Original Article

Down-regulation of LTBP2 suppresses the proliferation, migration and invasion in human prostate cancer cells

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Abstract: Latent transforming growth factor-beta binding protein 2 (LTBP2) is a member of the LTBPs family. It is dysregulated in many types of tumors and associated with proliferation, migration, and invasion. However, the potential involvement of LTBP2 in prostate cancer has not been fully explored. Therefore, in the present study, we analyzed the expression of LTBP2 in prostate cancer and explore its role in prostate cancer carcinogenesis. Our results demonstrated that the expression levels of LTBP2 at both mRNA and protein were significantly up-regulated in human prostate cancer tissues and cell lines. Moreover, functional studies established that down-regulation of LTBP2 suppressed the proliferation, migration and invasion of prostate cancer cells. Mechanistic investigation showed that down-regulation of LTBP2 significantly inhibited the levels of phospho-PI3K and phospho-Akt in LNCaP cells. In conclusion, the experimental results revealed that LTBP2 as an oncogene in prostate cancer, and knockdown of LTBP2 can efficiently inhibit prostate cancer cell proliferation, migration and invasion, at least partially through suppressing the PI3K/Akt signaling pathway. Thus, LTBP2 may be a potential therapeutic target for the treatment of prostate cancer.

Keywords: Prostate cancer, LTBP2, proliferation, invasion

Introduction

Prostate cancer is a malignant cancer of the prostate gland, and the most prevalent cancer type in the male population [1]. The incidence of prostate cancer has increased worldwide in recent years. Although various treatments for prostate cancer, such as chemotherapy, radical prostatectomy and hormone therapy, have been improved recently, the 5-year survival rate of patients with prostate cancer remains poor because most prostate cancer patients have metastasis at the time of diagnosis [2-4]. Thus, identification of molecular mechanisms underlying prostate cancer progression is crucial to develop new therapy options for prostate cancer patients.

Latent transforming growth factor-beta binding proteins (LTBPs) are multi-domain extracellular matrix (ECM) proteins with molecular masses of 150-220 kDa which share structural homology with fibrillins [5, 6]. LTBPs play vital roles in microfibril organization, elastic fiber assembly and tissue homeostasis [7-9]. LTBP2 is a member of the LTBPs family. It has been reported that LTBP2 promotes the assembly of microfibril bundles in cultured cells and organ cultured eyeballs [10]. In addition, previous studies have shown that LTBP2 was dysregulated in many types of cancers [11-13]. In cervical adenocarcinoma, LTBP2 was highly expressed in cervical adenocarcinoma tissues, and LTBP2 may promote the development of cervical adenocarcinoma and serve as a molecular marker of poor prognosis in cervical adenocarcinoma [12]. However, the potential involvement of LTBP2 in prostate cancer has not been fully explored. Therefore, in the present study, we analyzed the expression of LTBP2 in prostate cancer and explore its role in prostate cancer carcinogenesis.

Materials and methods

Tissue specimens

A total of 8 pairs of prostate cancer tissues and their matched adjacent normal prostate tis-
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Sues were collected from patients who had undergone radical prostatectomy between May 2015 and July 2015 at the Department of Urology, Huaihe Hospital of Henan University (Kaifeng, China). The resected tissues were immediately frozen in liquid nitrogen and stored at -80°C before use. This study was approved by the Ethnic Committee of Huaihe Hospital of Henan University, and written informed consent was obtained from each patient.

Cell culture

Human prostate cancer cell lines (LNCaP, DU145 and PC-3) and the immortalized normal prostate epithelial cell line (RWPE-1) were purchased from American Type Culture Collection (ATCC, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and 100 µg/ml penicillin, and 100 µg/ml streptomycin (Sigma, St. Louis, MO, USA). All cell lines were grown at 37°C in a humidified 5% CO₂ incubator.

Short hairpin RNA and cell transfection

The specific short hairpin RNA targeting LTBP2 (sh-LTBP2) and its negative control (sh-NC) were purchased from GenePharma (Shanghai, China). Prostate cancer cells at a density of 5×10⁴ cells/well were seeded into 96-well plates, and then transfected with sh-LTBP2 or sh-NC using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), respectively, according to the manufacturer’s instructions.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from prostate cancer tissues and cells using the RNeasy Micro Kit (Qiagen, Valencia CA) and transcribed to complementary DNA (cDNA) using the EasyScript First-Strand cDNA Synthesis SuperMix kit (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed using an ABI Prism 7500 System (Applied Biosystems) with the SYBR Green Supermix (Invitrogen, Carlsbad, CA, USA). PCR amplification was performed using the following primers: LTBP2, 5'-TTACAACAGAGACTCACT-3' (sense) and 5'-ACGACCAAGAGACAGAT-3' (antisense); and β-actin, 5'-TTAGTGGGTTAACACCTTTC-3' (sense) and 5'-ACCTTCCAGTTTTT-3' (antisense). The relative mRNA expression level of LTBP2 was calculated using the 2^ΔΔCt method [14].

Western blot

Total protein extracts were prepared using RIPA lysis buffer (Cell Signaling Technology, Darvers, MA, USA) according to the operating instructions. The equal amount of protein samples was separated on 12% SDS-PAGE gels and transferred to nitrocellulose membranes (Millipore Corp, Billerica, MA, USA). The membranes were blocked 5% skim milk in Tris-buffered saline with 0.1% Tween 20 (pH 7.6, TBST) for 1 h at room temperature, and then incubated with the following primary antibodies: anti-LTBP2, anti-p-PI3K, anti-PI3K, anti-p-Akt, anti-Akt and anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After being washed with TBST, membranes were incubated with secondary antibodies coupled to horseradish peroxidase at room temperature for 1 h. The target proteins were visualized using an ECL chemiluminescent detection system (Pierce, Rockford, IL, USA).

Cell proliferation assay

Cell proliferation was evaluated by the 3-(4,5-methylthiozol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the transfected cells (1×10⁴ cells/well) were seeded into 96-well plates and cultured at 24 h intervals for 4 days. Then 20 µl of MTT (5 mg/ml; Sigma) was added to each well and incubation continued at 37°C for additional 4 h. The culture media was removed and 200 µl DMSO (Sigma) was added to each well to completely dissolve the crystals. Absorbance at 490 nm was detected using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

Cell invasion assay

Cell migration assay was performed using Transwell® cell culture chambers (Corning Costar Corp., Cambridge, MA, USA). The lower compartment was filled with 600 µl of DMEM containing 10% FBS. The transfected cells (1×10⁵ cells/well) were resuspended in 0.1 ml of DMEM and placed in the upper part of the Transwell plate. After 24 h, the cells on the upper surface of the filters were removed with a cotton swab, and cells that had migrated to the lower surface were fixed with 100% methanol for 10 min and stained with 0.5% crystal violet solution for 30 min. The number of cells per five high power fields was counted using a microscope (Olympus, Tokyo, Japan). For cells invasion assays, the transfected cells (1×10⁶ cells/
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Statistical analysis

All statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as means ± standard deviation (SD). Statistical analysis involved using the Student’s t test for comparison of 2 groups or 1-way ANOVA for multiple comparisons. A p-value of 0.05 or less was considered significant.

Results

LTBP2 is frequently up-regulated in human prostate cancer tissues and cell lines

The mRNA expression levels of LTBP2 were initially measured in 8 pairs of prostate cancer tissues (PC) and their corresponding normal tissues (Normal) was evaluated using qRT-PCR. B. The protein expression of LTBP2 was evaluated by western blot. *P<0.05 vs. the Normal group.

C. The mRNA expression of LTBP2 in prostate cancer cell lines (LNCaP, DU-145 and PC-3) and the immortalized normal prostate epithelial cell line (RWPE-1) was measured by qRT-PCR. D. The protein expression of LTBP2 in prostate cancer cell lines was determined using western blot. Results were obtained from three independent experiments. *P<0.05 vs. the RWPE-1 group.

Figure 1. LTBP2 is frequently up-regulated in human prostate cancer tissues and cell lines. A. The mRNA expression of LTBP2 in 8 paired prostate cancer tissues (PC) and their corresponding normal tissues (Normal) was evaluated using qRT-PCR. B. The protein expression of LTBP2 was evaluated by western blot. *P<0.05 vs. the Normal group. C. The mRNA expression of LTBP2 in prostate cancer cell lines (LNCaP, DU-145 and PC-3) and the immortalized normal prostate epithelial cell line (RWPE-1) was measured by qRT-PCR. D. The protein expression of LTBP2 in prostate cancer cell lines was determined using western blot. Results were obtained from three independent experiments. *P<0.05 vs. the RWPE-1 group.
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Down-regulation of LTBP2 suppresses the proliferation of prostate cancer cells. LNCaP and DU-145 cells were transfected with sh-LTBP2 or sh-NC for 48 h, respectively. A. The transfection efficiency in LNCaP cells was confirmed by western blot assay. B. The transfection efficiency in DU-145 cells was confirmed by western blot assay. C. Cell proliferation was evaluated using the MTT assay in LNCaP cells. D. Cell proliferation was evaluated using the MTT assay in DU-145 cells. These data are from three independent experiments and presented as the mean ± SD. *P<0.05.

Figure 2. Down-regulation of LTBP2 suppresses the proliferation of prostate cancer cells. LNCaP and DU-145 cells were transfected with sh-LTBP2 or sh-NC for 48 h, respectively. A. The transfection efficiency in LNCaP cells was confirmed by western blot assay. B. The transfection efficiency in DU-145 cells was confirmed by western blot assay. C. Cell proliferation was evaluated using the MTT assay in LNCaP cells. D. Cell proliferation was evaluated using the MTT assay in DU-145 cells. These data are from three independent experiments and presented as the mean ± SD. *P<0.05.

samples and their corresponding non-tumor tissues by qRT-PCR. As shown in Figure 1A, the relative expression levels of LTBP2 were obviously up-regulated in prostate cancer tissues compared with the normal tissues. Moreover, western blot further demonstrated that the protein expression of LTBP2 was greatly increased in human prostate cancer tissues (Figure 1B). Similarly, up-regulation of LTBP2 was also observed in all tested prostate cancer cell lines compared with RWPE-1 cells (Figure 1C and 1D). Down-regulation of LTBP2 suppresses the proliferation of prostate cancer cells

To knockdown LTBP2 and examine loss-of-function, LNCaP and DU-145 cell lines, which have high endogenous LTBP2, were used in vitro following shRNA gene knockdown methods. Transfection with sh-LTBP2 significantly reduced the protein expression of LTBP2 in LNCaP and DU-145 cells, respectively (Figure 2A and 2B). Furthermore, we examined the effects of LTBP2 knockdown on prostate cancer cell pro-
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Down-regulation of LTBP2 suppresses the proliferation and invasion of prostate cancer cells through regulation of the PI3K/Akt signaling pathway

PI3K/AKT pathway plays a critical role in cancer progression and invasion. Thus, we examined the effect of LTBP2 knockdown on the activation of PI3K/Akt pathway in LNCaP cells. The results of Western blot analysis indicated that down-regulation of LTBP2 significantly inhibited the levels of phospho-PI3K and phospho-Akt in LNCaP cells, as compared with the sh-NC group (Figure 4A). Furthermore, we observed that the Akt activator (SC79) markedly reversed the LTBP2 knock-down effects on cell proliferation (Figure 4B) and invasion (Figure 4C).

Discussion

In the present study, our results showed high expression levels of LTBP2 in prostate cancer.
Down-regulation of LTBP2 suppressed the proliferation, migration and invasion of prostate cancer cells. Furthermore, down-regulation of LTBP2 significantly inhibited the levels of phospho-PI3K and phospho-Akt in LNCaP cells.

The dysregulation of LTBP2 is associated with tumorigenesis. Han et al. reported that both LTBP2 mRNA and protein levels were significantly higher in head and neck squamous cell carcinoma (HNSCC) tissues than in adjacent normal tissues, and high LTBP2 expression was related with clinical stage and lymph node metastasis [15]. Another study showed that LTBP2 was also highly expressed in cervical adenocarcinoma tissues, and knockdown of LTBP2 sharply suppressed the proliferation and migration of HeLa cells [12]. These findings reveal an oncogenic role for LTBP2. However, a tumor suppressive role of LTBP2 has also been reported. A study by Chen et al. confirmed that LTBP2 expression was significantly decreased...
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or lost in nasopharyngeal carcinoma biopsies and cell lines, and LTBP2 inhibited colony formation, anchorage-independent growth, migration in vitro and tumorigenicity in vivo nude mouse [16]. LTBP-2 expression was downregulated in human esophageal squamous cell carcinoma (ESCC) cell lines and tumor tissues [13]. These dual roles of LTBP2 could attribute to organ-specific actions and different cellular contexts. In this study, we showed that the expression levels of LTBP2 at both mRNA and protein were significantly up-regulated in human prostate cancer tissues and cell lines, and down-regulation of LTBP2 obviously suppressed the proliferation, migration and invasion of prostate cancer cells. These results suggest that LTBP2 may function as an oncogene involved in the development and progression of prostate cancer.

A growing body of evidence indicates that aberrant activation of the PI3K/Akt signaling pathway plays an important role in the development and progression of cancer including prostate cancer [17-19]. The PI3K/Akt pathway is frequently hyper-activated in prostate cancer [20, 21]. Akt (protein kinase B) is a major signaling molecule downstream of PI3K pathway. Akt activation regulates a variety of cellular functions, including proliferation, cell-cycle progression, invasion and apoptosis [22, 23]. Thus, it is reasonable to assume that inactivation of PI3K/Akt signaling pathway cascade is a potential good therapeutic approach to prostate cancer [24-26]. For example, the pan-AKT inhibitor (AZD5363) significantly delayed tumor growth and improved overall survival and progression-free survival in PTEN-deficient prostate cancer mice [27]. A recent study indicated that LTBP2 is closely related to the PI3K/Akt signaling pathway [12]. In the current study, we found that down-regulation of LTBP2 significantly inhibited the levels of phospho-PI3K and phospho-Akt in LNCaP cells. Furthermore, the Akt activator (SC79) markedly reversed the LTBP2 knock-down effects on cell proliferation and invasion. These results support the notion that down-regulation of LTBP2 inhibited the proliferation, migration and invasion of prostate cancer cells through the inactivation of PI3K/Akt signaling pathway.

In conclusion, the experimental results revealed that LTBP2 as an oncogene in prostate cancer, and knockdown of LTBP2 can efficiently inhibit prostate cancer cell proliferation, migration and invasion, at least partially through suppressing the PI3K/Akt signaling pathway. Thus, LTBP2 may be a potential therapeutic target for the treatment of prostate cancer.

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Disclosure of conflict of interest

None.

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