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The FOXP4-AS1/miR-3130-3p/SP4 feedback loop is associated with prostate cancer

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ABSTRACT

The vital function of mounting long noncoding RNAs (lncRNAs) in prostate cancer (PCa) has been illustrated in increasing reports. However, the roles of many lncRNAs in PCa have not been deciphered. A total of 62 pairs of PCa and adjacent normal tissue samples were provided by PCa patients undergoing surgery. Extensive assays were conducted in this study to investigate the role of FOXP4 antisense RNA 1 (FOXP4-AS1) in PCa tumorigenesis. This study elucidated that FOXP4-AS1 expression was elevated in PCa tissue samples and cell lines. Loss-of-function experiments revealed that depleted FOXP4-AS1 inhibited PCa cell proliferation *in vitro* and retarded tumor growth *in vivo*. Mechanically, FOXP4-AS1 functioned as a competing endogenous RNA (ceRNA) of miR-3130-3p, releasing SP4 from the inhibitory effect of miR-3130-3p. Rescue assays validated that FOXP4-AS1 modulated PCa progression via SP4. Interestingly, SP4 is known as a transcription factor and was predicted to bind with the promoter region of FOXP4-AS1. This current research confirmed that SP4 activated the transcription activity of FOXP4-AS1 and thus positively regulated its expression. To conclude, we discovered that FOXP4-AS1, miR-3130-3p, and SP4 constitute a feedback loop and contribute to PCa tumorigenesis, providing a new valuable diagnosis and therapeutic strategy for PCa.

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Introduction

Prostate cancer (PCa) is the malignancy with the highest morbidity rate and the third highest mortality rate in males on the globe, especially in those in developed countries (1, 2). Despite the improvements in diagnostic and therapeutic approaches to PCa, the overall 5-year survival rate of patients diagnosed with advanced PCa is still under 30% (1). Hence, investigation of the molecular mechanisms underlying PCa progression is of critical importance.

Long noncoding RNAs (lncRNAs) are endogenous cellular RNA transcripts with no or limited protein-coding ability, holding a length of over 200 nucleotides. Hitherto, scientific researchers have identified more than 10,000 lncRNAs in the human species. Many of the lncRNAs identified have been demonstrated to serve as oncogenic or tumor-suppressive lncRNAs in various human cancers. For example, long noncoding RNA SNHG15 promotes human breast cancer proliferation, migration and invasion by sponging miR-211-3p (3). Long noncoding RNA TCF7 promotes invasiveness and self-renewal of human non-small cell lung cancer cells (4). Long noncoding RNA ZEB1-AS1 epigenetically regulates the expressions of ZEB1 and downstream molecules in prostate cancer (5). LincRNA-p21 suppresses the development of human prostate cancer through the inhibition of PKM2 (6). FOXP4 antisense RNA 1 (FOXP4-AS1) is a lncRNA located in Chromosome 6. Previous studies have indicated that FOXP4-AS1 participates in colorectal cancer and osteosarcoma (7, 8). However, it is unclear whether FOXP4-AS1 has any regulatory effect on PCa.

In this study, we aimed at unmasking the role and the underlying mechanism of FOXP4-AS1 in PCa.

Materials and Methods

Tissue samples

A total of 62 paired PCa and adjacent normal tissue samples were all provided by PCa patients who had to undergo surgery at the Affiliated Haikou Hospital of Xiangya Medical College. Prior to surgery, no patients had received any specific clinical treatment. The tissue samples were snap-frozen by liquid nitrogen and preserved at the temperature of −196°C. Informed consent was signed by all patients and the Research Ethics Committee of Affiliated Haikou Hospital of Xiangya Medical College approved this research.

Cell lines and cell culture

Four human PCa cell lines (DU145, PC3, C4–2, LNCaP) and a normal prostate epithelial cell line (RWPE1) were obtained from ATCC (American Type Culture Collection; VA, USA). Cells were cultured in RPMI-1640 medium (Invitrogen; CA, USA) and added with 10% FBS (fetal bovine serum, Invitrogen), 100 U/mL penicillin, and 100 mg/mL streptomycin. The culture atmosphere was humidified and contained 5% CO_2 at 37°C.

Cell transfection

Three small hairpin RNAs (shRNAs) targeting FOXP4-AS1 (sh-FOXP4-AS1-1/2/3) and a negative control shRNA (sh-NC) were all provided by GenePharma (Shan-

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ghai, China). For FOXP4-AS1 overexpression, full-length FOXP4-AS1 cDNA was inserted into pcDNA3.1 vector (Invitrogen), constructing pcDNA3.1-/FOXP4-AS1 and an empty vector functioned as a negative control. For microRNA interference or overexpression, miR-3130-3p inhibitor, miR-3130-3p mimics and the corresponding inhibitor or mimics control were obtained from RioBio (Guangzhou, China). The aforementioned plasmids were transfected into PCa cells using Lipofectamine 3000 reagent (Thermo Fisher Scientific; MA, USA)

Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from tissues or cells using TRIzol Reagent (Thermo Fisher Scientific) and cDNAs were produced using PrimeScript RT Reagent Kit (TaKaRa; Dalian, China). Afterward, qPCR was carried out using SYBR GreenMaster Mix (TaKaRa). The sequences of the PCR primers used in this research were listed in Table 1. Data were calculated using the $2^{-\Delta\Delta Ct}$ method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used as internal controls.

Western blot

Cells lysis was performed with radioimmunoprecipitation assay lysis buffer (Beyotime; Shanghai, China) which was added with protease inhibitors. Total proteins were isolated using gel electrophoresis and transferred onto PVDF membranes (Millipore; MA, USA). After blocking with 5% skim milk, the membranes were incubated with primary antibodies against SP4 (ab151777, Abcam; MA, USA) and GAPDH (ab8245, Abcam). Afterward, the membranes were washed thrice with Tris-buffered saline and cultured with secondary antibodies. For visualization of the protein bands, enhanced chemiluminescence detection reagents (Applygen; Beijing, China) were utilized.

Cell proliferation assays

For the cell counting kit (CCK)-8 assay, transfected cells were placed in 96-well plates which were added with 10 mL of CCK-8 solution (Dojindo; Kumamoto, Japan) after incubation of 24, 48, 72 or 96 h. The absorbance value of each sample at 450 nm was measured using a microplate reader (Bio-Rad; CA, USA).

For the 5-ethynyl-2′-deoxyuridine (EdU) assay, pretreated PCa cells were cultured in EdU solution (Ribobio; Guangzhou, China) for 2 h. To probe EdU, Apollo was used. To indicate the nuclei, DAPI was used.

Caspase-3 activity assay

To measure Casepase-3 activity, a caspase-3 colorimetric assay kit (Abcam) was exploited strictly in line with the guidebook. The absorbance value was obtained using a microplate reader at 405 nm.

Nuclear/cytoplasmic fractionation

DU145 and PC3 cells were subjected to fractionation of nuclei and cytoplasm using a PARIS kit (Life Technologies) following the manufacturer's protocol. RT-qPCR was carried out to measure nuclear and cytoplasmic RNA.

Luciferase reporter assay

FOXP4-AS1 or SP4 fragment with the miR-3130-3p binding site was amplified before being subcloned into psiCHECK-2 vectors (Promega; WI, USA), producing FOXP4-AS1-WT or SP4-WT reporter vector. Additionally, FOXP4-AS1-Mut or SP4-Mut reporter vector was constructed via mutating the putative binding site of miR-3130-3p in FOXP4-AS1 or SP4 with a QuikChange Site-directed Mutagenesis Kit (Agilent, Santa Clara, CA). The luciferase plasmids were co-transfected with miR-3130-3p mimics or NC-mimics into PCa cells. Luciferase activity was measured by a dual-luciferase reporter assay system (Promega) 48 hours after transfection.

Tumor xenografts in animals

Male mice obtained from the Animal Center of Nanjing University (Nanjing, China) were preserved under specific pathogen-free conditions prior to experiments. DU145 cells processed with sh-FOXP4-AS1-1 or sh-NC were harvested followed by subcutaneous inoculation into the flanks of the mice. Tumor growth was measured every 3 days. The mice were stifled 15 days after injection.

RNA immunoprecipitation (RIP) assay

RIP assay was performed in line with the protocol of the Magna RIPTM RNA-binding protein immunoprecipitation kit (Millipore; MA, USA). Cells were collected and lysed in RIP lysate buffer. Then the cell extracts were cultured in RIP buffer which contained magnetic beads bound to Ago2 and IgG. Protease K was later used to digest protein, and the RNA precipitated obtained was subjected to RT-qPCR.

Chromatin immunoprecipitation (ChIP)

In the CHIP assay, an EZ-ChIP kit (Millipore) was utilized. Anti-IgG (negative control) and anti-SP4 were used for immunoprecipitation of chromatin fragments. At length, RT-qPCR analysis was performed to measure the chromatin DNA which has been precipitated.

Table 1. The sequences of the PCR primers used in this research.

Gene	Forward	Reverse
GADPH	5′-TATGATGATATCAAGAGGGTAGT-3′	5′-TGTATCCAAACTCATTGTCATAC-3′
U6	5′-CTCGCTTCGGCAGCACATATACTA-3′	5′-ACGAATTTGCGTGTCATCCTTGCG-3′
FOXP4-AS1	5′-GTGAGCTTCTGGGTTCGACA-3′	5′-ATTGAGGGTTAGGGCAGCAC-3′
SP4	5′-TTGCAGCAAGGCCAGCAGACC-3′	5′-GCTTCTTCTTTCCTGGTTCACTGCT-3′
miR-3130-3p	5′-ATTGCTGCACCGGAGACTG-3′	5′-GAACATGTCTGCGTATCTC-3′
miR-4999-5p	5′-TGCTGTATTGTCAGGTAGTG-3′	5′-GAACATGTCTGCGTATCTC-3′
miR-6890-3p	5′-CATGGGGTAGGGCAGAGTA-3′	5′-GAACATGTCTGCGTATCTC-3′
miR-423-5p	5′-GGGCAGAGAGCGAGAC-3′	5′-GAACATGTCTGCGTATCTC-3′
miR-4797-5p	5′-GACAGAGTGCCACTTACTG-3′	5′-GAACATGTCTGCGTATCTC-3′

Statistical analysis

Data were all presented as means ± SD of independent experiments in triplicate. Statistical analyses were carried out with SPSS version 17.0 software (Abbott Laboratories; IL, USA) and GraphPad Prism 6.0 software (CA, USA). Student's t-test or one-way analysis of variance was utilized to analyze differences between two or multiple groups. Spearman's rank correlation test was used to analyze correlation. Results with a P-value under 0.05 were considered statistically significant.

Results

FOXP4-AS1 is highly expressed in PCatissue specimens and cell lines

The expression pattern of FOXP4-AS1 in PCa was addressed in the first step of this research. Data from GEPIA (http://gepia.cancer-pku.cn/index.html) website indicated that FOXP4-AS1 was amplified in 492 PCa tissue specimens compared with 192 noncancerous tissue specimens (Figure 1A). We detected the expression level of FOXP4-AS1 in PCa tissue samples and normal tissue samples. According to the results of RT-qPCR, FOXP-AS1 expression was remarkably elevated in PCa tissue samples (Figure 1B). Consistently, the expression of FOXP4-AS1 in PCa cell lines was much higher than that in the normal RWPE1 cell line (Figure 1C). Thus it could be concluded that FOXP4-AS1 was upregulated in PCa tissues and cell lines.

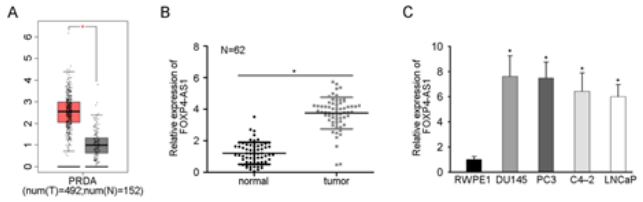

Figure 1. FOXP4-AS1 is highly expressed in PCatissue specimens and cell lines. (A) Data from GEPIA indicated the differential expression of FOXP4-AS1 in 492 PRAD tissue specimens and 152 normal tissue specimens. (B) RT-qPCR revealed the expression of FOXP4-AS1 in PCa tissues and normal tissues. (C) RT-qPCR unmasked the expression of FOXP4-AS1 in four PCa cell lines (DU145, PC3, C4–2, LNCaP) and a noncancerous cell line RWPE1. Experiments were all performed thrice. $^{*}P < 0.05$.

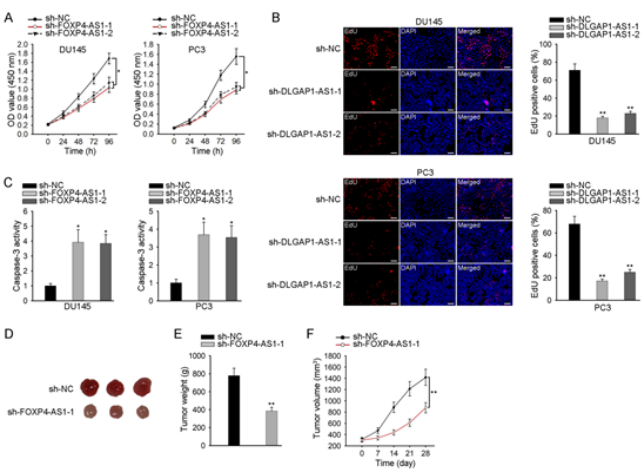

Figure 2. FOXP4-AS1 knockdown inhibits PCa cell growth in vitro and in vivo. (A) CCK-8 assay indicated the absorbance of PCa cells after transfection. (B) EdU assay demonstrated cell proliferation. (C) Casepase-3 activity assay detected Casepase-3 activity to reflect cell apoptosis. (D) In vivo xenograft mice assay was performed and the pictures were taken every three days after injection. (E) Tumors were excised and weighed. The data were presented as the mean tumor weight of each group. (F) Tumor volumes were measured and calculated following the formula: volume = (the longest diameter × the shortest diameter^2)/2. Experiments were all performed thrice. $^{*}P < 0.05$, $^{**}P < 0.01$.

FOXP4-AS1 knockdown inhibits PCa cell growth in vitro and in vivo

A cluster of loss–of–function assays in vitro were carried out to testify to the role of FOXP4-AS in PCa. Results of RT-qPCR indicated that FOXP4-AS1 expression in four DU145 and PC3 cells was dramatically decreased when after transfection of sh-FOXP4-AS1-1, sh-FOXP4-AS1-2 or sh-FOXP4-AS1-3 (Figure S1A). On account of the better knockdown efficacy, sh-FOXP4-AS1-1 and sh-FOXP4-AS1-2 were selected in the subsequent loss-of-function assays. CCK-8 illustrated that cell viability was notably impaired after FOXP4-AS1 depletion (Figure 2A). Analogously, the EdU assay demonstrated that cell proliferation was significantly suppressed when FOXP4-AS1 was silenced (Figure 2B). Afterward, cell apoptosis was assessed by conducting a Casepase-3 activity assay. The results showed that cell apoptosis was dramatically aggravated in the presence of silenced FOXP4-AS1 (Figure 2C). Consistently, *in vivo* xenograft assay indicated that tumor growth in vivo was apparently suppressed after FOXP4-AS1 knockdown (Figure 2D-F). In summary, FOXP4-AS1 depletion suppressed PCa cell growth in vitro and in vivo.

FOXP4-AS1 acts as a molecular sponge of miR-3130-3p in PCa cells

The mechanism underlying FOXP4-AS1 in PCa was then probed. To ascertain the subcellular localization of FOXP4-AS1, nuclear and cytoplasmic FOXP4-AS1 in DU145 and PC3 cells was measured and we discovered that FOXP4-AS1 was predominantly located in the cytoplasm, triggering us to assume its functioning as a molecular sponge (Figure 3A). DIANA tools (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=lncbasev2%2Findex-predicted) predicted a cluster of microRNAs (miRNAs) capable of binding to FOXP4-AS1. Among the miRNAs predicted, five miRNAs were chosen on account of higher binding stringency. After transfection of sh-FOXP4-AS1-1, the expression of miR-3130-3p was most significantly increased while the other four miRNAs didn't present such obvious amplification (Figure S1B). The expression of miR-3130-3p in PCa tissues was examined. As elucidated in Figure 3B, the expression of miR-3130-3p in PCa tissues was manifestly lower than that in the noncancerous tissues. Moreover, the expression of miR-3130-3p and FOXP4-AS1 was negatively correlated, as illustrated in Figure 3C. MiR-3130-3p in PCa cell lines was inhibited (Figure 3D). The putative binding site between FOXP4-AS1 and miR-3130-3p was predicted from DIANA tools and exhibited in Figure 3E. Luciferase reporter assay was then carried out and confirmed the binding within FOXP4-AS1 and miR-3130-3p (Figure 3F). RIP assay further validated the interaction between FOXP4-AS1 and miR-3130-3p (Figure 3G). The

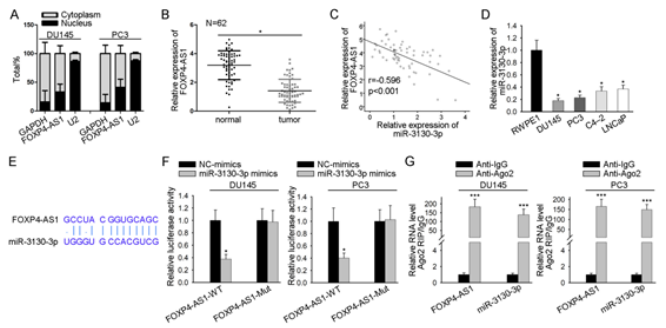

Figure 3. FOXP4-AS1 acts as a molecular sponge of miR-3130-3p in PCa cells. (A) Nuclear/cytoplasmic fractionation determined the subcellular distribution of FOXP4-AS1. (B) RT-qPCR measured the expression of miR-3130-3p in PCa and normal tissues. (C) Spearman's rank correlation test assessed the correlation between FOXP4-AS1 and miR-3130-3p expression. (D) RT-qPCR detected the expression of miR-3130-3p in four PCa cell lines and a normal cell line. (E) DIANA tools predicted the binding site between FOXP4-AS1 and miR-3130-3p. (F) Luciferase reporter assay detected the interaction between FOXP4-AS1 and miR-3130-3p. (G) RIP assay evaluated the binding of FOXP4-AS1 and miR-3130-3p. Experiments were all performed thrice. $^{*}P < 0.05$, $^{***}P < 0.001$.

role of miR-3130-3p in PCa cell growth was also detected. After transfection of miR-3130-3p mimics, cell proliferation was markedly suppressed, as tested by CCK-8 and EdU assays (Figure S1C-D). Additionally, cell apoptosis was facilitated in response to miR-3130-3p upregulation (Figure S1E). In conclusion, FOXP4-AS1 acts as a sponge of miR-3130-3p, which is a tumor suppressor in PCa.

FOXP4-AS1 sponges miR-3130-3p to deregulate SP4

TargetScan (http://www.targetscan.org/vert_71/) predicted that miR-3130-3p targeted the 3′-UTR of SP4 messenger RNA (mRNA). RT-qPCR was conducted and manifested that SP4 expression was apparently higher in PCa tissue specimens (Figure 4A). Besides, SP4 and FOXP4-AS1 expression were positively correlated while SP4 and miR-3130-3p expression was negatively correlated (Figure 4B). The expression of SP4 in PCa cell lines was next examined and the results indicated that SP4 was upregulated in PCa cell lines (Figure 4C-D). After FOXP4-AS1 knockdown, SP4 was decreased and the decreased expression was rescued by co-transfection of miR-3130-3p inhibitor (Figure 4E-F). The putative binding site between SP4 and miR-3130-3p was listed in Figure 4G. FOXP4-AS1 was significantly upregulated after treatment of pcDNA3.1/FOXP4-AS1 (Figure S2A). Luciferase reporter assay confirmed the predicted binding site and illustrated that SP4 luciferase reporter activity was suppressed in response to miR-3130-3p overexpression and that tendency was alleviated by FOXP4-AS1 overexpression (Figure 4H). Afterward, the RIP assay implied the enrichment of FOXP4-AS1, miR-3130-3p and SP4 in the anti-Ago2 group, suggesting their co-existence in RNA-induced silencing complex (RISC). Taken together, FOXP4-AS1 deregulates SP4 from miR-3130-3p in PCa cells.

FOXP4-AS1 regulates PCa progression via SP4

To confirm whether FOXP4-AS1 regulates PCa cell growth via the FOXP4-AS1/miR-3130-3p/SP4 axis, rescue assays were confirmed. SP4 was dramatically upre-

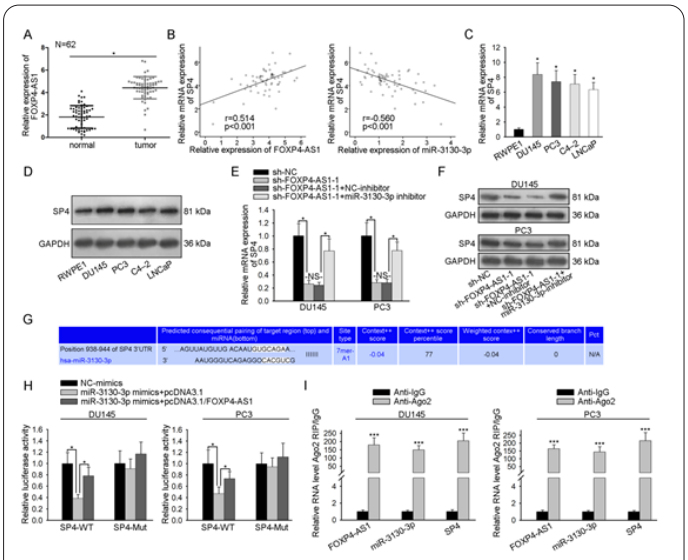

Figure 4. FOXP4-AS1 sponges miR-3130-3p to deregulate SP4. (A) RT-qPCR decided the mRNA levels of SP4 in PCa and non-tumor tissues. (B) Spearman's rank correlation test determined the correlation between SP4 mRNA and FOXP4-AS1 or miR-3130-3p expression. (C) RT-qPCR detected the mRNA levels of SP4 in four PCa cell lines and a normal cell line. (D) Western blot examined the protein levels of SP4 in four PCa cell lines and a normal cell line. (E) RT-qPCR examined the variation of SP4 mRNA level. (F) Western blot tested the variation of SP4 protein level. (G) TargetScan predicted the binding sequence in SP4 with miR-3130-3p. (H) Luciferase reporter assay detected the interaction between SP4 and miR-3130-3p or FOXP4-AS1. (I) RIP assay validated the enrichment of FOXP4-AS1, miR-3130-3p and SP4 in the anti-Ago2 group. Experiments were all performed thrice. $^{*}P < 0.05$, $^{***}P < 0.001$.

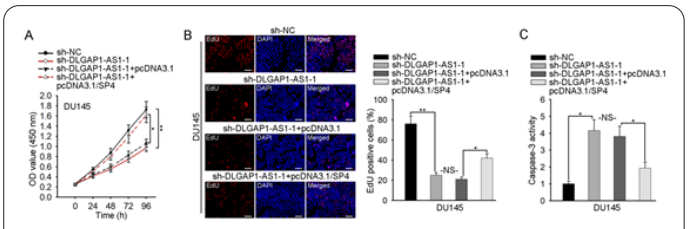

Figure 5. FOXP4-AS1 regulates PCa progression via SP4. (A) CCK-8 assay tested cell viability after transfection. (B) EdU assay evaluated cell proliferation. (C) Casepase-3 assay measured Casepase-3 activity to reflect cell apoptosis. Experiments were all performed thrice. $^{*}P < 0.05$, $^{**}P < 0.01$.

gulated in cells processed with pcDNA3.1/SP4 (Figure S2B-C). The declined cell growth resulting from FOXP4-AS1 depletion was revived by SP4 overexpression (Figure 5A-B). The aggravated cell apoptosis was also mitigated by SP4 overexpression (Figure 5C). Overall, SP4 is a prerequisite for FOXP4-AS1 to modulate PCa progression.

SP4 binds to the promoter region of FOXP4-AS1, forming a positive feedback loop

Considering the transcription role of SP4, we hypothesized that SP4 might in return regulate FOXP4-AS1 at the transcription level. Through UCSC (http://www.genome.ucsc.edu/cgi-bin/hgGateway), we found SP4 was predicted to regulate the transcription activity of FOXP4-AS1 and the binding motif was presented in Figure 6A. Next, we detected the impact of SP4 on FOXP4-AS1 expression. SP4 was knocked down using shRNAs targeting SP4

(sh-SP4-1/2). The knockdown efficiency was examined by RT-qPCR and western blot (Figure S2D-E). Affiliated with the better knockdown efficiency, sh-SP4-1 was selected in the subsequent assay. FOXP4-AS1 was obviously decreased in cells depleting SP4 (Figure 6B). ChIP analysis revealed the binding affinity of SP4 with the promoter region of FOXP4-AS1 (Figure 6C). Luciferase reporter assay showed that SP4 knockdown suppressed the relative luciferase activity of the FOXP4-AS1 promoter (Figure 6D). To sum up, this research concluded that SP4 could bind to the promoter region of FOXP4-AS1, thus positively regulating its transcription activity and constituting the FOXP4-AS1/miR-3130-3P/SP4feedback loop (Figure 6E).

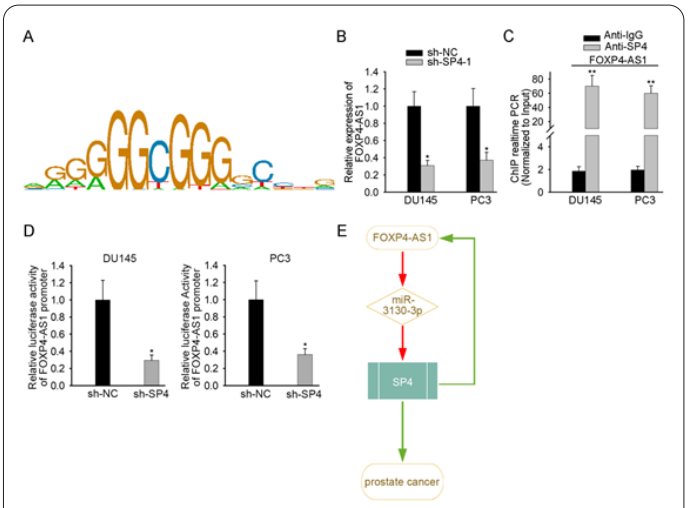

Figure 6. SP4 binds to the promoter region of FOXP4-AS1, forming a positive feedback loop. (A) UCSC genome browser predicted the binding motif of SP4 and FOXP4-AS1. (B) RT-qPCR detected FOXP4-AS1 expression in PCa cells treated with sh-SP4-1 or sh-NC. (C) ChIP assay determined the binding of SP4 and FOXP4-AS1 promoter. (D) Luciferase reporter assay detected the interaction between SP4 and FOXP4-AS1 promoter. (E) A concept map summarizing the results of the whole research. Experiments were all performed thrice. $^{*}P < 0.05$, $^{**}P < 0.01$.

Discussion

PCa is a malignancy with a high incidence rate afflicting females in the USA. As estimated, 164,690 new PCa cases will be diagnosed and 29,430 patients will die from PCa in 2018 (8). It might provide new insight into PCa diagnosis and therapeutics to decipher novel molecular mechanisms involved in PCa progression.

Large quantities of lncRNAs have been reported to possess a close relationship with PCa progression. For example, long noncoding RNA SChLAP1 accelerates the proliferation and metastasis of prostate cancer via targeting miR-198 and promoting the MAPK1 pathway (10). Androgen-induced long noncoding RNA (lncRNA) SOCS2-AS1 promotes cell growth and inhibits apoptosis in prostate cancer cells (11). Long noncoding RNA LINC01296 is associated with poor prognosis in prostate cancer and promotes cancer cell proliferation and metastasis (12). FOXP4-AS1 has been unveiled as an oncogenic lncRNA in colorectal cancer and osteosarcoma (7, 8). Herein we unmasked the elevated expression levels of FOXP4-AS1 in PCa tissue specimens and cell lines. Moreover, PCa cell growth was retarded not only in vitro but also in vivo as a result of depleted FOXP4-AS1.

LncRNAs have been frequently reported to act as competing endogenous RNAs (ceRNAs) to sequester miRNAs and thus deregulate the downstream targets of the miRNAs (13). For instance, long noncoding RNA PVT1 facilitates cervical cancer progression via negative regulating of miR-424 (14). Long non-coding RNA UICLM promotes colorectal cancer liver metastasis by acting as a ceRNAfor microRNA-215 to regulate ZEB2 expression (15). SNHG1 lncRNA negatively regulates miR-199a-3p to enhance CDK7 expression and promote cell proliferation in prostate cancer (16). The cytoplasmic distribution of FOXP4-AS1 provided the prerequisite for FOXP4-AS1 to act as a ceRNA. MiR-3130-3p has been reported as a tumor suppressor in endometrial cancer (17). Our study demonstrated that miR-3130-3p was downregulated in PCa tissues and cell lines. The interaction between FOXP-AS1 and miR-3130-3p was also validated. In addition, PCa cell growth was suppressed after introducing miR-3130-3p mimics into PCa cells.

Specificity protein 4 (SP4), belonging to the SP family, is known as a transcriptional activator (18-20). The association between SP proteins and PCa progression has been elucidated in increasing reports (21-23). This research confirmed that FOXP4-AS1 promoted SP4 expression via sequestering miR-3130-3p. Besides, SP4 was a prerequisite for FOXP4-AS1 to modulate PCa cell proliferation. Moreover, SP4 could in return positively regulate FOXP4-AS1 expression by activating its transcription activity.

Overall, FOXP4-AS1 contributed to PCa cell growth via sequestering miR-3130-3p and thus disinhibiting SP4, which in return transcriptionally activated FOXP4-AS1. Our study indicates a new valuable diagnosis and treatment strategy for PCa.

Conflicts of interest

The authors declare that no conflicts of interest exist.

Funding

None.

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