PTCSC1 Promotes Bladder Cancer Progression by AKT Pathway

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Research

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Abstract

In bladder cancer the expression of PTCSC1 is elevated, but how PTCSC1 influences tumor progression are still unclear. We found that in human bladder cancer PTCSC1 is upregulated. The study showed that the expression level of PTCSC1 is higher in bladder cancer tissue than that of PTCSC1 in para-carcinoma tissue. Furthermore, PTCSC1 promoted bladder cancer cell migration and invasion, and decreased expression of PTCSC1 inhibited the expression of matrix metalloprotease MMP2 and MMP9, and increased the expression level of E-cadherin. PTCSC1 promoted tumor growth in a mouse model of human bladder cancer. Additionally, PTCSC1 shRNA caused a significant decrease in p-AKT expression in T24 cells and BIU-87 cells, but the overexpression of PTCSC1 got an opposite result. All the results showed that PTCSC1 can influence bladder cancer cell migration and invasion ability by the AKT pathways. PTCSC1 may be an effective therapeutic target in bladder cancer.

Background

The human genome is actively transcribed which was revealed by advances in functional genomics; but, most of the transcripts are non-coding RNA including long non-coding RNAs [1]. Unlike microRNAs, lncRNAs are larger than 200bp in length. Increasing evidence suggests that lncRNAs could be the important play of different cellular processes. Many study confirmed that lncRNAs may have an effect on gene expression by various mechanisms. The phosphoinositide-3-kinase (PI3K)–protein kinase B/AKT (PI3K-PKB/AKT) pathway responds to growth factors, cytokines and other cellular stimuli, which play the key role in cell signalling. AKT activated transfers signaling and regulates well-known MDM2/p53, Foxo and NF- κ B and other downstream targets. As a result, AKT plays a pivotal effect in cell survival, proliferation, growth, angiogenesis, metabolism and cell migration and other diverse cellular processes [2]. Lots of research indicates that in many cancers AKT is often dysregulated [3]. In particular, it is not known whether lncRNAs are involved in the regulation of AKT activity in bladder cancer. Although the good deal of human long non-coding RNAs (lncRNAs) were studied nowadays, their physiological functions for the majority of lncRNAs is unknown. Koirala P et al found that lncRNA PTCSC1 is a positive regulator for AKT. LncRNA PTCSC1 and DHX9 upregulation in breast cancer contribute to breast tumour progression, which were significant players in the AKT pathway [4]. Our study showed PTCSC1 increased bladder cancer migration and invasion ability. Decreased expression of PTCSC1 inhibited the expression of matrix metalloprotease MMP2 and MMP9 and increased the E-cadherin expression. PTCSC1 promoted tumor growth in a mouse model of human bladder cancer. Additionally, PTCSC1 shRNA caused a significant decrease in p-AKT expression in T24 cells and BIU-87 cells. In conclusion, our study shows PTCSC1 as key players in bladder tumour progression.

Materials And Methods

Sample collection

Fresh bladder tissues (n = 20) and peri-cancerous tissues (n = 20) were gotten from patients who were pathologically diagnosed as bladder cancer and underwent surgical resection at the Hospital from September 2015 to September 2020. The specimens were placed in cryogenic vials containing inactivated RNase within 15 min and preserved in liquid nitrogen. Patients did not have other diseases. The Ethics Committee of the Hospital approved the study, and the informed consent were signed by patients.

Cell Culture And Transfection

T24 cells and BIU-87 cells were bought from the American Type Culture Collection (ATCC; Manassas, VA, USA), and the cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) which contains 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), L penicillin and streptomycin. And L penicillin and streptomycin concentration were both 100 U/mL. Cells that were logarithmically grown and in good condition were inoculated into 6 well plates before transfection with 1×10^5 cells/well. Cell abundance during transfection was about 75%. Transfection was performed by Lipo fectamine TM 2000 for 48 h.

Construction Of Stable Ptcsc1 Knockdown Cell Lines

Short hairpin RNAs (shRNAs) targeting PTCSC1 were inserted into pLL3.7 vectors. And the shPTCSC1 sequences was in supplementary table1. The recombinant pLL3.7 vectors and pRSV-Rev, pMDLg/pRRE, and pMD2.G packaging vectors were cotransfected in the 293T cells. After 48 and 72 h supernate of 293T cells was obtained respectively. The supernate is lentiviruses with infection. The supernate of 293T cells was filtered with 0.45 Impoly(vinylidene) difluoride (PVDF) filters. Viruses from supernate were obtained by ultracentrifugation (40 min at 80 000 g). The virus precipitate was stored in RPMI 1640 at 80°C. Infectious titers were determined by counting the number of GFP expressing colonies. The virus and polybrene(10 g/mL) (Sigma-Aldrich) were added to the T24 cells and BIU-87 cells respectively.

After 24 h, the stale supernatant was thrown away and fresh culture solution was added in. Cells infected with virus were identified by western blot analysis at 96 h. Then flow cytometry (BD FAS Aria III Cell Sorter, Beckman Coulter, Miami Lakes, FL, USA) was used to identify and collect cells with GFP.

Verification Of Ptcsc1 Transcript

5'RACE using a previous described procedure, followed by PCR with primers AKO-polyT adaptor, AK0-adaptor and AK0-5Race-3.2 (Supplementary table1). For 3'RACE Poly adenosine by poly A polymerase (NEB) was added first and then reverse transcribed using AKO-polyT adaptor primer. PCR used primers AK0-3Race-5.1 and AK0-adaptor-5.1 (Supplementary table1). The resulting PCR product was cloned into pCR8 for DNA sequencing. The above procedures and sequences of primers were from references[4].

Construction Of Cell Lines Stably Overexpressing Ptcsc1

The full length PTCSC1 sequence was cloned into the Lentivirus expression vector pCDH-CMV-MCS-EF1-GFP (SBI) to generate pCDH-CMV-PTCSC1-EF1-GFP plasmid. The of primer sequences were showed in supplementary table1.

The pCDH-CMV-PTCSC1-EF1-GFP plasmid and pCMV-VSV-G Packaging Mix (Addgene) were co-transfected in 293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's guidelines. The pCDH-CMV-MCS-EF1-GFP (SBI) plasmid and pCMV-VSV-G Packaging Mix (Addgene) were co-transfected in 293T cells using Lipofectamine 2000 (Invitrogen). Infectious supernate were gathered at 48h and 72 h after transfection and lentiviruses were filtered through 0.45 µm polyvinylidene fluoride filters. Infectious supernate were concentrated by ultracentrifugation (1.5 h at 80,000 g). The virus containing pellet was dissolved in 1640 and stored at -80°C. T24 cells and BIU-87 cells were infected with different groups concentrated virus respectively. To obtain cell lines stably expressing PTCSC1, T24 cells and BIU-87 cells with GFP-expressing colonies were subsequently sorted respectively by Flow cytometry (BD FAS Aria III Cell Sorter, Beckman Coulter) by Green Fluorescence Protein (GFP).

Cancer cells of every group migration assay and invasion assay

Migration and invasion ability of T24 cells and BIU-87 cells were detected by transwell insert chambers with 8 µm porous membranes (Corning Inc., Corning, NY, USA).

Cancer cells of every group without FBS were in the upper chamber, and medium with 10% FBS was in the lower chamber. The every group cells were cultured for 24 h. The cancer cells on the upper surface of the upper chamber were erased using a cotton swab, and the upper chamber was washed with phosphate buffer. Furthermore, the cancer cells on the lower surface of the chamber were immersed in 4% paraformaldehyde .

Once the surface was not wet, the cancer cells were dyed with 1% crystal violet for about 3 min. The migrating cancer cells were those on the lower surface of the chamber. Migrating cancer cells were counted at x100 or x200 magnification in eight fields of view. Matrigel gel was diluted with serum-free cold cell medium DMEM (10mg/ml to 5 mg/ml). About 100ul of diluted matrigel gel were added on the upper chamber. The remaining steps of invasion assay were the simliar to migration assay.

Rna Extraction And Real Time Polymerase Chain Reaction (Rt-pcr)

Total RNA of cancer cells or tissue was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). And the total RNA was quantified using UV spectrophotometer.

D260 nm/D280 nm value was calculated for selecting qualified RNA samples, which were saved at -20°C for use. Extracted RNA was reversely transcribed into cDNA and amplified by Real Time quantitative Polymerase Chain Reaction (RT-qPCR) using SYBR Premix Ex Taq II kit (TaKaRa, Otsu, Shiga, Japan).

PCR reaction conditions were as follow: pre-denaturation at 95°C for 30 s, 95°C for 5 s, and 60°C for 31 s, for 40 cycles in total. The relative levels of every group RNA were analyzed quantitatively with the 2- $\Delta\Delta C_t$ method. The expression of GAPDH of every sample was choosed as an internal reference. The above experiments were repeated for three times.

Western Blot

The protein in total from cancer cells of every group was extracted using RIPA Lysis Buffer (RIPA; Beyotime, Shanghai, China). The protein were quantified by bicinchoninic acid (BCA) method (Pierce, Waltham, MA, USA) and loaded for electrophoresis. The sample protein were transferred on a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The PVDF was blocked in 5% skim milk for 2 hours, soaked with primary antibodies at 4°C for 12h and secondary antibodies for 2 h. Protein bands were exposed by enhanced chemiluminescence (ECL) and the relative expression of protein was measured by Image J Software (NIH, Bethesda, MD, USA).

Xenograft Animal Experiments

seven weeks old nude mice were purchased from Beijing HFK Bioscience (Beijing, China). The mice were divided into eight groups randomly (n = 6 per group). The every group cancer cells were injected subcutaneously into the right dorsal flanks of the mice at 5×10^6 cells every animal respectively. The xenograft sizes were measured every three days with a caliper. Tumor size was calculated in the light of the following way: $V \text{ (mm}^3\text{)} = \text{length} \times \text{width}^2/2$. The mice were put to death using CO₂ inhalation followed by cervical dislocation. The animal study were carried out according to the institutional guidelines for animal care and approved by the Institutional Animal Care and Use Committee of the Chinese Academy of Medical Sciences.

Statistical analysis

Statistical analyses were performed using SPSS software (version 13.0). The t-test was used for analyzing the differences between the two groups. A $p < 0.05$ indicated the significant difference.

Results

High expression of PTCSC1 in bladder cancer cells and bladder cancer patients

The cellular expression of PTCSC1 in bladder cancer cell was higher than that in bladder cells by the qRT-PCR assay (Fig. 1A). PTCSC1 expression in 20 bladder cancer patients was detected. Identically, bladder cancer tissues (n = 20) compared to adjacent non-tumor bladder cancer tissues had a higher level of PTCSC1 expression by the qRT-PCR assay (Fig. 1B). After transfection with a siRNA to decrease the expression of endogenous PTCSC1 the migration of bladder cancer cells was dramatically diminished (Fig. 1C).

PTCSC1 promotes migration and invasion in T24 cells

PTCSC1 were over expressed in T24 cells (Fig. 2A). ShRNA induced a corresponding down regulation in PTCSC1 (Fig. 2B). The cell migration and invasion ability that was influenced by knockdown and overexpression of PTCSC1 were detected using transwell assay. PTCSC1 overexpression in T24 cells increased migration and invasion ability compared with control group (Fig. 2C). PTCSC1 knocked down with shRNA in T24 cells reduced migration and invasion (Fig. 2D). We further examined the expression of migration and invasion related proteins in T24 cells. Western blotting assays demonstrated that the expression of MMP2 and MMP9 was increased, and the expression of E-cadherin was inhibited when the expression of PTCSC1 was overexpressed (Fig. 2E). Western blotting assays demonstrated that the expression of MMP2 and MMP9 was inhibited, and the expression of E-cadherin was increased when the expression of PTCSC1 was knocked down (Fig. 2F).

PTCSC1 promotes migration and invasion in BIU-87 cells

PTCSC1 were over expressed in BIU-87 cells (Fig. 3A). ShRNA induced a corresponding down regulation in PTCSC1 (Fig. 3B). The cancer cells migration and invasion ability were measured by transwell assay. PTCSC1 overexpression in BIU-87 cells increased migration and invasion ability compared with control group. PTCSC1 knocked down with shRNA in BIU-87 cells reduced migration and invasion (Fig. 3D). We further examined the expression of migration and invasion related proteins in BIU-87 cells. Western blotting assays demonstrated that the expression of MMP2 and MMP9 was increased, and the expression of E-cadherin was inhibited when the expression of PTCSC1 was overexpressed (Fig. 3E). Western blotting assays demonstrated that the expression of MMP2 and MMP9 was inhibited, and the expression of E-cadherin was increased when the expression of PTCSC1 was knocked down (Fig. 3F).

Ptcsc1 Promotes Bladder Cancer Growth In Vivo

To explore further, the function of PTCSC1 in tumor progression in vivo, T24 cells with stably overexpressed or knocked down PTCSC1 were injected subcutaneously into nude mice (n = 5). Tumor growth was monitored every week. After 5 weeks, tumors were collected for calculation of tumor size and weight. Moreover, T24 tumors derived from cells transfected with PTCSC1 overexpression vectors were significantly larger than control tumors (Fig. 4A, B, C). And the BIU-87 tumors had the same results with T24 tumors (Fig. 4G, H, I). Tumors that formed in T24 cells stably transfected with PTCSC1-shRNA were

smaller compared with control tumors (Fig. 4D, E, F). And the BIU-87 tumors had the same results with T24 tumors (Fig. 4G, J, K). Thus, lncRNA PTCSC1 promoted bladder cancer cell growth in vivo.

Ptcsc1 Promotes Bladder Cancer Progression Through Akt Pathway

The AKT signaling pathways have been widely reported to be among the most important signaling pathways that participate in a regulatory network during cell migration and invasion in various cancers[5–7]. The expression level of p-AKT was evaluated by western blot analysis. The results showed that PTCSC1 overexpression led to an obvious increase in p-AKT expression in T24 cells and BIU-87 cells, compared with the vector control groups (Fig. 5A, 4C). The results showed that PTCSC1 shRNA led to an obvious decrease in p-AKT expression in T24 cells and BIU-87 cells, compared with the vector control groups (Fig. 5B, 4D). This finding indicated that the activation of the AKT pathway might participate in the effect of PTCSC1 on T24 cells and BIU-87 cells migration and invasion.

Dicussion

Each year, bladder cancer is diagnosed about more than 430 000 patients worldwide, making it the fourth most common cancer in men and the 11th most common cancer in women[8].

Various studies suggest that the molecular mechanisms involved in cancer progression are not only related to protein coding genes but also to noncoding RNAs. lncRNAs have attracted an increasing amount of research interest because of their dysregulation in various cancers and their pivotal roles in cancer progression[9]. The lncRNAs greatly affect the prognosis, growth, survival, epithelial-to-mesenchymal transition, and metastasis of bladder cancer cells[10, 11]. For instance, Yang et al showed that lncRNA UCA1 regulates the PI3K pathway to participate in the cell cycle regulation of bladder cancer cells[12]. Zhu et al found that lncRNA ANRIL overexpression promotes proliferation and inhibits apoptosis in bladder cancer [13]. Chen et al indicated that lncRNA-n336928 level is associated with the advanced stage, grade, and over all survival in bladder cancer, suggesting its potential as a prognostic biomarker[14]. Cao et al showed that lncRNA GAS5 plays antitumor roles to suppress the proliferation and cell cycle progression of BC cells[15]. In addition, Li et al showed that lncRNA UCA1 regulates the miR-195/ARL2 axis, thereby affecting the mitochondrial function of bladder cancer cells[16]. These studies emphasized the importance of lncRNAs in bladder cancer and implied that lncRNAs might be a promising therapeutic target.

The PI3K/Akt/mTOR pathway denotes a prominent survival pathway which is aberrantly activated in many cancers. Phosphorylation of Akt has been correlated with poor prognosis in various cancers including liver[17]. Amplification, overexpression or mutations in PI3K subunits usually occur in tumors. Being a direct downstream target for Akt, mTOR is also over-activated in various tumors and associated with malignant phenotype[18]. Numerous mechanisms contribute to the PI3K/Akt/mTOR pathway

activation as reviewed elsewhere[17]. Targeting the PI3K/Akt/mTOR pathway in combinatorial therapy may prove to be an effective strategy to cope with cancer. Combining chemo- or radio-therapy with tyrosine kinase inhibitors (TKIs) may become the rationale in conventional treatment. Many clinical trials with Akt pathway inhibitors and other combination agents have been in progress.

In our study PTCSC1 enhanced bladder carcinoma cell migration and invasion ability, and decreased expression of PTCSC1 inhibited the expression of matrix metalloprotease MMP2 and MMP9, and increased the expression of E-cadherin. PTCSC1 promoted tumor growth in a mouse model of human bladder cancer. Additionally, PTCSC1 shRNA caused a significant decrease in p-AKT, expression in T24 and BIU-87 cells. Consequently, the role of PTCSC1 in bladder cancer related to AKT pathway will be addressed in our further research.

Conclusions

A novel function of PTCSC1 in promoting cell migration and invasion was identified. The present discovery showed that PTCSC1 has a significant effect on bladder by AKT pathway and that targeting PTCSC1 might be an important therapeutic strategy in the treatment of bladder cancer.

Declarations

Consent for publication:

Not applicable

Availability of data and materials:

All data generated or analysed during this study are included in this published article.

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Authors' contributions:

Xingkang Jiang performed the experimental animal models, molecular experiment and was a major contributor in writing the manuscript. Haokun Ren analyzed and interpreted the patient data. Zheng Zhang did the molecular experiment. Wei Zhang design project and performed molecular experiment. All authors read and approved the final manuscript.

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Declaration of Conflicting Interests:

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Statement:

This study was approved by the human ethics and research ethics committees of the Second Hospital of Tianjin Medical University (approval no. KY2020K108). All sick persons signed the consent form.

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Figures

Figure 1

AK023948 is expressed highly in bladder cancer. A. QRT-PCR assay showing AK023948 expression levels in SV-HUC-1 cells and the four indicated bladder cell lines. B. AK023948 expression was analyzed by qRT-qPCR in bladder tissues and normal tissues. SD is represented with bars (N = 20, T = 20). C. The effect of AK023948 uaRNA knockdown by siRNA on cell mobility in bladder cancer cells was investigated using a transwell assay (scale bar: 100 μ m).

Figure 2

AK023948 promotes migration and invasion in T24 cells. A. AK023948 expression in T24 cells with stable AK023948 overexpression and was evaluated by qRT-qPCR analysis. B. AK023948 expression in T24 cells with stable AK023948 knockdown and was evaluated by qRT-qPCR analysis. C. The effect of stably overexpressed AK023948 on migration (upper panel) and invasion (down panel) of T24 cells was investigated using a transwell assay (scale bar: 100 μ m). D. The effect of AK023948 stably knocked down with shRNA on migration (upper panel) and invasion (down panel) of T24 cells was investigated using a transwell assay (scale bar: 100 μ m). E. Western blotting analysis of MMP2, MMP9 and E-cadherin in AK023948 over expression T24 cells. F. Western blotting analysis of MMP2, MMP9 and E-cadherin in AK023948 knocked down T24 cells.

Figure 3

AK023948 promotes migration and invasion in BIU-87 cells. A. AK023948 expression in BIU-87 cells with stable AK023948 overexpression and was evaluated by qRT-qPCR analysis. B. AK023948 expression in BIU-87 cells with stable AK023948 knockdown and was evaluated by qRT-qPCR analysis. C. The effect of stably overexpressed AK023948 on migration (upper panel) and invasion (down panel) of BIU-87 cells was investigated using a transwell assay (scale bar: 100μm). D. The effect of AK023948 stably knocked down with shRNA on migration (upper panel) and invasion (down panel) of BIU-87 cells was investigated using a transwell assay (scale bar: 100μm). E. Western blotting analysis of MMP2, MMP9 and E-cadherin in AK023948 over expression BIU-87 cells. F. Western blotting analysis of MMP2, MMP9 and E-cadherin in AK023948 knocked down BIU-87 cells.

Figure 4

AK023948 inhibits bladder cell growth in vivo. The growth of AK023948 overexpressed or knocked down T24 cells and BIU-87 cells injected subcutaneously into nude mice (n= 5). A, G. Images of tumors that formed in the nude mice injected overexpression AK023948 T24 cells and BIU-87 cells respectively. B, H. Growth curves for xenograft tumors that formed in the nude mice injected overexpression AK023948 T24 cells and BIU-87 cells respectively. C, I. Tumor weights of xenograft AK023948 overexpression tumors of T24 cells and BIU-87 cells were measured respectively. D, G. Images of tumors that formed in the nude mice injected knocking down AK023948 T24 cells and BIU-87 cells respectively. E, J. Growth curves for the xenograft AK023948 knockdown tumors. F, K. Tumor weights of the xenograft AK023948 knockdown tumors were measured. *P< 0.05 and **P<0.01

Figure 5

AK023948 promotes bladder cancer progression through AKT pathway A, C. The analysis of the effect of AK023948 overexpression in T24 cells and BIU-87 cells on AKT pathway regulation by western blot analysis. B–D. The analysis of the effect of AK023948 knockdown in T24 cells and BIU-87 cells on AKT pathway regulation by western blot analysis. Data are presented as mean $\pm$ SD ($n = 3$). $**P < 0.01$ (Student's t test)

Supplementary Files

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