LncRNA AC245100.4 affects the tumorigenesis of prostate cancer via regulating the STAT3/NR4A3 axis

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Research

Keywords: LncRNA, Prostate cancer, NR4A3, STAT3, Proliferation, Migration, Apoptosis

Posted Date: December 22nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-131948/v1>

Abstract

Background

Prostate cancer (PCa) is the second most common cancer and the fifth leading cause of cancer deaths among men globally. However, the molecular mechanisms leading to the progression have not been fully elucidated.

Methods

The expression and location of AC245100.4 were examined by RT-qPCR and nuclear-cytoplasmic separation assay. RNA-seq analysis was performed to identify the downstream of AC245100.4. RNA immunoprecipitation was performed to identify the proteins those bind to AC245100.4. Western blotting was performed to quantify the expression of proteins. Finally, a series of gain- or loss-functional assays were done to prove the precise role of AC245100.4 and NR4A3 in PCa.

Results

We identify a critical lncRNA AC245100.4, which is significantly up-regulated in prostate cancer tissues and cells. Knockdown of AC21500.4 can significantly inhibit prostate cancer progression in vitro and in vivo. Further RNA-seq analysis shows that NR4A3 may be the potential target of AC245100.4. Mechanistically, AC245100.4 de-regulates NR4A3 transcriptionally via increasing p-STAT3, which is a transcriptional repressor of NR4A3.

Conclusion

Our results demonstrated that AC245100.4 was a critically oncogenic lncRNA in PCa via inhibiting NR4A3 and paved a promising avenue to combat PCa progression by targeting AC245100.4 or NR4A3.

Background

Prostate cancer (PCa) is the second most common cancer and the fifth leading cause of cancer deaths among men. In 2018, It is estimated that approximately 1.3 million new cases related to prostate cancer with 359,000 deaths at the same time¹. Studies have shown that tumor progression is closely related to the tumor microenvironment², non-coding RNAs (ncRNAs) such as miRNAs³ and long non-coding RNAs (lncRNAs)^{4,5} and various protein molecules^{6,7}. Therefore, unveiling underlying mechanism of PCa progression is imperative in clinic.

Accumulating evidence has improved our awareness that ncRNA is an important component of complex regulatory networks in vivo^{8,9}, including miRNA and lncRNA. Among them, lncRNA is a kind of RNA

transcripts with a length of more than 200nt and limited coding protein potential, which is differentially expressed in many specific normal and cancerous tissues¹⁰. Recently, lncRNA regulates gene expression via various ways, including epigenetics^{11, 12}, transcriptional activation¹³, post-transcriptional regulation¹⁴ and translation regulation¹⁵. In PCa, lncRNA rapidly receives significance for their vital roles in many pathophysiological processes^{16–18}. Although the expression and function of several lncRNAs have been investigated in PCa, the mechanisms of most lncRNAs in PCa are still unclear.

STAT3 belongs to the nuclear transcription factor which regulates various physiological and pathological processes, such as cell proliferation, differentiation, apoptosis and metastasis¹⁹. Studies have shown that some lncRNAs play significant role in the activation of JAK/STAT signaling pathway, thereby affecting tumorigenesis and development^{20–22}. Recent research reports that p-STAT3 can bind to the promoter of Nuclear receptor subfamily 4A3 (NR4A3, also known as NOR-1) to inhibit the transcription of NR4A3, thereby inhibiting cell apoptosis and promoting cell proliferation²³. However, the role of NR4A3 has not been reported in PCa.

Although the function of most lncRNAs is still blurry, the accumulating evidence for their involvement in many biologic processes provides compelling arguments in supporting of their significance. In our previous study, we found that an up-regulated lncRNA AC245100.4 could bind to HSP90 to promote the proliferation of prostate cancer²⁴. In this study, we identified the vital role of AC245100.4 on regulating the progression of PCa via inhibiting NR4A3. Mechanistically, AC245100.4 de-regulates NR4A3 transcripts via decreasing p-STAT3, which acts as a transcriptional repressor of NR4A3. In summary, these results suggest that further research is needed to explore non-coding genes to provide new targets for the diagnosis and prognosis of prostate cancer.

Methods

Cell culture

Human prostate epithelial cells, RWPE-1 and human prostate cancer cell lines (DU145, PC3, 22RV1, LNCAP) were purchased from the American Type Culture Collection (Manassas, VA, USA). All the cell lines were cultured in DMEM medium supplemented with 10% serum and 1% penicillin-streptomycin at 37°C with 5% CO₂.

Real-time quantitative PCR (RT-qPCR)

Trizol reagent (TaKaRa, Japan) was used for extraction of total RNA. The Prime Script® RT Reagent Kit with gDNA Eraser (TaKaRa, Japan) was performed for reverse transcription according to the manufacturer's instructions. The relative expression of RNA was calculated by 2^{-ΔΔCT} method.

Cell Counting Kit-8 (CCK-8)

Firstly, PC3 and DU145 cells were seeded into 96 wells. Then CCK-8 kit (Beyotime, Nantong, China) was performed to detect the proliferation of PCa cells according to the manufacturer's instructions. Readings of Absorbance were measured with a plate reader at 450 nm.

Colony Formation Assay

PC3 and DU145 cells were seeded into 96 wells. After transfection, cells were incubated for 14 days after transfection. Methanol was performed to fix cells and crystal violet was performed to stain the cells. Finally, the colonies was observed and counted ($\times 50$ cells).

Wound healing migration assay

Primarily, PC3 and DU145 cells were seeded into wells. Vertical wounds were created using 200- μ l pipette tips in each plate after transfection. The wounds were observed in each well were imaged in 0 h or 24 h incubation.

Transwell Assay Cell migration assays

Transwell chambers (Corning, NY, USA) were performed to detect the migration of PC3 and DU145 cells. Serum-free medium was filled into upper chambers, and 10% serum medium was filled into the lower chambers. The cells migrated from upper chambers to the lower chambers were fixed and stained by crystal violet. Finally, the cells in lower chambers were observed and imaged.

Xenograft mouse model

All animal experiments were approved by the Animal Care and Use Committee of Harbin Medical University. Approximately 1×10^7 sphere cells were resuspended in media, followed by subcutaneous implantation in flanks of male BALB/c nude mice (4–6 weeks old). Tumor volumes were determined using a caliper according to the standard formula: $V = (\text{length} \times \text{width}^2)/2$. Five weeks later, the mice were sacrificed and tumors were analyzed. To pulmonary metastasis, approximately 5×10^6 DU145 cells were injected into in male BALB/c nude mice by tail intravenous. The mice were sacrificed after 5 weeks and lung tissue were analyzed.

Immunoprecipitation assays

Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Milipore, Darmstadt, Germany) was used to detect the interaction between STAT3 and AC245100.4 according to the manufacturer's instructions. Primarily, cells were washed with PBS for 3 times, then resuspended in a lysis buffer for 5 min. secondly, Cell lysate was incubated at 4°C with magnetic beads and 10 μ g STAT3 (CST, USA) or IgG antibodies, with overnight shaking. The magnetic beads were separated to obtain the RNA-Protein complex. Finally, RNA was extracted by Trizol and detected by RT-qPCR assay.

Chromatin immunoprecipitation assays

Simple-ChIP Enzymatic Chromatin IP kit (CST, USA) was used to detect the interaction between p-STAT3 and promoter of NR4A3 according to the manufacturer's instructions. Anti-p-STAT3 (ser 705) (CST, USA)

was employed in ChIP assay, with goat anti-rabbit IgG used as a negative control.

Statistical analysis

All experiments were repeated for three times. Graph-Pad prism version 7 was used for statistical analysis. For comparisons between two groups, the Student's t test or Chi-squared test was used. $P < 0.05$ was considered to be statistically significant.

Results

AC245100.4 is up-regulated in prostate cancer tissues and cells.

AC245100.4 is transcribed from the positive strand of a genomic DNA sequence located on chromosome 1q21.1 (from 143699511 to 143717173) with length of 1555 bp. In this study, the expression of AC245100.4 was higher in prostate cancer tissues compared with normal prostate tissues from TCGA data (Fig. 1A). To prove whether the lncRNA is associated with prostate cancer, we detected the expression of AC245100.4 in common tumor cells. The results showed that AC245100.4 has relatively high expression in PCa, especially in PC3 and DU145 cells (Fig. 1B&C), and it was mainly located in cytoplasm (Fig. 1D). These results suggested that AC245100.4 may be closely related to prostate cancer and may play a major biological role in the cytoplasm.

AC245100.4 regulates the proliferation, migration and apoptosis of PCa cells in vitro.

It has now become widely accepted that lncRNAs function as crucial regulators at each step during genetic information processing in the living cell^{25, 26}. In this study, we down-regulated the expression of AC245100.4 in PC3 and DU145 cell, then detect the biological function of the cells. The results showed that the proliferation and migration of above cells were inhibited through interference of AC245100.4 (Fig. 2A-D). Furthermore, we found that the percentage of apoptosis was increased via knockdown of AC245100.4 (Fig. 2E&F). Meantime, the protein expression of Bcl-2 was decreased and the expression of activated caspase-3 was increased compared with control cells (Fig. 2G). These results suggested that AC245100.4 was closely related to prostate cancer and plays an important biological role as an oncogene.

AC245100.4 regulates the proliferation and migration PCa cells in vivo.

To explore the effect of AC245100.4 on tumor proliferation and metastasis in vivo, DU145 cells with sh-AC245100.4 or sh-ctrl were inoculated into male nude mice subcutaneously. The smaller subcutaneous tumors were found in the sh-AC245100.4 group were significantly compared with the sh-ctrl group (Fig. 3A&B). consistently, the mean volume and weight of tumors were substantially lower in the group of sh-AC245100.4 (Fig. 3C&D). Tumors formed from sh-AC245100.4 DU145 cells suggested the weak positivity for Ki-67 than tumors from the sh-ctrl (Fig. 3E). Moreover, the metastatic nodules formed in sh-AC245100.4 were less than in sh-ctrl group (Fig. 3F). These observations were consistent with the results

in vitro. Taken together, these findings suggested that the interference of AC245100.4 inhibited tumor proliferation and metastasis of PCa cells in vivo.

AC245100.4 regulates the expression of NR4A3.

To date, the specific function of lncRNAs in the regulation of tumor progression is not well known. Thus, to explore the precise mechanism of lncRNAs in PCa progression could increase the understanding of the specific mechanisms of PCa tumorigenesis. In this research, the second-generation sequencing (Next generation sequencing, NGS) was used to explore the mechanism of AC245100.4. There were 117 up-regulated genes and 72 down-regulated genes in the condition of AC245100.4 silence (Fig. 4A and 4B). GO functional classification of differential genes shows that differential genes mainly exist in cell process, biological regulation, biological process regulation and other molecular functions (Fig. 4C&D). KEGG pathway analysis showed that differential genes were mainly enriched in cell communication, signal transduction, molecular folding and classification degradation, cancer, metabolism, immunity and other pathways (Fig. 4E&F). Among 189 different genes, we further performed RT-qPCR to detect the relative mRNA expression of several genes related to tumor progression via the knockdown of AC245100.4 (Znf177, APIP, GDI2, YOD1, NR4A3). Interestingly, NR4A3 as a significantly up-regulated gene caught our attention (Supplement.1). Then, we found suggested that the knockdown of AC245100.4 could increase the expression of NR4A3 (Fig. 4G&H). Moreover, the same results could be found in xenograft mouse model by immuno-histochemical staining (Fig. 4I). Anyway, the expression of NR4A3 was upregulated via the silence of AC245100.4. However, the correlation between NR4A3 and AC245100.4 and the role of NR4A3 on progression of PCa are still a fog.

Overexpression of NR4A3 inhibits proliferation and induces apoptosis in PCa.

Previous research has found that heterogeneously expressed NR4A3 could decrease the growth of non-small cell lung cancer²⁷. In addition, lncRNA LINC00467, as an oncogenic lncRNA, it was a highly expressed in Hepatocellular carcinoma by repressing NR4A3²⁸. Therefore, whether AC245100.4 can regulate the expression of NR4A3, and the relationship between NR4A3 and the occurrence and development of prostate cancer, which still need to be explored. TCGA data shows that NR4A3 was lower expressed in prostate cancer tissues than its in normal prostate tissues (Fig. 5A). Also, the same result was found in prostate cancer cells and normal prostate cells (Fig. 5B). Therefore, the above results indicate that NR4A3 may be associated with prostate cancer. Given that NR4A3 was lower expressed in prostate cancer cells, we over-expressed NR4A3 in PC3 and DU145 cell lines (Supplement.2). At the same time, the proliferation of above cells was decreased compared with the control cells (Fig. 5C&D). Importantly, the apoptosis rates were increased in the PC3 and DU145 cells by overexpression of NR4A3 (Fig. 5E&F). Meantime, the Bcl-2 protein was down-regulated and the activated caspase-3 protein was up-regulated in above cells (Fig. 5G).

To study the role of NR4A3 on tumor growth and metastasis in vivo, DU145 cells with NR4A3 plasmid or control vector were inoculated into nude mice. Five weeks later, the volume and weight of tumors were

substantially smaller in the NR4A3 group than its in the control group (Fig. 5H, I&J). The formed tumors in NR4A3 overexpression group had lower positivity for Ki-67 compared with the control group (Fig. 5K). Taken together, these results demonstrated NR4A3 may play the role of tumor suppressor in PCa.

Knockdown of AC245100.4 increases the expression of NR4A3 by regulating the phosphorylation status of STAT3.

Previous studies have shown that NR4A3 was transcriptionally silenced by STAT3 in the gastric cancer²⁹. Meantime, several lncRNAs have been revealed to regulate phosphorylation of STAT3 in a direct binding manner or other manners^{27, 30}. Therefore, we suspected that AC245100.4 increased phosphorylation of STAT3 by direct binding, thus silenced NR4A3. As shown in the results, the protein level of p-STAT3 was decreased by knockdown of AC245100.4, while there was no change in total STAT3 (Fig. 6A). Also, the relative expression of STAT3 had no change via the knockdown of AC245100.4 (Fig. 6B). IL6 is believed to mediate STAT3 tyrosine phosphorylation and cryptotanshinone is a STAT3 inhibitor³¹. To further determine whether STAT3 could influence the expression of AC245100.4, PC3 and DU145 cells were stimulated with IL6 or cryptotanshinone. However, we didn't observe the change of the expression of AC245100.4 after with IL-6 or cryptotanshinone treatment (Fig. 6C&D). These findings indicated that STAT3 is the downstream of AC245100.4. Further RIP assays suggested that AC245100.4 was directly bound to STAT3 (Fig. 6E). To investigate whether the knockdown of AC245100.4 could influence the enrichment of p-STAT3 in the promoter of NR4A3. As shown in Fig. 6F, ChIP assays suggested that the knockdown of AC245100.4 prominently suppressed the enrichment of p-STAT3 in the promoter of NR4A3. In a word, AC245100.4 regulating the expression of NR4A3 was accomplished by directly bound to STAT3 and inducing its phosphorylation status.

Silence of NR4A3 attenuates the tumor suppressive roles of AC245100.4 knockdown in PCa.

To investigate whether the tumor-promoting role of AC245100.4 was accomplished by NR4A3 in PCa, we designed three interfering segments of NR4A3 and selected the segment with the best interference efficiency for subsequent experiments (Fig. 7A&B). We co-transfected interfering fragments of NR4A3 and AC245100.4 in PC3 and DU145 cells. Next, we found that the silence of NR4A3 reversed the inhibition of cell viability caused by AC245100.4 knockdown (Fig. 7C&D). Besides, the increasing protein level of NR4A3 was verified in the group of the silence of AC245100.4, however, the results were reversed by treating with IL6 (Fig. 7E). In brief, these results proved convincingly that NR4A3 drops off the tumor promoting role of AC245100.4 in PCa and that NR4A3 may act as a potential tumor suppressor in PCa.

Discussion

PCa is one of the most common malignant tumors in men worldwide³². Relatively, the incidence and mortality of PCa are lower in China. However, with the aging of the population, and the changing in diet and lifestyles, PCa is becoming a serious urinary malignancy affecting the health of Chinese males³². It has appeared a lot of studies about PCa, but the pathogenesis of PCa has not been well elucidated. With

the further development of research and biotechnology, especially the emergence of high-throughput sequencing technology, a large number of lncRNAs were found, which are an important part of the complex regulatory network in the body³³. Therefore, it will be helpful for providing a novel theoretical foundation in the prevention and treatment of PCa by revealing the underlying mechanisms in these regulatory transcripts.

lncRNAs, the novel class of ncRNAs with more than 200nt in length, play significant roles in controlling gene expression^{34, 35}. Biologically, lncRNAs have intriguing properties with important regulatory function and present as promising biomarkers and therapeutic targets³⁶. Nevertheless, the significance of lncRNAs on the oncogenesis and progression of PCa is still not clear. Given that lncRNAs are important components of complex regulatory networks in vivo and regulate different genes by various ways, RNA-seq assays were used to explore specific regulatory networks of AC245100.4 in PCa. As shown in RNA-seq analysis, there are 189 differential expression genes after the silence of AC245100.4. Interestingly, NR4A3 as a significantly up-regulated gene caught our attention.

NR4A3 belongs to the nuclear receptor (NR) family 4A group (NR4A), which regulates the expression of specific genes through a ligand-dependent manner. Recently, studies have shown that NR4A3 can participate in the processes of many diseases, especially tumors^{37 38}. In the current study, lower expression of NR4A3 was found in PCa tissues and cells compared with normal prostate tissues and cells. Consistently, functional studies also showed that the overexpression of NR4A3 inhibited the growth of PCa cells. In addition, we found that the knockdown of AC245100.4 upregulated NR4A3 expression in PCa cells. Furthermore, rescue experiment showed that the silence of NR4A3 reversed the repressing role caused by AC245100.4 deletion on PCa progression. Therefore, these results suggested that the inhibitory effect of AC245100.4 silence on PCa is partially accomplished by NR4A3.

Previous study have reported that STAT3 binds to the promoter of NR4A3 and represses its transcriptional activity in gastric cancer cells²⁹. Consistently, we found that p-STAT3 is enriched in the promoter of NR4A3. Our data further found that the stimulation of STAT3 by IL6 reversed the increasing expression of NR4A3 caused by AC245100.4 knockdown, which supported that the process AC245100.4 regulates the transcription of NR4A3 was mediated by STAT3. AC245100.4 regulates the transcriptional regulation of NR4A3 was mediated by p-STAT3. In the previous study, several lncRNAs have been reported to directly interact with STAT3 to alter the phosphorylation level of STAT3^{39, 40}. In the present study, we discovered that AC245100.4 directly interacted with STAT3 to change its phosphorylation level. However, how AC245100.4 regulates phosphorylation level of STAT3 and whether there are other genes regulated by STAT3 in the results of RNA-seq are still ambiguous and need further investigation.

Conclusion

In summary, we identified a critical lncRNA AC245100.4, which is markedly up-regulated in prostate cancer tissues and cells and the knockdown of AC21500.4 significantly inhibits the progression of prostate cancer in vitro and in vivo. Moreover, NR4A3 acts as a potential target of AC245100.4 in the

progression of prostate cancer. Mechanistically, the silence of AC245100.4 up-regulates NR4A3 transcriptionally via decreasing p-STAT3, which acts as a transcriptional repressor of NR4A3 (Fig. 8). These results suggest that AC245100.4 may act as a tumor promoter and it may be potential therapeutic strategies for PCa. Moreover, further research is needed to explore non-coding genes to provide new targets for the diagnosis and prognosis of PCa.

Declarations

Ethical Approval and Consent to participate

All animal experiments were approved by the Animal Care and Use Committee of Harbin Medical University.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of supporting data

The data sets supporting the results of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgement and Funding

This study was supported financially by the Fundamental Research Funds for the Provincial Universities (Grant No. 2018-KYYWF-0432) and the Postdoctoral Scientific Research Developmental Fund of China (Grant No. 212538).

Authors' contributions

Xiaoguang Yu, Jiabin Zhao: conceptualization; Weiyu Yang, Shan Jiang: formal analysis; Nan Wang, Rou Li, Xu Yang: data curation; Chi Liu: validation and writing - original draft; Hui Xie, Ping Lin: writing - review and editing. All authors edited and approved the manuscript.

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Figures

Figure 1

The expression of AC245100.4 was up-regulated in prostate cancer tissues and cells. (A) AC245100.4 expression in prostate adenocarcinoma tissues from TCGA database. (B) The expression of AC245100.4 in various cancer cells. (C) The relative AC245100.4 expression in prostate cancers cells and prostate cells. (D) The expression levels of AC245100.4, U6 and GAPDH in the nuclear and cytoplasm of PC3 and DU145 cells were detected by q-PCR after nuclear-cytoplasmic separation. *P < 0.05, ** P < 0.01.

Figure 2

AC245100.4 regulates the proliferation, migration and apoptosis of PCa cells in vitro. (A&B) The effect of AC245100.4 on the proliferation was tested by CCK-8 and colony formation assay in PC3 and DU145 cells. (C) The effect of AC245100.4 on the migration was detected by Transwell in PC3 and DU145 cells. (D) The role of AC245100.4 on the migration was detected by wound healing in PC3 and DU145 cells. (E&F) The apoptosis rates were detected by flow cytometry analysis and the representative images in PC3 and DU145 cells were shown. (G) After repression of AC245100.4, the protein expression changes of Bcl-2 and cleaved caspase-3 were determined by western-blot. * $P < 0.05$, ** $P < 0.01$.

Figure 3

AC245100.4 regulates the proliferation and migration of PCa cells in vivo. (A&B) The tumors were removed from the tumor-bearing mice. (C) The tumor volume was recorded every week. (D) Tumor weight of tumor-bearing mice. (E) Effects of AC245100.4 on morphology and expression of Ki67 in tumor-bearing mice were detected by HE stain and immunohistochemistry (20×). (F) The effects of AC245100.4 on the pulmonary metastasis of nude mice were detected by HE stains (20×). *P<0.05, ** P<0.01.

Figure 4

AC245100.4 regulates the expression of NR4A3. (A) The expression heat-map of DEG from RNA-seq; (B) The DEG expression volcano distribution map. (C&D) Significantly different genes were enriched in terms associated with cancer by enrichment analysis of GO after knockdown of AC245100.4. (E&F) The most enriched pathways GO analysis after knockdown of AC245100.4. (G&H) The expression of NR4A3 was detected by RT-qPCR and WB after knockdown of AC245100.4. (I) The effects of AC245100.4 on the

expression of NR4A3 in tumor-bearing mice were detected by immunohistochemistry (20×). * P<0.05, ** P<0.01.

Figure 5

Overexpression of NR4A3 inhibits proliferation and induces apoptosis in PCa. (A) The expression of NR4A3 in prostate cancer and normal tissues from TCGA database. (B) The relative NR4A3 expression in prostate cancer cells and normal cells were detected by qRT-PCR. (C&D) The proliferation of PC3 and

DU145 cells was determined by CCK-8 and colony formation after overexpression of NR4A3. (E&F) The apoptosis rates were detected by flow cytometry analysis and the representative images in PC3 and DU145 cells were shown. (G) After overexpression of AC245100.4, the protein expression changes of Bcl-2 and cleaved caspase-3 were determined by western-blot. (H) The tumors were removed from the tumor-bearing mice. (I) The tumor volume was recorded every week. (J) Tumor weight of tumor-bearing mice. (K) Effects of NR4A3 on morphology and expression of Ki67 in tumor-bearing mice by HE stains and immunohistochemistry (20×). * P<0.05, ** P<0.01, *** P<0.001.

Figure 6

Knockdown of AC241500.4 increases the expression of NR4A3 by regulating the phosphorylation status of STAT3. (A) The protein level of STAT3 and p-STAT3 in PC3 and DU145 cells were determined by western blot. (B) The relative STAT3 expression in PC3 and DU145 cells were determined by qRT-PCR. (C&D) The relative expression of AC245100.4 was detected after stimulation with 50ng/ml IL6 and 5μM Crypto for 24h respectively. (E) The interaction of STAT3 and AC245100.4 were performed by RIP. (F) The

enrichment of p-STATS in the promoter of NR4A3 was detected by ChIP assays to detect * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Figure 7

Silence of NR4A3 attenuates the tumor suppressive roles of AC245100.4 knockdown in PCa. (A&B) The interference efficiency of si-NR4A3 in PC3 and DU145 cells were determined by qRT-PCR and western blot. (C&D) The proliferation of PCa cells was determined by CCK8 and colony formation assays after co-interference of AC245100.4 and NR4A3. (E) Western blot was used to explore the protein level of NR4A3 after knockdown of AC245100.4 and adding of IL6. * $P<0.05$, ** $P<0.01$. *** $P<0.001$.

Figure 8

A scheme of the proposed mechanisms. AC245100.4 is markedly up-regulated in PCa and the knockdown of AC21500.4 significantly inhibits the progression of PCa. Moreover, the silence of AC245100.4 up-regulates NR4A3 transcriptionally via decreasing p-STAT3, which acts as a transcriptional repressor of NR4A3.

Supplementary Files

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