical committee. Mice were maintained under specific pathogen-free conditions. BALB/C nude mice were randomly divided into two groups: group A (LNCaP-LV-shNC tumor tissue group) and group B (LNCaP-LV-shTARBP1 tumor tissue group). LNCaP cells infected with LV-shNC or LV-shTARBP1 (2×10^6 cells) were subcutaneously injected into the armpit of each nude mouse. After 24 days, the nude mice were sacrificed, and tumor tissues were excised. The size of tumors was measured using a ruler and body weight of mice were weighed using a scale.

Western Blot Analysis for Tumor Tissues

Tissues resected from 4-week-old tumors grown in the nude mice were washed with cold phosphate-buffered saline, minced, and dissolved in cell lysis buffer (Cell Signaling Technologies, Danvers, MA, USA) with protease inhibitors. The total protein concentration was examined using Quick Start Bradford reagent (Bio-Rad, Hercules, CA, USA). Whole-cell extracts (50 μg) were separated on 10–15% sodium dodecyl sulphate–polyacrylamide gel electrophoresis gels and moved to the membranes (Millipore, Billerica, MA, USA). These membranes were then incubated with primary antibodies, which were determined using a Horse Radish Peroxidase (HRP)-conjugated Goat Anti-Rabbit IgG (ThermoFisher, Waltham, Massachusetts, USA). Bands were detected by West Glow FEMTO Chemiluminescent Substrate (BIOMAX, Seoul, South Korea). The primary antibodies targeted TARBP1 (Cell Signaling Technologies, Massachusetts, USA) and β-actin (wanleibio, Shenyang, China) as a loading control.

Statistical Analysis

A two-tailed Student's t-test was employed for statistical analyses of the data. Significant results were determined at a $P< 0.05$.

Results

TARBP1 Expression in Prostate Cancer Cells

We examined messenger ribonucleic acid (mRNA) expression and protein expression of endogenous TARBP1 in the prostate cancer cell LNCaP using quantitative real-time polymerase chain reaction and Western blot analysis respectively. We found that TARBP1 mRNA and protein has been shown to be expressed in LNCaP cells.

To assess whether TARBP1 has a role in growth of prostate cancer, we transfected a lentivirus vector LV-shTARBP1 and a control non-silencing LV-shNC, a negative control shRNA into LNCaP cells to suppress expression of endogenous TARBP1. Through qRT-PCR and Western blot analysis, we validated that the expression levels of TARBP1 was decreased in TARBP1-knockdown prostate cancer cell compared with cells transfected with negative control LV-shNC RNA (Fig.1A, B).

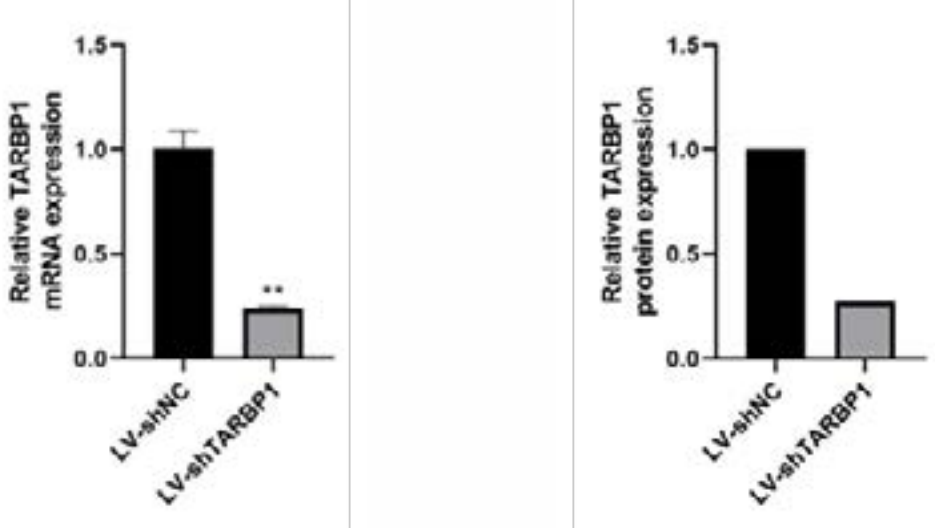

Figure 1. (A) The relative mRNA expressions of TARBP1 in the LNCaP determined by qRT-PCR. LV-shTARBP1 and LV-shNC infected LNCaP cells respectively. (B) Expression of TARBP1 protein in the LNCaP were examined by Western blot analysis. LV-shTARBP1 and LV-shNC infected LNCaP cells respectively.

Results of Animal Experiment

We analyzed the role of TARBP1 in tumor progression in vivo condition. In this experiment, injections were performed to 12 nude mice and six mice were included in each group (A and B). Tumors were resected from mice 24 days after orthotopic inoculation of LV-shNC or LV-shTARBP1 cancer cells. The tumor growths measured using a ruler were significant smaller in the group B (LNCaP-LV-shTARBP1) compared with group A (LNCaP-LV-shNC). Knocking down TARBP1 significantly suppressed the tumor progression of BALB/C nude xenograft in vivo. Furthermore, body weight of mice in LV-shNC group increased significant slowly compared with LNCaP-LV-shTARBP1 (Fig. 2A, B).

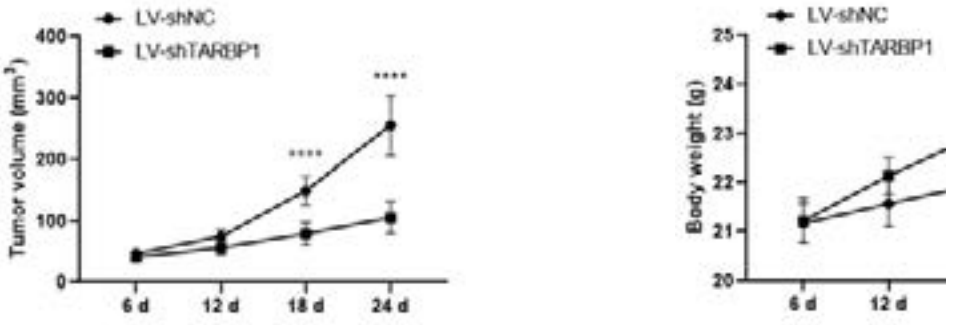

Figure 2. (A) Comparison of tumor sizes between the group B (LNCaP-LV-shTARBP1) and group A (LNCaP-LV-shNC). Knocking down TARBP1 significantly suppressed the tumor progression of BALB/C nude xenograft. (B) Comparison of body weight of the nude mice between the group B (LNCaP-LV-shTARBP1) and group A (LNCaP-LV-shNC). Body weight of mice in group B increased slowly compared with group A. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$).

Moreover, the result of western blot analysis for TARBP1 in tumor tissues from xenograft nude mice bearing orthotopic LNCaP tumors shows that the expression of TARBP1 was also significantly decreased in the LNCaP-LV-shTARBP1 group compared with LNCaP-LV-shNC group ($P < 0.05$). (Fig 3).a

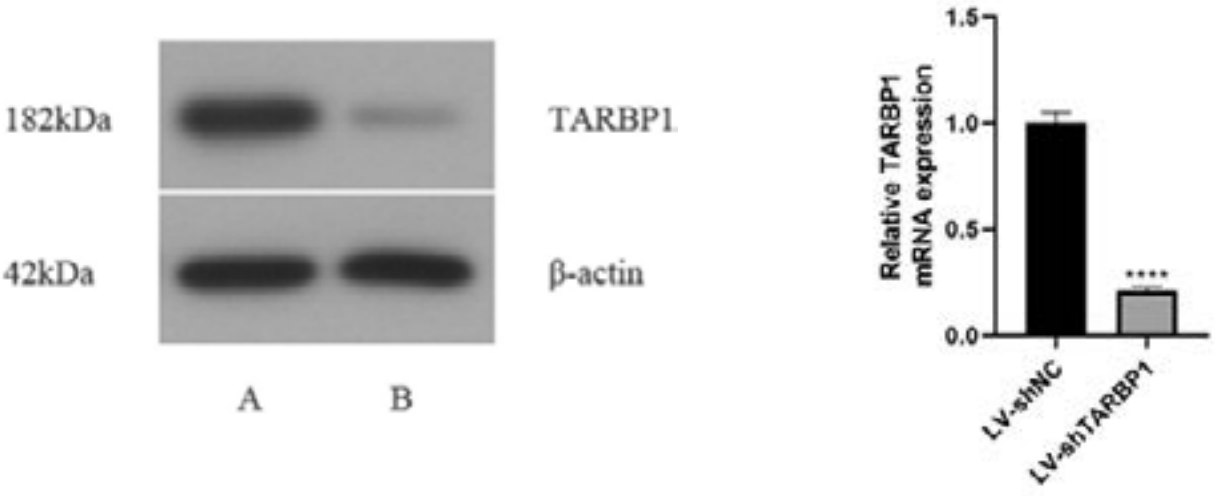

Figure 3. Western blot results of TARBP1 in tumor tissues showed that the protein expression levels of TARBP1 in tumor tissues of the LV-shTARBP1 group were significantly lower than the LV-shNC group.

The results from in vitro cell growth and in vivo xenograft nude mice demonstrated that inhibition of TARBP1 expression could down-regulate the growth of cancer and TARBP1 is critical for prostate cancer growth.

Discussion

Prostate cancer ranks as the second most commonly diagnosed cancer in men and stands as the fifth leading cause of cancer-related death globally. In its early stages, it often presents without noticeable symptoms and typically progresses slowly.[12] Prostate cancer incidence and mortality rates worldwide associate with increasing, with the mean age at diagnosis approximately 65 years. Prostate cancer cases are initially suspected due to elevated serum concentrations of prostate-specific antigen (PSA). PSA is a glycoprotein produced by epithelial cells of the prostate gland under both normal and pathological conditions. Nonetheless, as elevated PSA levels may also be observed in benign prostatic hyperplasia, prostatitis, and other non-malignant conditions, histopathological confirmation via prostate biopsy remains the gold standard for confirming the diagnosis.

The etiology of prostate cancer remains incompletely understood and continues to be the focus of extensive research. The well-established risk factors for prostate cancer include advanced age, ethnicity, genetic predisposition, and a positive family history, high intake animal fats and red meat, along with reduced consumption of fruits, vegetables, vitamins, and coffee as well as obesity, sedentary lifestyle, chronic inflammation, hyperglycemia, infectious agents, and environmental exposures to toxic chemicals or ionizing radiation.[13-18]

Prostate cancer exhibits a significant genetic predisposition, with inherited factors contributing to as much as 60% of the overall risk. This hereditary risk is attributable to both common genetic variants and pathogenic mutations within genes associated with moderate and high risk for the disease.[19,20]

Numerous germline pathogenic variants and single nucleotide polymorphisms have been identified as being associated with an increased risk of prostate cancer. Germline genetic testing may be recommended to evaluate prostate cancer risk and to guide therapeutic decisions in individuals diagnosed with the disease. Molecular biology research is still being conducted extensively to improve the diagnosis and treatment of prostate cancer.

Transactivation response RNA-binding protein 1 (TARBP1) is a protein involved in RNA binding and regulation of gene expression, particularly in post-transcriptional gene silencing mechanisms. It belongs to the family of double-stranded RNA-binding proteins and plays a role in RNA interference (RNAi) pathways. Although TARBP1 is less well-studied than its homolog TARBP2 (also known as TRBP), it may still participate in RNA interference pathways and interact with components of the RNA-induced silencing complex.[21,22]

TARBP1 has been found to be upregulated in hepatocellular carcinoma (HCC), with its expression levels showing a significant correlation with both pathological grade and clinical stage of the disease. Additionally, TARBP1 is overexpressed in non-small cell lung cancer (NSCLC), and its expression is significantly associated with pathological grade, clinical stage, and histological subtype. Both these studies suggest that TARBP1 may be an independent prognostic biomarker in patients with HCC or NSCLC.[9,10]

In this study, we investigated the expression of TARBP1 in PCa cell line and in tumor tissues from xenograft nude mice bearing orthotopic LNCaP tumors. Our results showed that the expression of TARBP1 was significantly decreased in the knock-down TARBP1 cancer cell line and tumor compared with control cell and tumor tissue. Moreover, tumor growth and a lose of body weight were slowly compared with control mice with PCa.

Expression of TARBP1 similar our results have also been reported in some cancers such as hepatocellular carcinoma, non-small cell lung cancer and epithelial skin cancers. [9,11,23]

The observed differences in TARBP1 expression between tumor tissues and knock-down TARBP1 tumor tissue showed its role in involving tumorgenesis, suggesting its involvement in cancer initiation, progression, and possibly therapeutic targeting. Future studies should further explore the precision interaction between TARBP1 and other signaling pathways.

Conclusion

In this study, we revealed that the TARBP1 highly expressed in prostate cancer and its role in promoting tumor growth. In summary, these results showed that TARBP1 could serve as a potential therapeutic target for prostate cancer treatment and it is also a biomarker for tumor diagnosis and prognosis.

Conflict of Interest

There were no conflicts and interest.

Author Contribution

L.W, H.L and Sh.A conceived the project and designed the research. L.W, H.L, B.Ts, G.B, and Sh.A contributed to study conception, planning experiments and technical support. L.W, H.L and Sh.A conducted data analysis and data interpre-tation. L.W, H.L and Sh.A participated in the result discussion and technical support. L.W, H.L and Sh.A wrote the manuscript. All authors read and approved the final.

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