LncRNA AC008972.1 as a Novel Therapeutic Target for Prostate Cancer

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Abstract

Background: Prostate cancer is the leading cause of disease and death in men. Long non-coding RNAs (lncRNAs), microRNA (miRNAs) and mRNAs networks mediate prostate cancer progression. Here, we aim to investigate functions of lncRNA AC008972.1/miR-143-3p/thousand-and-one-amino acid 2 kinase (TAOK2) in prostate cancer.

Methods: The expression levels of lncRNA AC008972.1, miR-143-3p and TAOK2 are detected in prostate cancer tissues and cell lines by RT-qPCR. PC3 and LNCaP cells are used to establish lncRNA AC008972.1-knockdown, miR-143-3p-overexpressing, and TAOK2-down-regulated cells. Cell viability is examined by MTT and cell proliferation is detected by clone formation assay. Cell migration and invasion are tested by wound scratch assay and transwell chamber assay. The rate of apoptosis was analyzed by flow cytometry. The protein expression is detected by western blot assay. The target is validated by RNA binding protein immunoprecipitation (RIP) assay and dual luciferase activity assay. A mouse xenograft model was conducted to investigate the oncogenic effect of lncRNA AC008972.1 on prostate cancer.

Results: High expression of lncRNA AC008972.1 was associated with low overall survival in prostate cancer. Down-regulation of lncRNA AC008972.1 delayed prostate cancer process by inhibiting cell viability, proliferation, migration and invasion, as well as altering protein expression—whereas cell apoptosis was markedly promoted. lncRNA AC008972.1 negatively regulated miR-143-3p expression and miR-143-3p overexpression promoted prostate cancer process in vitro. TAOK2 expression was decreased by miR-143-3p through the complementary targeting of TAOK2 mRNA. Down-regulation of lncRNA AC008972.1 mitigated prostate cancer process in vitro based on miR-143-3p/TAOK2 node. Furthermore, the data of xenograft model experiment showed that inhibition of lncRNA AC008972.1 suppressed tumor growth *in vivo*.

Conclusions: Collectively, knockdown of lncRNA AC008972.1 inhibits prostate cancer cell growth based on down-regulation of TAOK2 induced by miR-143-3p. Here, we identify that lncRNA AC008972.1 exerts essential roles in the progression of prostate cancer and serves as a novel therapeutic target for prostate cancer.

Introduction

As the leading cause of disease and death in men, 1.6 million men are diagnosed with prostate cancer and 366,000 men die from prostate cancer every year [1]. Epidemiological studies of prostate cancer have disclosed lifestyle factors and individual biology influence the survival rate of prostate cancer and the risk of developing prostate cancer in multiple ways [1, 2]. Advances in prostate cancer diagnosis and treatment have ameliorated the capacity for stratifying patients by risk, thus allowing clinicians to recommend treatment according to cancer prognosis and patient preference [3, 4]. Chemotherapy effectively improves survival rate compared with androgen deprivation therapy [3-5]. Additionally, drugs

such as abiraterone and enzalutamide improve outcomes for men with metastatic prostate cancer that shows resistance to conventional hormone therapy [3, 4].

As transcripts of more than 200 nucleotides, long non-coding RNAs (lncRNAs) are unable to code proteins. Their biological contributions have been associated with malignant tumors by organizing nuclear domains, mediating transcription in cis or trans, thus regulating mRNA processing and post-transcriptional management, and modulating protein activity [6-8]. Results from differential screens of the expression of genes show that 61 lncRNAs are differentially expressed in prostate cancer, such as lncRNA AC008972.1, lncRNA LINC00844 and lncRNA PCAT1 [9-11]. Some of these lncRNAs have been well acknowledged as diagnostic, therapeutic or prognostic molecules [12, 13]. However, the biological roles of lncRNA AC008972.1 have not been precisely investigated. Furthermore, prior to clinical translation, it is significant to extensively understand the functional mechanism of lncRNAs.

As a class of small non-protein coding endogenous 19-20 nucleotide long small-stranded RNAs, microRNAs (miRNAs) are evolutionarily conserved in mammalian genome [14, 15]. In prostate cancer, the lncRNA-miRNA-mRNA networks mediate sequence-specific DNA binding, gene transcription and signaling transduction [9]. MiR-143-3p exhibits tumor suppressive effects in gallbladder carcinoma [16], prostate cancer [17], as well as ovarian cancer [18]. Thousand-and-one-amino acid 2 kinase (TAOK2) has been validated to play roles as mitogen-activated protein kinase kinase kinase (MAPKKK) to induce the activation of downstream kinases, exhibiting specificity for stress-elicited activation of p38 mitogen-activated protein kinase (MAPK) in prostate cancer [19-22]. Here, we aim to investigate the existence and functions of lncRNA AC008972.1/miR-143-3p/TAOK2 in prostate cancer.

Materials And Methods

Patients

This study obtained the approval from the Ethics Committee of Jiangsu Province Hospital of Chinese medicine (NO. NL-129-02). We obtained the informed written consents from all patients for utilizing the pathologic tissue specimens. The clinical characterization and tissues of patients with prostate cancer were collected from Affiliated Hospital of Nanjing University of Chinese Medicine (Nanjing, China). Tumor grades were classified according to the World Health Organization's Classification. All men in the cohort were followed for 100 months. All experimental procedures were implemented in accordance with the Declaration of Helsinki of 1975 (revised in 2000). The clinicopathologic findings were shown in **Table 1**. lncRNA AC008972.1 expression in tissues was examined by reverse transcription-quantitative PCR (RT-qPCR).

Animal study

All animal experiments were performed with the approval of the Animal Ethics Committee of Jiangsu Health Vocational College (No. JHVC-IACUC-2020-B002). 8-week-old male nude mice were randomly divided into two groups: sh-NC or sh-lncRNA AC008972.1 group (3 in each group). The PC3 cells

transfected with sh-NC vector or sh-lncRNA AC008972.1 vector were injected subcutaneously into mice. The tumor size was monitored every week. After four weeks, the mice were sacrificed. The tumor volumes were calculated by using the following formula: Tumor volume = (width² × length)/2. Tumor tissues were fixed for H&E (Solarbio, China), IHC (Solarbio, China) and Tunel assay (Promega) following the manufacturer's instruction.

Cell lines

Cell lines RWPE-1, PC3 and DU145 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). P4E6 cells were purchased from the European Collection of Authenticated Cell Cultures (ECACC, Salisbury, UK), and LNCaP from the Cell Lines Service (CLS, Eppelheim, Germany). Human prostate epithelial RWPE-1 line was cultured in keratinocyte serum free medium (K-SFM) (Gibco, Rockville, MD, USA) added with 0.05 mg/mL bovine pituitary extract and 5 ng/mL human recombinant epidermal growth factor (EGF) (Sigma-Aldrich, St. Louis, MO, USA). P4E6 cells were cultured in stemline keratinocyte medium II (Sigma-Aldrich) supplemented with 2 mmol/L glutamine and 2% fetal bovine serum (FBS). PC3 cells were maintained in F-12K medium containing 10% FBS. LNCaP cells were maintained in Earle's BSS medium containing 2 mmol/L L-glutamine and 10% FBS. DU145 cells were cultured in Eagle's minimum Essential medium containing 10% FBS. All cells were incubated in 95% air and 5% CO₂ at 37°C under 90-95% humidity. The medium was supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin (Sigma-Aldrich).

Cell transfection

To artificially modulate lncRNA AC008972.1, miR-143-3p, or TAOK2 expression, transfection was carried out in PC3 and LNCaP cells. In short, PC3 and LNCaP cells were seeded in a 6-well plate with a density of 10⁵ cells/mL and pre-incubated for 24 h. Transfection was carried out using Lipofectamine RNAiMAX reagent (Thermo Fisher Scientific, Somerset, NJ, USA) according to the manufacturer's instructions. The cells were transfected with 10 pmol/mL of lncRNA AC008972.1 short-hairpin RNA (sh-lncRNA AC008972.1), or negative control (sh-NC) (Sigma-Aldrich). To up-regulate or down-regulate miR-143-3p, the cells were transfected with miR-143-3p mimic (NC mimic as a negative control) or miR-143-3p inhibitor (NC inhibitor as a negative control) (Sigma-Aldrich). To down-regulate TAOK2 expression, shRNA for TAOK2 was transfected into PC3 and LNCaP cells, with sh-NC as a negative control (Sigma-Aldrich).

Cell viability

All cells were collected 48 h after transfection. Cell viability was assayed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method. The cells were incubated with 200 µL of 0.5 mg/mL MTT (Sigma-Aldrich) prepared in phosphate-buffered saline at 37°C. After 4 h, the supernatant was removed, and the formazan crystals were dissolved in 100 µL of dimethyl sulfoxide. The absorbance of each well was detected at 540 nm using a microplate reader (BioTek Instruments, Winooski, VA, USA).

Colony formation

PC3 and LNCaP cancer cells were seeded in 6-well plates at a density of 100 cells each well. The transfected cells were kept at 37°C in 5% CO₂ for 14 days, and the medium was changed every 2 days. The formed colonies were fixed using 70% ethanol, followed by staining with 0.5% crystal violet. The colony consisting of 50 cells were counted using Image J software (NIH, Bethesda, MD, USA). The experiment was carried out in triplicate.

Scratch assay

After transfection, PC3 and LNCaP cells were cultured in 6-well plates for 10 h. Then, two parallel wounds were created with a 200-μL pipette tip. The debris was cleaned with phosphate buffer saline (PBS). The cells were continually cultured for 48 h. Wound closure was monitored using a microscope (Olympus, Tokyo, Japan) and imaged with ImageJ software.

Transwell assay

Migration of PC3 and LNCaP cells were measured using Transwell Permeable Supports (BD Biosciences, San Jose, A, USA). After transfection, the cells were seeded in a density of 2×10^4 cells/well into the top chamber of 8 mm-pore size Transwell chambers and pre-incubated in low serum (0.5% FBS) conditions for 24 h. The bottom chamber was coated with 20% (w/v) FBS in PBS as a chemoattractant overnight at 4°C. The cells were allowed to migrate for 72 h at 37°C. Any PC3 and LNCaP cells that had migrated through the membrane and stuck to the lower surface of the membrane were fixed with 4% (w/v) paraformaldehyde and stained with 0.5% (w/v) crystal violet (Sigma-Aldrich) for visualization. The migrated cells were counted under a microscope. Invasion assay was performed with the Transwell chambers coated with Matrigel (Corning, Cambridge, MA, USA).

Flow cytometry assay

To detect apoptosis, cells were harvested, following by staining with annexin V-FITC/PI in the dark. The rate of apoptosis was analyzed by flow cytometry. All determinations were performed independently in triplicate.

RNA extraction and RT-qPCR

Total RNA from tissues and cells were extracted using QIAzol Lysis reagent (QIAGEN, Hilden, Germany) and miRNeasy kit (QIAGEN). DNase digestion was performed. Cell fractionation was carried out using the NE-PER nuclear extraction kit (Thermo Fisher Scientific) to obtain cytoplasmic RNA and nuclear RNA. cDNA was generated using SuperScript III (Invitrogen, Carlsbad, CA, USA) and random primers (Invitrogen). qPCR was performed on an Applied Biosystems 7900HT Real-Time PCR system. The amplification was implemented with Power SYBR Green MasterMix (Applied Biosystems) and specific primers for lncRNA AC008972.1, miR-143-3p, TAOK2, U6 and GAPDH (**Table 2**). U6 was an internal control for miR-143-3p, and GAPDH for lncRNA AC008972.1 and miR-143-3p.

Western blot

Protein extracts were prepared using RIPA lysis buffer (50mM Tris salt buffer, pH = 7.4, 150mM NaCl, 1% NP 40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with proteinase and phosphatase inhibitors (Roche, Mannheim, Germany). Protein content was assayed using the Bradford protein assay kit (BioRad, Hercules, CA, USA). Appropriately 10 µg of protein was loaded onto sodium dodecyl sulfate-polyacrylamide gels for separation, followed by electroblotting onto nitrocellulose membranes (Amersham, Buckinghamshire, UK). The membranes were then blocked with 5% non-fat dry milk in 20 mmol/L Tris-HCl (pH 8.0) containing 137 mmol/L NaCl and 0.1% Tween 20 (TBS-T) for 1 h at room temperature before exposure to the primary antibodies against E-cadherin (#24E10, Cell Signaling Technology, Danvers, MA, USA), vimentin (#D21H3, Cell Signaling Technology), TAOK2 (#NP_001157246.1, USBiological Life Sciences, Salem, MA, USA) and β-actin (#4970, Cell Signaling Technology) at 1:1,000 dilution overnight at 4°C. Protein blots were then incubated with a 1:5,000 dilution of horseradish peroxidase-conjugated goat anti-rabbit antibody (#7074, Cell Signaling Technology) for 1 h at room temperature. The proteins were visualized with SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) and high-performance chemiluminescence film (GE Healthcare, Buckinghamshire, UK). The densitometric analysis of proteins was performed using ImageJ software.

Dual luciferase activity

PC3 and LNCaP cells were seeded into 24-well plates with a density of 1×10^5 cells/well. To confirm that miR-143-3p targets lncRNA AC008972.1, the cells were transfected with 10 ng of wild-type (lncRNA AC008972.1-WT) or mutant (lncRNA AC008972.1-Mut) pMIR-lncRNA AC008972.1 plasmid carrying firefly luciferase, 20 pmol of miR-143-3p mimic or NC mimic, and 10 ng of pRL-TK plasmid (Promega, Madison, WI, USA). Further, to investigate the targeting relationship between miR-143-3p and TAOK2, the cells were transfected with miR-143-3p mimic or NC mimic, pMIR-TAOK2-WT or pMIR-TAOK2-Mut, and pRL-TK plasmid. The transfection was carried out using Lipofectamine RNAiMAX reagent according to manufacturer's protocol. Firefly and renilla luciferase activity was examined using Dual-Glo luciferase reporter assay system (Promega). Firefly luciferase activity was normalized with renilla luciferase activity. Each transfection was performed in triplicate and repeated thrice.

RNA binding protein immunoprecipitation (RIP) assay

RIP assay was carried out using EZMagna RIP kit (Millipore, Billerica, MA, USA) according to the manufacturer's protocol. PC3 and LNCaP cells at 80%-90% confluency were collected and lysed in RIPA buffer. Cell extract was incubated with RIP buffer containing magnetic beads linked to anti-Ago2 antibody (Millipore) or normal mouse IgG (Millipore). To remove the protein, the extract was incubated with proteinase K at 55°C for 30 min. RNA content was detected with a NanoDrop (Thermo Fisher Scientific). The immunoprecipitated lncRNA AC008972.1 and miR-143-3p were quantified by qRT-PCR.

Statistical analysis

Statistical analysis was done with GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA). Student's t-test was used to analyze the statistical significance between two groups. One-way ANOVA followed by Tukey test was used to perform comparison between multiple groups. Data were presented as mean $\pm$ standard deviation (SD). p-values less than 0.05 were considered to be significant.

Results

High expression of lncRNA AC008972.1 is associated with low overall survival in prostate cancer

To investigate lncRNA AC008972.1 expression in prostate cancer, prostate cancer tissues and para-cancerous tissues were exposed to RT-qPCR. We confirmed that lncRNA AC008972.1 was enriched in prostate cancer tissues compared with para-cancerous tissues ($p < 0.01$) (**Figure 1A**). Besides, patients with grade III-IV prostate cancer showed higher expression of lncRNA AC008972.1 relative to patients with grade I-II prostate cancer ($p < 0.01$) (**Figure 1B**). Overall survival was significantly lower in patients with high expression of lncRNA AC008972.1 compared with those with low expression of lncRNA AC008972.1 ($p < 0.001$) (**Figure 1C**). Compared with human prostate epithelial RWPE-1 cell line, the expression of lncRNA AC008972.1 was apparently higher than those in prostate cancer cells P4E6, PC3, LNCaP and DU145 cells ($p < 0.01$ or $p < 0.001$) (**Figure 1D**), among which the PC3 and LNCaP cells exhibited the highest lncRNA AC008972.1 expression. Hence, the PC3 and LNCaP cell lines were selected for the subsequent experiment. In conclusion, high expression of lncRNA AC008972.1 was associated with prostate cancer process.

Down-regulation of lncRNA AC008972.1 delays prostate cancer process in vitro

Next, sh-lncRNA AC008972.1 was introduced into PC3 and LNCaP cells, and the expression of lncRNA AC008972.1 was significantly decreased relative to sh-NC group ($p < 0.01$) (**Figure 2A**). Then we evaluated the biological roles of lncRNA AC008972.1 in prostate cancer cell lines. Knockdown of lncRNA AC008972.1 decreased cell viability ($p < 0.01$) (**Figure 2B**), inhibited colony formation ($p < 0.01$) (**Figure 2C**), blocked wound closure ($p < 0.01$) (**Figure 2D**) and prohibited migration and invasion ($p < 0.01$) (**Figure 2E**). Furthermore, knockdown of lncRNA AC008972.1 promoted cell apoptosis ($p < 0.001$) (**Figure 2F**). Additionally, lncRNA AC008972.1 knockdown increased E-cadherin expression while decreasing vimentin expression ($p < 0.001$) (**Figure 2G**). Up-regulation of E-cadherin increased the sensitivity of prostate cancer to paclitaxel [23]. Generally, up-regulation of vimentin reflected higher tumor grade, metastasis and shorter survival in malignant tumors [24, 25]. Thus, the results indicated that knockdown of lncRNA AC008972.1 delayed prostate cancer process in vitro.

lncRNA AC008972.1 negatively modulates miR-143-3p expression

Compared to the cytoplasm, lncRNA AC008972.1 expression was downregulated in the nuclei of PC3 and LNCaP cells ($p < 0.05$ or $p < 0.01$) (**Figure 3A**), implying that lncRNA AC008972.1 might function as a cytoplasmic RNA. Predicted by TargetScan Online Tool (http://www.targetscan.org/vert_72/), lncRNA AC008972.1 shared the complementary sequence with miR-143-3p (**Figure 3B**). Next, we transduced miR-

143-3p mimic into PC3 and LNCaP cells, and miR-143-3p was significantly enhanced in PC3 and LNCaP cells ($p < 0.01$) (**Figure 3C**). To confirm the targeting relationship between lncRNA AC008972.1 and miR-143-3p, we performed dual luciferase activity assay and RIP assay. Results from dual luciferase activity assay showed that miR-143-3p decreased the expression of lncRNA AC008972.1 by targeting the complementary sequence ($p < 0.05$) (**Figure 3D**). RIP results demonstrated the association between lncRNA AC008972.1 and miR-143-3p ($p < 0.001$) (**Figure 3E**). Additionally, we detected the down-regulation of miR-143-3p in prostate cancer tissues compared with non-tumor tissues ($p < 0.01$) (**Figure 3F**), as well as in prostate cancer cells P4E6, PC3, LNCaP and DU145 cells compared with human prostate epithelial RWPE-1 cells ($p < 0.05$ or $p < 0.01$) (**Figure 3G**). Furthermore, lncRNA AC008972.1 knockdown decreased the expression of miR-143-3p in PC3 and LNCaP cells ($p < 0.01$) (**Figure 3H**), and this linear negative regulatory relationship of lncRNA AC008972.1 on miR-143-3p expression was continually demonstrated in PC3 cells ($R = 0.43$, $p = 0.016$) (**Figure 3I**). Here, we verified that binding of lncRNA AC008972.1 and miR-143-3p resulted in down-regulation of miR-143-3p.

MiR-143-3p overexpression inhibits prostate cancer process in vitro

Since modulation of lncRNA AC008972.1 expression resulted in changes in miR-143-3p expression, we hypothesized that miR-143-3p was implicated in the roles of lncRNA AC008972.1 in cell phenotypes. We noticed a decrease in cell viability in PC3 and LNCaP cells transfected with miR-143-3p mimic compared to those transfected with NC mimic, especially 72 h after transfection ($p < 0.01$) (**Figure 4A**). What's more, miR-143-3p overexpression prohibited colony formation ($p < 0.001$) (**Figure 4B**), retarded wound closure ($p < 0.05$ or $p < 0.01$) (**Figure 4C**), restrained migration and invasion ($p < 0.01$) (**Figure 4D**) and stimulated cell apoptosis ($p < 0.001$) (**Figure 4E**). Compared with NC mimic, miR-143-3p mimic induced E-cadherin expression, while reducing vimentin expression ($p < 0.01$) (**Figure 4F**). In a word, miR-143-3p overexpression repressed the progression of prostate cancer in vitro.

MiR-143-3p decreases TAOK2 expression by complementary targeting of TAOK2 mRNA

Similarly, TAOK2 mRNA shared the complementary elements with miR-143-3p, as predicted by TargetScan (**Figure 5A**). Experimental confirmation was carried out by dual luciferase activity assay. Obviously, miR-143-3p mimic decreased luciferase activity, suggesting that TAOK2 mRNA was targeted by miR-143-3p ($p < 0.01$) (**Figure 5B**). Compared with non-tumor tissues, relative mRNA expression of TAOK2 was elevated in prostate cancer tissues ($p < 0.01$) (**Figure 5C**). Consistently, TAOK2 transcription was increased in prostate cancer cells P4E6, PC3, LNCaP and DU145 cells compared with human prostate epithelial RWPE-1 cells ($p < 0.05$ or $p < 0.01$) (**Figure 5D**). More importantly, we confirmed the decrease in TAOK2 transcript ($p < 0.01$) (**Figure 5E**) and protein ($p < 0.01$) (**Figure 5F**) levels following miR-143-3p overexpression. However, the linear modulatory role of miR-143-3p on TAOK2 transcripts was not significant in PC3 cells ($R = 0.4$, $p = 0.268$) (**Figure 5G**). Thus, the obtained results showed that miR-143-3p reduced TAOK2 transcription by complementary targeting of TAOK2 mRNA.

Down-regulation of lncRNA AC008972.1 mitigates prostate cancer process in vitro based on miR-143-3p/TAOK2 node

Considering the modulatory role of lncRNA AC008972.1 in miR-143-3p expression, as well as miR-143-3p in TAOK2 expression, we speculated that lncRNA AC008972.1 functioned in prostate cancer process based on miR-143-3p node. Notably, lncRNA AC008972.1 knockdown decreased cell viability ($p < 0.01$) (**Figure 6A**), decreased colony formation ($p < 0.01$) (**Figure 6B**), blunted wound closure ($p < 0.01$) (**Figure 6C**), restrained migration and invasion ($p < 0.01$) (**Figure 6D**) and promoted cell apoptosis ($p < 0.001$) (**Figure 6E**), while miR-143-3p inhibitor obviously counteracted the effects of lncRNA AC008972.1 knockdown ($p < 0.01$). Interestingly, simultaneous transfection with sh-lncRNA AC008972.1, miR-143-3p inhibitor and sh-TAOK2 mitigated the effects of sh-lncRNA AC008972.1 and miR-143-3p inhibitor on cell viability, colony formation, wound closure, migration, invasion and cell apoptosis ($p < 0.01$) (**Figure 6A-6E**). Further, lncRNA AC008972.1 knockdown decreased vimentin expression, while increasing E-cadherin ($p < 0.01$) (**Figure 6F**) expression, which was restricted by miR-143-3p inhibitor, suggesting that miR-143-3p expression mediated by lncRNA AC008972.1 knockdown restricted vimentin expression and elevated E-cadherin expression ($p < 0.01$) (**Figure 6F**). However, vimentin were decreased while E-cadherin was increased in PC3 cells transfected with sh-lncRNA AC008972.1, miR-143-3p and sh-TAOK2 compared with those transfected with sh-lncRNA AC008972.1 and miR-143-3p ($p < 0.01$) (**Figure 6F**). Together, lncRNA AC008972.1 functioned in prostate cancer cell phenotypes by modulating the miR-143-3p/TAOK2 node.

Down-regulation of lncRNA AC008972.1 mitigates prostate cancer process *in vivo*

To investigate the oncogenic effect of lncRNA AC008972.1 on prostate cancer, an *in vivo* study was conducted. PC3 cell lines were transfected with sh-lncRNA AC008972.1 vector or sh-NC vector, and subsequently implanted into nude mice to construct mouse xenograft model. The tumors volume ($p < 0.01$) and weight ($p < 0.001$) of sh-lncRNA AC008972.1 group were significantly down-regulated compared with sh-NC group (**Figure 7A-7C**). Furthermore, the Hematoxylin and Eosin (HE) staining and immunohistochemistry assay (IHC) results illustrate that the tumors developed from sh-lncRNA AC008972.1 cells displayed alterations in shape and the reduction of Ki-67 compared with tumors formed from sh-NC vector-transfected cells ($p < 0.001$). While the Tunel assay result shows that the percentage of apoptotic bodies was increased incredibly in the tumors from sh-lncRNA AC008972.1 cells ($p < 0.001$) (**Figure 7D**). Finally, we verified the lncRNA AC008972.1/miR-143-3p/TAOK2 regulatory axis *in vivo*. The qRT-PCR was conducted to detect the RNA relative expression level. The expression of lncRNA AC008972.1 was shut down in tumors derived from sh-lncRNA AC008972.1 cells ($p < 0.001$). On the contrary, miR-143-3p was up-regulated due to lncRNA AC008972.1 knocking down ($p < 0.001$), which led to the reduction of TAOK2 ($p < 0.001$) (**Figure 7E**). Taken together, Knockdown of lncRNA AC008972.1 regulates TAOK2 by reducing the sponge effect on miR-143-3p to inhibit tumor development and promote tumor cell apoptosis.

Discussion

Here we detected that lncRNA AC008972.1 high expressed in prostate cancer was associated with the low overall survival rate. LncRNA AC008972.1 knockdown mitigated prostate cancer cell growth. Further, we documented a critical function of miR-143-3p together with its downstream targeting gene TAOK2.

Additionally, we characterized how lncRNA AC008972.1/miR-143-3p/TAOK2 axis played roles in prostate cancer process in vitro. Mechanically, knockdown of lncRNA AC008972.1-induced miR-143-3p led to down-regulation of TAOK2, which contributed to bluntness of prostate cancer cell growth.

Whole genome transcriptomic analysis has revealed that lncRNA transcripts are implicated in physiological and pathological processes such as prostate cancer [26]. Accumulating studies have evidenced that the pathology and progression of prostate is generally associated with the aberrant expression of lncRNAs [13, 27, 28]. Currently, we firstly detected the up-regulation of lncRNA AC008972.1 in prostate cancer, and this high expression was more significant in grade III-IV prostate cancer. Besides, the high expression of lncRNA AC008972.1 presented a low overall survival rate. Consequently, lncRNA AC008972.1 showed the promise as a predictive, diagnostic and prognostic biomarker in prostate cancer. However, the functional role of lncRNA AC008972.1 remained partially understood. Here, we proved that knockdown of lncRNA AC008972.1 decreased prostate cancer cell viability, restrained colony formation, inhibited wound closure, and abated migration and invasion. Further, knockdown of lncRNA AC008972.1 increased E-cadherin expression. E-cadherin, as an epithelial-mesenchymal transition transcription factor, plays roles in metastasis and drug resistance of prostate cancer [29]. Of note, sh-lncRNA AC008972.1 decreases the expression of vimentin that is associated with a higher likelihood of metastasis.

It was noticed that miR-143-3p was down-regulated in prostate cancer tissues compared to that in non-tumor tissues, which was consistently reported by previous studies [30, 31]. What's more, the fact that lncRNA AC008972.1 shared the complementary sequence with miR-143-3p suggested the links between lncRNA AC008972.1 and miR-143-3p expression. Here, we demonstrated that lncRNA AC008972.1 negatively modulated miR-143-3p generation by targeting miR-143-3p. In addition, prostate cancer cell proliferation and migration was abrogated by miR-143-3p [30-32], which is consistently confirmed in vitro in our study. It has been reported that the modulatory networks consisting of lncRNAs and miRNAs are implicated in the molecular mechanisms and tumorigenesis in prostate cancer [9]. It was worth noting that miR-143-3p up-regulation mediated by lncRNA AC008972.1 knockdown restrained prostate cancer cell growth, implying that miR-143-3p was a critical mediator for knockdown of lncRNA AC008972.1 to inhibit prostate cancer.

TAOK2 has been characterized as MAPKKs activating downstream MKK3 and MKK6, which exhibits specificity for stress-induced activation of p38 MAPK in prostate cancer [19-22]. Currently, we observed significant up-regulation of TAOK2 in prostate cancer tissues, which had not been reported previously. It was also verified that miR-143-3p mimic lessened TAOK2 at mRNA and protein levels. Of note, TAOK2 mRNA is predicted to possess the complementary sequence with miR-143-3p [33], which is further experimentally verified. Studies have provided evidences miR-143-3p inhibits cancer progression through targeting its downstream genes and regulating protein expression [18, 34, 35]. However, whether miR-143-3p inhibited prostate cancer cell growth via mediating TAOK2 expression lacked the exact evidences. Here, we confirmed that knockdown of lncRNA AC008972.1 inhibited prostate cancer process depending on miR-143-3p-mediated down-regulation of TAOK2.

Conclusions

Summarily, lncRNA AC008972.1 is highly expressed in prostate cancer, which indicates low overall survival rates. Knockdown of lncRNA AC008972.1 inhibits prostate cancer cell growth based on miR-143-3p-induced down-regulation of TAOK2. Our research provides a potential therapeutic target for prostate cancer, despite more detailed mechanisms need further research.

Declarations

Consent for publication

All authors agree with the content of the manuscript.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

All animal experiments were performed with the approval of the Animal Ethics Committee of Jiangsu Health Vocational College (No. JHVC-IACUC-2020-B002). All clinical specimens used in this study obtained the approval from the Ethics Committee of Jiangsu Province Hospital of Chinese medicine (NO. NL-129-02). Informed consent was obtained from all patients included in this study.

AUTHOR CONTRIBUTIONS

JL, HY and QZ conceived the project and designed the experiments. JL, HX and HY performed the study, analyzed the data, and wrote the paper. QW, RS, WM, TM and JL performed the study. JL and WM analyzed the data. HY and QZ analyzed the data and critically revised the manuscript.

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Conflict of Interest

The authors declare no competing interests.

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Tables

Table 1 Demographic and clinical characteristics of prostate cancer patients and control subjects

LINC00313			
Clinical parameters	Low expression (n=13)	High expression (n=17)	P value
Age			0.173
>60	6	12	
≤60	7	5	
FIGO			0.009
I~II	9	4	
III~IV	4	13	
Tumor Size			0.056
>2 cm	8	11	
≤2 cm	5	6	
Lymph nodes status			0.138
Negative	7	6	
Positve	6	11	

Table 2 The sequence of specific primers for RT-qPCR

Gene symbol	Specific primers
LncRNA AC008972.1	Forward: 5'-CCTTCATTTCGCATTCTTCTG-3'
	Reverse: 5'-ACCATCCACGCTCTTCCTAT-3'
miR-143-3p	Forward: 5'-TGAGATGAAGCACTG-3'
	Reverse: 5'-GTGCAGGGTCCGAGGT-3'
TAOK2	Forward: 5'-CCGGGATGTCCGGAATAGTG-3'
	Reverse: 5'-CTCCTCTTCCTCCTCTGGGG-3'
U6	Forward: 5'-TCTTTCTCGAGCAAGGTCGGGCAGGAAGAGGGCCTA-3'
	Reverse: 5'-AAAAC TCGAGGAATTCGGGCCCGGTGTTTCGTCCTTTCCACAAG-3'
GAPDH	Forward: 5'-ATGGGGAAGGTGAAGGTCG-3'
	Reverse: 5'-GGGGTCATTGATGGCAACAATA-3'

Figures

Figure 1

Overall survival curve (Kaplan-Meier) is related to LncRNA AC008972.1 expression. (A) LncRNA AC008972.1 was highly expressed in tumor tissues relative to non-tumor tissues ($p < 0.01$); (B) Grade III-IV prostate cancer showed high expression of LncRNA AC008972.1 compared with grade I-II prostate cancer. LncRNA AC008972.1 mRNA expression was measured using RT-qPCR. Relative ratios were shown as fold changes compared with non-tumor tissues or I + II stages ($p < 0.01$); (C) There was an association between low OS and high LncRNA AC008972.1 expression ($p < 0.001$); (D) LncRNA AC008972.1 was abundance in prostate cancer cells (P4E6, PC3, LNCaP, and DU145) compared with normal prostate epithelial cells (RWPE-1). Relative ratios were shown as fold changes compared with LncRNA AC008972.1 expression of RWPE-1 cells ($p < 0.01$).

Figure 2

LncRNA AC008972.1 loss restricts proliferative activity, invasion and migration, and epithelial-mesenchymal transition of PC3 and LNCaP cells. (A) Quantification of LncRNA AC008972.1 48 h after transfection with shRNA for LncRNA AC008972.1 ($p < 0.01$). (B) Cell viability of PC3 and LNCaP cells was examined 0, 24, 48 and 72 h after transfection with sh-NC or sh-LncRNA AC008972.1 ($p < 0.01$). (C) Colony formation by crystal violet staining, (D) wound closure by scratch assay, (E) migration and invasion by 24-transwell assay, (F) cell apoptosis by flow cytometry, and (G) E-cadherin and vimentin by Western blot were performed 48 h after transfection with shRNA for LncRNA AC008972.1 ($p < 0.01$).

Figure 3

LncRNA AC008972.1 negatively modulates miR-143-3p expression. (A) Expression of LncRNA AC008972.1 was examined in cytoplasm and nuclei of PC3 and LNCaP cells ($p < 0.05$ or $p < 0.01$); (B) LncRNA AC008972.1 shares the complementary sequence with miR-143-3p; (C) Quantification of miR-143-3p 48 h after transfection with NC mimic or miR-143-3p mimic ($p < 0.01$); (D) Dual luciferase activity assay and (E) RIP assay were performed to validate the targeting relationship between LncRNA AC008972.1 and miR-143-3p in PC3 and LNCaP cells ($p < 0.05$ or $p < 0.001$); (F) miR-143-3p was low expressed in tumor tissues compared to non-tumor tissues ($p < 0.01$); (G) miR-143-3p was down-regulated in prostate cancer cells (P4E6, PC3, LNCaP, and DU145) relative to normal prostate epithelial cells (RWPE-1) ($p < 0.05$ or $p < 0.01$ vs RWPE-1 cells). (H-I) miR-143-3p was detected in PC3 and LNCaP cells 48 h after transfection with sh-NC or sh-LncRNA AC008972.1 ($p < 0.05$ or $p < 0.01$).

Figure 4

miR-143-3p inhibits prostate cancer process in vitro. (A) Cell viability of PC3 and LNCaP cells was examined 0, 24, 48 and 72 h after transfection with NC mimic or miR-143-3p mimic ($p < 0.01$). (B) Colony formation by crystal violet staining, (C) wound closure by scratch assay, (D) migration and invasion by 24-transwell assay, (E) cell apoptosis by flow cytometry, and (F) E-cadherin and vimentin by Western blot were performed 48 h after transfection with NC mimic or miR-143-3p ($p < 0.05$ or $p < 0.01$).

Figure 5

miR-143-3p decreases TAOK2 expression by complementary targeting of TAOK2 mRNA. (A) miR-143-3p shares the complementary sequence with TAOK2; (B) Dual luciferase activity assay was performed to validate the targeting relationship between TAOK2 and miR-143-3p in PC3 and LNCaP cells ($p < 0.001$); (C) TAOK2 was highly expressed in tumor tissues relative to non-tumor tissues ($p < 0.01$); (D) TAOK2 transcription was increased in prostate cancer cells (P4E6, PC3, LNCaP, and DU145) relative to normal prostate epithelial cells (RWPE-1) ($p < 0.05$ or $p < 0.01$ vs RWPE-1 cells); TAOK2 (E) mRNA and (F-G) protein expression was examined in PC3 and LNCaP cells 48 h after transfection with sh-NC or sh-IncRNA AC008972.1 ($p < 0.01$ or $p = 0.268$).

Figure 6

Down-regulation of lncRNA AC008972.1 mitigates prostate cancer process in vitro based on miR-143-3p/TAOK2 node. (A) Cell viability of PC3 and LNCaP cells was examined 0, 24, 48 and 72 h after transfection with the indicated plasmids. (B) Colony formation by crystal violet staining, (C) wound closure by scratch assay, (D) migration and invasion by 24-transwell assay, (E) cell apoptosis by flow cytometry, and (F) E-cadherin and vimentin by Western blot were in the cells transfected with NC mimic or miR-143-3p 48 h after transfection. ** $p < 0.01$, (sh-lncRNA AC008972.1 + NC inhibitor) vs (sh-NC + NC inhibitor); ## $p < 0.01$, (sh-lncRNA AC008972.1 + miR-143-3p inhibitor) vs (sh-lncRNA AC008972.1 + NC inhibitor); \$\$\$ $p < 0.01$, (sh-lncRNA AC008972.1 + miR-143-3p inhibitor + sh-TAOK2) vs (sh-lncRNA AC008972.1 + miR-143-3p inhibitor).

Figure 7

Down-regulation of lncRNA AC008972.1 mitigates prostate cancer process *in vivo*. (A) Sh-lncRNA AC008972.1 or sh-NC PC3 cell lines were implanted into nude mice to construct mouse xenograft model. (B) Tumor volume, (C) Tumor weight, (D) H&E, IHC and TUNEL assay, (E) the expression of lncRNA AC008972.1, miR-143-3p and TAOK2 were detected by qRT-PCR. ** $p < 0.01$, *** $p < 0.001$, sh-lncRNA AC008972.1 vs sh-NC.