Long Noncoding RNA TCONS_00027385 Acts as a miR-874-5p Sponge to Suppress the Progression of Prostate Cancer Through Regulating ASCC2 Expression

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Keywords: Prostate cancer, TCONS_00027385, proliferation, apoptosis, miR-874-5p, ASCC2

DOI: https://doi.org/10.21203/rs.3.rs-728951/v1
Abstract

Background: A novel pyrrolo indole alkaloids, named Robustanoids A, was isolated from Coffea canephora beans, and it inhibits proliferation of prostate cancer (PCa) cells. However, the molecular mechanism linking Robustanoids A to the tumorigenesis of PCa is not yet clear.

Methods: We investigated the expression of lncRNAs in PCa cells with Robustanoids A and control group by microarray analysis. The expression level of TCONS_00027385 in PCa tissues and cell lines was detected by qRT-PCR. Additionally, we conducted functional experiments to investigate the biological effects of TCONS_00027385 on the development of PCa both in vitro and in vivo. Furthermore, bioinformatic analysis, luciferase reporter experiment, RIP assay, pulldown assay, and protein chip were performed to investigate the oncogenic molecular mechanisms of TCONS_00027385.

Results: In our current study, we focused on TCONS_00027385, which was up-regulated in PCa tissues and cell lines. The high expression of TCONS_00027385 was related to the progression of PCa. Function assays revealed that silencing TCONS_00027385 inhibited PCa cell proliferation and induced apoptosis, while over-expression of TCONS_00027385 remarkably played an opposite role. A deeper investigation showed that TCONS_00027385 acted as a sponge for hsa-miR-874-5p in PCa, and ASCC2 was a target of miR-874-5p in the downstream. Moreover, a positive association between TCONS_00027385 with ASCC2 and a negative relationship between miR-874-5p and TCONS_00027385 (or ASCC2) were also founded. According to the rescue assay, inhibiting ASCC2 could partially suppress the oncogenic effect on cell proliferation and apoptosis in PCa caused by the overexpression of TCONS_00027385.

Conclusion: TCONS_00027385 acted as a competing endogenous RNA (ceRNA) for miR-874-5p to regulate the expression of ASCC2. TCONS_00027385 regulated the miR-874-5p/ASCC2 axis to promote PCa progression.

Background

Prostate cancer (PCa) is the one of most frequent malignancy involving uncontrolled cell growth, and its high mortality imposes a heavy burden to patients, family and society (1–3). Furthermore, in some geographical regions such as North America, Africa and East Asia, the mortality rate of PCa has increased for at least two decades (4–6). Despite significant advance has been made in early diagnosis, it is often unable to be detected in a timely manner. In addition, a high relapse rate after androgen deprivation therapy and chemotherapy also resulted a high mortality in advanced PCa (7). Therefore, effective treatment strategies for PCa are urgently needed. Recently, emerging researches has emphasized that long noncoding RNAs (lncRNAs) might play as new biomarkers for cancer diagnosis as well as potential targets for cancer treatment (8).

LncRNAs have been found to play important roles in various physiological as well as pathological processes (9, 10). Today, thousands of highly expressed lncRNAs have already been identified in humans, which are characterized by high tissue-specification and stable structures (11–13). Expression
disturbance of lncRNA expression might lead to abnormal expression of genes and promote tumorigenesis and carcinogenesis (14–16). Recently, emerging researches suggested that lncRNAs might also participate in PCa, including cell proliferation, metastasis, migration, and invasion (17, 18), however, the detailed associations between lncRNAs with PCa are largely unknown and need to be addressed.

Our previous research discovered a novel pyrrolo indole alkaloid (Robustanoids A) from *C. canephora* (a kind of robusta coffee beans) (19), which repressed proliferation and promoted apoptosis of PCa cells. However, the antitumor mechanism triggered by Robustanoids A in PCa cells has never been elucidated. Therefore, based on our experimental results, we first analyzed the expression profiles of lncRNAs by Robustanoids A in PCa cell through microarrays. We focused on hsa- TCONS_00027385 located on chromosome 19, and founded that the expression of TCONS_00027385 increased in PCa tissues and cell lines, which was related to the proliferation and apoptosis of PCa cells *in vitro* and tumor growth *in vivo*. Mechanistically, we discovered that TCONS_00027385 might act as a sponge of miR-874-5p, which could further upregulate the expression of activating signal co-integrator complex 2 (ASCC2). Our current evidence above suggested that TCONS_00027385 might play the role of a potential biomarker in the occurrence of PCa and provided a novel target in PCa clinical treatment.

### Materials And Methods

#### Cell culture and reagents

The cell lines used in this study provided by Stem Cell Bank of Chinese Academy of Sciences and were cultured at 37 °C with 5 % CO₂ and 95 % air, as the instructions from the manufacturer. Briefly, cells of WPMY-1, VCaP and DU-145 were maintained in Dulbecco's Modified Eagle's Medium (12800017, GIBCO). Cells of 22RV1 and LNCaP were maintained in RPMI 1640 medium (31800022, GIBCO) and PC-3 were cultured in F12K medium (21127022, GIBCO). Cells were cultured in the complete mediums containing the above mediums respectively, 10 % fetal bovine serum (10099141C, GIBCO), 100 U/ml penicillin, and 100 μg/ml streptomycin.

**Robustanoids A treatment on 22RV1 cells**

Robustanoids A (Fig. 1a) was isolated and purified from *C. canephora*. 22RV1 cells were plated in 6-well plates followed by treated with 10 μM of Robustanoids A for 48 hr. In the control group, cells received 0.1% DMSO, which was tantamount to the amount in the experimental group.

**Microarray analysis**

After Robustanoids A treatment, cells were frozen by liquid nitrogen as soon as possible. According to Arraystar’s protocol (Rockville, MD, USA), samples were prepared and microarray hybridization were performed. Data were further remained after deletion of repeat sequences and noncoding RNAs (ncRNAs) less than 200 bp. Subsequently, hybridization was performed in Arraystar Human LncRNA Microarray V5.0, intended for comprehensive analysis of human lncRNAs and protein coding transcripts, while
Agilent Scanner G2505C (Jamul, CA, USA) was used to scan data. LncRNAs which were meeting the condition of fold-changes $\geq 2$ and $P$-values < 0.05 were considered to be significantly differentially expressed.

**Patients and specimens**

PCa clinical samples and the corresponding normal samples were provided by 100 PCa patients who accepted surgically resection in the Urology Department of the First Affiliated Hospital of Xinjiang Medical University in 2020 and 2021 (the ethical number: 20210301-92). The enrolled patients were pathologically confirmed and did not have androgen deprivation treatment, chemotherapy, radiotherapy, or other anticancer treatment before operation. All individuals participating in this study signed informed consents, in addition, all procedures were carried out in accordance with the ethical standards of the First Affiliated Hospital of Xinjiang Medical University.

**Subcellular fractionation**

Nuclei and cytoplasm were separated using the PARIS™ Kit (AM1921, Thermo Fisher Scientific) based on guides from the manufacturer.

**Fluorescence in situ hybridization (FISH)**

The FISH test was conducted in 22RV1 and DU-145 cells according to the manufacturers’ instructions. The 5’FAM-labeled TCONS_00027385 probe (Additional file 1: S 1) in the current study was delegated to GenePharma (Shanghai, China) to design and synthesize. In brief, before permeabilization, cells were fixed in 4% paraformaldehyde (PFA) for 20 min. Subsequently, samples were incubated overnight in a corresponding probe at 37 °C. Finally, DAPI (D9542, Sigma-Aldrich) was used to stain the cell nuclei. Observation and photography of staining results were conducted in the fluorescence microscope (Zeiss LSM 880, Germany).

**5’ and 3’ RACE**

Based on the manufacturer’s instructions, a SMARTer™ RACE cDNA amplification kit (Clontech, Palo Alto, CA) was used to perform 5’ and 3’ RACE analysis methods to investigate the transcriptional start and stop sites of TCONS_00027385 (see the Additional file 1: S 2 for details).

**siRNA synthesis and transfection**

Specifical siRNAs which targeted TCONS_00027385 were delegated to Tsingke hz-synth department (Hangzhou, China) to synthesize, while miR-874-5p inhibitors and miR-874-5p mimics were delegated to Tsingke Biotechnology Co., Ltd. (Beijing, China) to synthesize. Transfections were performed using Lipofectamine 3000 (L3000075, Thermo Fisher Scientific) based on the manufacturer’s guide. The transfected cells were incubated in the corresponding medium for 48 or 72 hr after transfection (see the Additional file 1: S 3 & Table 1 for detail).
Colony formation test

Seeding transfected cells at 1000 cells per 10 cm plate. After 18–21 days of incubation, the cell culture medium was washed with PBS (PS102S, EpiZyme), followed by fixing in ethanol for 20 min and then staining in crystal violet for another 20 min. Finally, colonies were photographed for further count.

MTT assay

The transfected cells were seeded in 96-well plates with a density of $1 \times 10^5$ cells/well. After the treatment, cells were added and incubated with 20 μl MTT solution (5 mg/ml) (ST1537, Beyotime) for another 4 hr. Subsequently, after carefully removing supernatants, 100 μl DMSO was added for crystal dissolution. Finally, calculating the relative value of absorbance at 490 nm measured by a microplate reader (Bio-Rad, USA) to determine the proliferation of the cells.

EdU incorporation test

Using an EdU Apollo DNA *in vitro* kit (C0078L, Beyotime), cell proliferation was measured by ethynyl-2-deoxyuridine incorporation test. In short, after the cells were transfected with corresponding vector and cultured, they were added and incubated with 50 μM EdU in 100 μl at 37 °C for 2 hr. Finally, cells observance were performed by a fluorescence microscopy (Zeiss LSM 880, Germany). The result was based on at least three repeats.

TUNEL assay

Cells were seeded in dishes specific for confocal microscope (801002, NEST Biotechnology), and cultured in serum-free medium for 24 hr. Subsequently, fixing cells in 4 % PFA at room temperature for 20 min. Washing cells in 4 °C PBS and permeabilizing cells in 0.1 % Triton X-100 in 0.1 % sodium citrate for 10 min on ice, they were performed TUNEL staining mixture by the *in situ* Cell Death Detection kit, TMR red (11684817910, Roche) at 37 °C in the dark for 1 hr. Finally, cells were then rinsed in PBS and stained in DAPI solution for nuclei location. A LSM 880 (Zeiss, Germany) confocal microscope was used to observe fluorescence.

Apoptosis detection

The cells were collected with trypsin without EDTA and resuspended in 490 μl binding buffer. Subsequently, samples were incubated in the dark with 5 μl Annexin V-FITC and 5 μl PI (BD 559763, BD Biosciences) for 20 mins, and then detected apoptosis stage using a FACSCalibur (BD, Biosciences, USA) within 1 hr.

Western blot detection

After cell proteins were extracted, they were separated on 10% SDS-PAGE gels, and then transferred to PVDF membranes (ISEQ00010, Millipore). Next, the membranes were blocked with 5% skimmed milk
powder, and incubated with corresponding specific antibody at 4 °C overnight. On the next day, after incubating with appropriate secondary antibody, the expression level of protein in the samples were detected by an ECL detection system (ChemiScope 3200 Mini, China), with GAPDH as a control. In Additional file 1: S4, we have presented all the antibodies used in this experiment.

Lentivirus-induced overexpression and knockdown

To achieve TCONS_00027385 knockdown (KD) in 22RV1 and DU-145 cells, lentiviral particles within shRNAs against TCONS_00027385 or the corresponding control were obtained from Genechem (Shanghai, China). According to a previous research (20), three potential shRNAs (TCONS_00027385-KD1, TCONS_00027385-KD2, and TCONS_00027385-KD3) and a control one (TCONS_00027385-con) were designed for synthesis; the detailed sequences were presented in additional file 1: Table 2. Using polybrene transfection reagent for lentivirus transduction, and using an enhanced green fluorescent protein (EGFP) to verify and estimate the efficiency of transfection (additional file 1: Figure 1-3). Subsequentially, screening positive transformants with puromycin (MA0318, Meilun Biotechnology), the selected clones were amplified and the KD efficiency measured by qRT-PCR was analyzed. The details of experimental method for stably over-expressing cell lines can be found in additional file 1: S5 & Figure 4.

RIP test

Based on instruction for manufacturer, using the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, MA) to perform the RIP test. Lysing the cells and incubating with Ago2 and IgG. After the cell was mixed with anti-IgG and anti-Ago2 in RIP buffer (Millipore), the RNAs in precipitation were preserved to further sequential analyze.

Luciferase reporter experiment

Using the online database, including TargetScan (http://www.targetscan.org/vert_72), ENCORI (http://starbase.sysu.edu.cn), and miRWalk (http://mirwalk.umm.uni-heidelberg.de) for prediction of binding sites. After synthesizing the wild-type and mutant fragments related with the binding site of miR-874-5p in the 3′-UTR of TCONS_00027385 and ASCC2, they were inserted into the vector of pmirGLO (Tsingke Biotechnology, China). Additionally, they were mixed with miR-874-5p mimics or inhibitors and co-transfected into 293T cells, respectively. The luciferase activity was investigated then estimated through a dual-luciferase reporter system (Promega, Madison, WI).

Quantification of proteins by antibody array

The high-throughput protein profiles were performed using the protein array platform (AAH-APO-1-8, RayBiotech Life) based on the manufacturer’s guide. Using centrifugation to clarify cell samples (control group and sh-1 group), and before application to the arrays, a total protein concentration within the working range was obtained. Details for the experimental methods can be found in additional file 1: S6.

Zebrash experiments
The zebrafish experiments were completed on the zebrafish platform of Zhejiang University School of Medicine (the ethical number: 2021-20515#). After fertilization, zebrafish (Danio rerio) eggs were cultured in Danieau's solution at 28 °C according to standard laboratory conditions. At 48 hr after fertilization, the membranes out of the embryos were carefully removed with forceps, and the embryos were anesthetized in 0.04 mg/ml tricaine. The microinjection was performed to the embryos after transferred to a modified agarose gel. Using a Pneumatic Picopump and a manipulator (WPI) to inject approximately 400 DU-145 cells on the ventral end of the Cuvier Duct, where which entered embryo's heart. The injections were repeated in at least 15 embryos in each group. The survival rate less than 85 % in the control group was regarded to the demarcation line for abandonment. After implantation, embryos were cultured at 33 °C (21). Every other day, the growth of tumor was observed through a SMZ18 fluorescence microscope (Nikon, Japan) while green pixels data were quantified by NIS-Elements imaging software (D 4.50.00).

**Tumor xenograft model**

After 22RV1 cells (1×10^7 cells) with or without TCONS_00027385-KD1 (sh-1) were suspended in 200 μl PBS, they were injected subcutaneously into nude mice of 4-6 weeks old in each side. 30 days after injection, the mice were sacrificed for measurements of tumors in the terms of maximum (L) and minimum (W) length and weight. This animal experiment had been approved by the Animal Care and Use Committee of Zhejiang University (the ethical number: 2021-20515#).

**Statistical analysis**

Analyzing the data of at least three in-dependent tests with the GraphPad Prism 7.04 software (La Jolla, USA) and expressing them in the form of mean ± S.D. The student's t-test was performed to compare the differences between two groups, while the survival rate was calculated through Kaplan–Meier survival analysis. In addition, analysis of Cox proportional hazard model multivariate was conducted to measure the significance of TCONS_00027385 expression and clinicopathological characteristics on overall survival. Statistically significant was defined as P-value < 0.05.

**Results**

Expression of TCONS_00027385 increased in PCa and predicted a poor prognosis

Microarray analysis showed IncRNA expression variations in Robustanoids A (+) group and control group. The box plot, scatter plot, and volcano plot demonstrated the expression variations of IncRNA between two groups (Fig. 1b-d). A total of 1366 differentially expressed IncRNAs were screened out, with a condition of the folding changes greater than 2.0, and the P-value < 0.05. Among them, 816 IncRNAs were upregulated while 550 were downregulated (Additional file 3). In addition, the cluster heat map showed the more than 2.5-fold changes in differentially expressed IncRNAs (Fig. 1e). Incidentally, Table 1 had listed the IncRNAs of top 10 up-regulated and down-regulated. Expressions of these 20 IncRNAs were verified by RT-PCR, and 18 IncRNAs were validated successfully. First, we found that the expression of TCONS_00027385 in Robustanoids A (+) group was downregulated by sevenfold (P < 0.001). Next,
compared with the normal samples, TCONS_00027385 was significantly up-regulated in PCa tissues (Fig. 1f). Furthermore, the up-regulated expression of TCONS_00027385 was remarkably related to age, tumor size, and clinical stage (Fig. 1g-h), however, no obvious relationship was found between lymph node metastasis or tumor differentiation (The results were not shown). Kaplan–Meier survival curves showed that compared to the group with low TCONS_00027385 level, high TCONS_00027385 expression was associated with a lower overall survival rate (Fig. 1i). Additionally, compared to prostate stromal immortalized cell line, the TCONS_00027385 expression was upregulated remarkably of PCa cell lines (Fig. 1j). In general, the expression of TCONS_00027385 increased in PCa, which might be related to clinical progress and poor prognosis in PCa patients. Based on the human reference genome (GRCh37/hg19) in the UCSC genome database (http://genome.ucsc.edu), TCONS_00027385 is located at chr19:47742377-47747476. Thus, we measured and found the genomic length of the lncRNA TCONS_00027385 was 1152 bp (see the Additional file 2: S 1 for detail).

**TCONS_00027385 decreased proliferation and facilitated apoptosis in PCa cells**

In order to investigate whether TCONS_00027385 might participate in proliferation and apoptosis of cells, we conducted experiments on gain and loss of function. The utility of TCONS_00027385 down-expression (shRNA & siRNA) and TCONS_00027385 over-expression were confirmed in RT-PCR assays (Fig. 2a-c). Cell proliferation and apoptosis detection assays were also conducted. The MTT, colony formation assays and EdU assay indicated that knocking-down TCONS_00027385 reduced proliferation in 22RV1 and DU-145 cells, while over-expression of TCONS_00027385 remarkably played an opposite role (Fig. 2d-h). Moreover, TUNEL assays and flow cytometry were utilized to prove that knocking-down TCONS_00027385 significantly promoted cell apoptosis (Fig. 2i-j). For further confirmation, we used protein chip to measure the expression of apoptosis markers in PCa cells. As expected, chip analysis showed differential expression proteins (DEPs) variations in Robustanoids A (+) group and control group. A total of 19 differentially expressed proteins has been screened out, with the adjusted $P$-value less than 0.05 and the folding change more than 1.2 or less than 0.83 (absolute logFC > 0.263) (Table 3). The scatter plot and volcano plot demonstrated the changes in expressions of protein between the two groups (Fig. 3a-b). The PCA and heatmap were performed on all DEPs (Fig. 3c-d), with plotting the first two principal components to show the difference between the two groups. Using R package “clusterProfiler”, we conducted analysis on the protein function annotation Gene Ontology (GO) and KEGG pathway. The three subtypes in GO analysis containing biological process (Fig. 3e), molecular function (Fig. 3f), and cellular component (Fig. 3g). KEGG analysis relating genomic information to high-level functional information, was a systematic analysis of gene function (Fig. 3h). In summary, TCONS_00027385 decreased PCa cell proliferative and promoted apoptosis capacities.

**TCONS_00027385 knockdown inhibited tumor growth**

To further investigate the bio-functions of TCONS_00027385 on the growth of tumor, we performed experiments on subcutaneous xenograft tumor models and zebrafish. In our established zebrafish model, representative fluorescence microscopic images of both the experimental and the control groups are
presented in Fig. 3i. The sh-TCONS_00027385 (sh-1) fish bodies had a declining fluorescence intensity, however, we observed an opposite result in the TCONS_00027385 over-expression group (Additional file 2: S 3-4). In addition, in the pectoral region of nude mice, 22RV1 cells were implanted subcutaneously, with eight mice in negative control group and sh-1 group, respectively. For the next four weeks, the volumes of tumors were measured every 7 days. As we expected, the silence of TCONS_00027385 remarkably inhibited tumor growth \textit{in vivo} (Figure 3j-k). Therefore, the above results indicated that TCONS_00027385 might be able to regulate the progression of PCa.

**TCONS_00027385 played a role of a sponge for miR-874-5p**

In order to investigate intrinsic mechanism of TCONS_00027385 on PCa carcinogenesis, we located the expression of TCONS_00027385 subcellularly. The results indicated that TCONS_00027385 was mainly dispersed in cytoplasm (Fig. 4a-b), suggesting TCONS_00027385 might perform the biological function by sponging miRNA. Then, using Miranda software and Targetscan, we determined a candidate microRNA (miR-874-5p) as well as predicted the probable targets of TCONS_00027385 (Fig. 4c-d) in the downstream. The results from luciferase reporter experiment verified the luciferase activity of WT-TCONS_00027385 was significantly reduced by miR-874-5p mimics, however, it did not change remarkably in Mut-TCONS_00027385 (Fig. 4 e). In the other hand, the RIP test further showed that compared to the IgG group, TCONS_00027385 and miR-874-5p were accumulated in beads linked to Ago2 (Fig. 4f). In addition, the overexpression of WT-TCONS_00027385, but not Mut-TCONS_00027385, reduced the expression of miR-874-5p in PCa cells (Fig. 4g). Furthermore, over-expressing TCONS_00027385 greatly reduced expression level of miR-874-5p in both 22RV1 and DU-145 cells, while silence of TCONS_00027385 resulted an increase in expression level of miR-874-5p, with shRNA NC group acting as an internal control (Fig. 4h-i). Subsequently, we further found the level of miR-874-5p in PCa tissues was lower than which in normal condition, with a negative association between expression levels of TCONS_00027385 expression with miR-874-5p (Fig. 4j). To excavate more evidence, we conducted \textit{in vivo} experiments. The expression level of miR-874-5p in the tumors from nude mice collected previously was higher in the TCONS_00027385 knock-down cell line, but in the cells over-expressing TCONS_00027385, the expression level was lower (see the Additional file 2: S 2 for detail). In addition, TCONS_00027385 encouraged the proliferation and apoptosis of cells at least in part through sponging miR-874-5p (Fig. 2e-f). Above all, TCONS_00027385 accelerated the progress of PCa by sponging miR-874-5p.

**ASCC2 was a target of miR-874-5p in the downstream in PCa**

Through base pairing with 3'UTR, the post-transcriptional effects of miRNAs often resulted protein synthesis inhibition (22). Thus, in order to determine the detailed regulation mechanism of TCONS_00027385 in PCa, we searched TargetScan database and found that ASCC2 might be the target of miR-874-5p in the downstream (Fig. 4k). In order to confirm this prediction, we conducted a luciferase reporter experiment, and the results turned out that ASCC2 acted as a direct target of miR-874-5p (Fig. 4l). Furthermore, the expression of ASCC2 was down-regulated by silence of TCONS_00027385, and
miR-874-5p mimics could partially offset the corresponding increases of the expression of ASCC2 caused by the over-expression of TCONS_00027385 in PCa cells (Fig. 4m-p). Consistently, we observed the same result in the protein level (Fig. 5a-b). The above observation indicated that TCONS_00027385 could influenced the expression of ASCC2 through its interaction with the miR-874-5p.

We also tested the level of ASCC2 in tumor tissues. Comparing with neighboring normal tissues, ASCC2 was remarkably increased in PCa tissues, and we found a positive association between the expression level of ASCC2 with TCONS_00027385, and a negative relationship between the expression of ASCC2 and the level of miR-874-5p (Fig. 5c). Moreover, we tested the expression of ASCC2 \textit{in vitro}, while the transfection efficiency has been determined previously (Fig. 5d-e). As expected, by knocking down TCONS_00027385, the expression of ASCC2 in 22RV1 and DU-145 cells was significantly reduced, while an increase in expression was observed in cells over-expressing TCONS_00027385 (Fig. 4m-n). So far, we have verified that ASCC2 acted as a direct target of miR-874-5p. Nevertheless, the effects of ASCC2 in prostate cancer has far from been completely clarified.

**ASCC2 inhibition suppressed the tumorigenesis effects of TCONS_00027385**

At last, we carried out functional experiments to examine the effects of ASCC2 by exploring the effects of TCONS_00027385 mediated by ASCC2 in aspect of tumor growth promotion. To investigate whether the inhibition of ASCC2 could reverse the carcinogenic effect of TCONS_00027385, we conducted ASCC2 knocking-down in 22RV1 and DU-145 cells. According to rescue experiments, silence of ASCC2 partially reversed the effect on cell proliferation and apoptosis caused by overexpression of TCONS_00027385 (Fig. 5f-i). In summary, these results indicated that TCONS_00027385 competitively binded to miR-874-5p and subsequentially resulted in an up-regulation of the expression of ASCC2 (Fig. 5j), becoming a key tumor-promoting factors for PCa.

**Discussion**

Recently, because of the high incidence and poor prognosis, PCa has received widespread attention (23–25). Emerging researches have demonstrated that lncRNAs acted an essential role in the occurrence and development of PCa (26–28). For example, IncRNA-PCAT1 disrupted the complex of PHLPP/FKBP51/IKKa and promoted AKT and NF-κB signaling pathways. The expression of IncRNA-PCAT1 was positively correlated with the progress of CRPC (29). The function of LINC00844 was trans, affecting the transcription of genes regulated by androgen, and preventing the migration and invasion of prostate cancer cell (30). As a new AR translational regulator, Inc-LBCS inhibited the castration resistance of prostate cancer by connected with hnRNPK (31). Our current study discovered that the expression of TCONS_00027385 was remarkably up-regulated in PCa tissues and cell lines, while it was related to the development and prognosis in PCa patients, indicating TCONS_00027385 might be involved in the progression of PCa. Additionaly, we founded that TCONS_00027385 promoted the proliferation and apoptosis of PCa cells \textit{in vitro} and tumor growth \textit{in vivo}. However, the basic molecular mechanisms hasn't been elucidated.
Recently, emerging researches have confirmed that the ceRNA mechanism took part in various diseases (32–37), which has made great progress in PCa. For instance, the lncRNA UCA1 exerted carcinogenic activity through sponging miR-143 and then upregulating the expression of MYO6 in PCa (38). The lncRNA FOXP4-AS1 played a role as a ceRNA to sponge miR-3184-5p and the regulate FoxP4 in post-transcription (39). The lncRNA BLACAT1 could regulate proliferation and metastasis capabilities of prostate cancer cells, and might able to be the ceRNA to modulate the expression level of DVL3 through sponging miR-29a-3p (40). However, the impact of TCONS_00027385 on the development of PCa is still unexplored. Therefore, it is worth exploring whether the network of ceRNA involving miR-874-5p and TCONS_00027385 existed in PCa cells. In the current study, subcellular fractionation experiments showed that TCONS_00027385 was mainly existed in cytoplasm, providing the potential for TCONS_00027385 to function as a ceRNA in the course of disease in PCa. Then, the online database ENCORI (41), miRWalk (42, 43) and TargetScan (44–46) were used to postulate the downstream target (miR-874-5p) in downstream for TCONS_00027385. Luciferase reporter test, RIP test, and pull-down test were utilized to verify the connection between TCONS_00027385 and miR-874-5p. The TCONS_00027385 over-expressing decreased the level of miR-874-5p, while silence of TCONS_00027385 increased the expression level of miR-874-5p. Further experiments showed miR-874-5p had the effect of inhibiting cell apoptosis in the occurrence and development of PCa, indicating a tumor promotion impact of TCONS_00027385 dependent on miR-874-5p.

The ceRNA mechanism pointed out that because of miRNAs competitively bind to lncRNAs, the expression of mRNA would increase. The upstream miRNA of ASCC2 in PCa was found by means of bioinformatics analysis. Although, ASCC2 was first reported in the study of Lee YH (47), the researches on it in human cancers were still limited (48). In our study, ASCC2 was predicted to be a direct target of miR-874-5p through TargetScan. The luciferase reporter experiment and RIP test further enhanced the postulation of direct binding between TCONS_00027385 and miR-874-5p. ASCC2, as an oncogene, whose expression level had a positive relationship with TCONS_00027385, and a negative correlation with miR-874-5p. Moreover, according to rescue trials, silence of ASCC2 partially abolished the tumorigenic effects of TCONS_00027385.

Conclusions

In conclusion, our current research indicated that the new ceRNA network of TCONS_00027385/miR-874–5p/ASCC2 axis contributing to the development of PCa, which might reveal a novel biomarker of PCa and also provide new enlightenment for PCa treatment.

Abbreviations

BP, biological process;
CC, cellular component;
DEPs, differential expression proteins;

Dpi, days post injection;

EdU, Ethynyl-2-deoxyuridine;

GO, Gene Ontology;

KEGG, Kyoto Encyclopedia of Genes and Genomes;

MF, molecular function;

MTT, 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide;

PCa, prostate cancer;

qRT-PCR, quantitative real-time polymerase chain reaction;

RIP, RNA immunoprecipitation;

SD, standard deviation;

TUNEL, TdT-mediated dUTP Nick-End Labeling;

Declarations

Ethics approval and consent to participate

The use of human tissue in this study was approved by the ethics committee of the first affiliated hospital of Xinjiang medical university (Approval number: 20210301-92). Informed consent was obtained from all patients. The animal experiments were approved by the Zhejiang University ethics committee of experimental animals (Approval number: 2020-17105#; 2021-20515#).

Consent for publication

Not applicable.

Availability of data and materials

All of the source data are available from the corresponding authors upon reasonable request. Microarray data was listed in Additional file 3.

Competing interests

The authors declare that they have no competing interests.

Funding
This work was financially supported by China Postdoctoral Science Foundation Funded Project (Grant No. 2018M642444), Key Research and Development Program of Guangdong Province (Grant No. 2019B020212001) and Xinjiang Uygur Autonomous Region Natural Science Foundation (2020D01C158; 2018D01C167).

**Authors’ contributions**

Jianxin Han, Wei Li, Hengqing An, and Ying Zhang designed all experiments, Yanpei Gu and Wei Li performed bioinformatics analyses, Ruiying Qiu and Ning Tao collected prostate cancer samples, Jianxin Han, Ning Tao, Yanpei Gu, Fan Xue, Zhenlei Zhao, Yali Yan, Hongrui Xiao and Lihuan Chen performed all experiments. The manuscript was wrote by Jianxin Han and revised by Ying Zhang, Wei Li and Hengqing An. The authors read and approved the final manuscript.

**Acknowledgements**

The authors are indebted to Dr. Yingniang Li and other staff at the zebrafish platform of Zhejiang University School of Medicine for their help with the animal experiments. We also thank Dr. Yunqing Li from the Testing & Analysis Center of Agriculture, Life and Environment science, Zhejiang University for assistance with the photograph through confocal laser. We are also deeply indebted to all of the participants, including Dr. Xiaqian Chen, Dr. Yunhong Li, and Dr. Fanghuan Zhu for their enthusiastic participation in this study. In the end, Dr. Jianxin Han wants to say to Ms. Lihuizi Liu, you had me at “hello”.

**References**

1. Dulinska-Litewka J, Sharoni Y, Halubiec P, Lazarczyk A, Szafranski O, McCubrey JA, et al. Recent Progress in Discovering the Role of Carotenoids and Their Metabolites in Prostatic Physiology and Pathology with a Focus on Prostate Cancer-A Review-Part I: Molecular Mechanisms of Carotenoid Action. Antioxidants (Basel, Switzerland). 2021;10(4):585-627.

2. Hernando Polo S, Moreno Munoz D, Rosero Rodriguez AC, Silva Ruiz J, Rosero Rodriguez DI, Counago F. Changing the History of Prostate Cancer with New Targeted Therapies. Biomedicines. 2021;9(4):392-412.

3. Hofbauer LC, Bozec A, Rauner M, Jakob F, Perner S, Pantel K. Novel approaches to target the microenvironment of bone metastasis. Nature reviews Clinical oncology. 2021; doi: 10.1038/s41571-021-00499-9.

4. Dess RT, Spratt DE. Why the UK Should Consider Gene Expression Testing in Prostate Cancer. Clinical Oncology. 2020;32(3):149-155.

5. Schutz FAB, Sirachainan E, Kuppusamy S, Hoa NTT, Dejthevaporn T, Bahadzor B, et al. Optimizing outcomes for patients with metastatic prostate cancer: insights from South East Asia Expert Panel. Therapeutic Advances in Medical Oncology. 2021;13.
6. Okoye JO. High mortality risk of prostate cancer patients in Asia and West Africa: A systematic review. Avicenna journal of medicine. 2020;10(3):93-101.

7. Mandair D, Rossi RE, Pericleous M, Whyand T, Caplin ME. Prostate cancer and the influence of dietary factors and supplements: a systematic review. Nutrition & Metabolism. 2014;11:30-41.

8. Chan JJ, Tay Y. Noncoding RNA:RNA Regulatory Networks in Cancer. International Journal of Molecular Sciences. 2018;19(5):1310-1336.

9. Batista PJ, Chang HY. Long Noncoding RNAs: Cellular Address Codes in Development and Disease. Cell. 2013;152(6):1298-1307.

10. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, et al. The landscape of long noncoding RNAs in the human transcriptome. Nature Genetics. 2015;47(3):199-208.

11. Karreth FA, Pandolfi PP. ceRNA Cross-Talk in Cancer: When ce-bling Rivalries Go Awry. Cancer Discovery. 2013;3(10):1113-1121.

12. Chen Y, Zitello E, Guo R, Deng Y. The function of LncRNAs and their role in the prediction, diagnosis, and prognosis of lung cancer. Clinical and translational medicine. 2021;11(4): 367-389.

13. Flippot R, Beinse G, Boileve A, Vibert J, Malouf GG. Long non-coding RNAs in genitourinary malignancies: a whole new world. Nature Reviews Urology. 2019;16(8):484-504.

14. Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. Molecular Cancer. 2011;10:38-55.

15. Smillie CL, Sirey T, Ponting CP. Complexities of post-transcriptional regulation and the modeling of ceRNA crosstalk. Critical Reviews in Biochemistry and Molecular Biology. 2018;53(3):231-245.

16. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature. 2014;505(7483):344-352.

17. Pickard MR, Mourtada-Maarabouni M, Williams GT. Long non-coding RNA GAS5 regulates apoptosis in prostate cancer cell lines. Biochim Biophys Acta. 2013;1832(10):1613-1623.

18. Mitobe Y, Takayama K-i, Horie-Inoue K, Inoue S. Prostate cancer-associated lncRNAs. Cancer Letters. 2018;418:159-166.

19. Han J, Niu ST, Liu Y, Gan L, Wang T, Lu CD, et al. Robustanoids A and B, two novel pyrrolo [2,3-b] indole alkaloids from Coffea canephora: isolation and total synthesis. Organic Chemistry Frontiers. 2018;5(4):586-589.

20. Dubrovska A, Kim S, Salamone RJ, Walker JR, Maira SM, Garcia-Echeverria C, et al. The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(1):268-273.

21. Haldi M, Ton C, Seng WL, McGrath P. Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish. Angiogenesis. 2006;9(3):139-151.
22. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA Translation and Stability by microRNAs. In: Kornberg RD, Raetz CRH, Rothman JE, Thorner JW, editors. Annual Review of Biochemistry, Vol 79. Annual Review of Biochemistry. 79. Palo Alto: Annual Reviews; 2010. p. 351-379.

23. Xu YH, Deng JL, Wang G, Zhu YS. Long non-coding RNAs in prostate cancer: Functional roles and clinical implications. Cancer Letters. 2019;464:37-55.

24. Bayat H, Narouie B, Ziaee SM, Mowlaj S J. Two long non-coding RNAs, Prcat17.3 and Prcat38, could efficiently discriminate benign prostate hyperplasia from prostate cancer. Prostate. 2018;78(11):812-818.

25. Grasso CS, Wu YM, Robinson DR, Cao XH, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012;487(7406):239-243.

26. Kamada S, Takeiwa T, Ikeda K, Horie-Inoue K, Inoue S. Long Non-coding RNAs Involved in Metabolic Alterations in Breast and Prostate Cancers. Frontiers in Oncology. 2020; doi: 10.3389/fonc.2020.593200.

27. Hua JT, Chen S, He HH. Landscape of Noncoding RNA in Prostate Cancer. Trends in Genetics. 2019;35(11):840-851.

28. Xu T, Lin Cm, Cheng Sq, Min J, Li L, Meng Xm, et al. Pathological bases and clinical impact of long noncoding RNAs in prostate cancer: a new budding star. Molecular Cancer. 2018;17(1):103-120.

29. Shang Z, Yu J, Sun L, Tian J, Zhu S, Zhang B, et al. LncRNA PCAT1 activates AKT and NF-κB signaling in castration-resistant prostate cancer by regulating the PHLPP/FKBP51/IKKα complex. Nucleic Acids Research. 2019;47(8):4211-4225.

30. Lingadahalli S, Jadhao S, Sung YY, Chen M, Hu L, Chen X, et al. Novel IncRNA LINC00844 Regulates Prostate Cancer Cell Migration and Invasion through AR Signaling. Molecular Cancer Research. 2018;16(12):1865-1878.

31. Gu P, Chen X, Xie R, Xie W, Huang L, Dong W, et al. A novel AR translational regulator IncRNA LBCS inhibits castration resistance of prostate cancer. Molecular Cancer. 2019;18(1):109-123.

32. Liang Y, Song X, Li Y, Chen B, Zhao W, Wang L, et al. LncRNA BCRT1 promotes breast cancer progression by targeting miR-1303/PTBP3 axis. Molecular Cancer. 2020;19(1):85-105.

33. Wu Y, Zhang Y, Zheng X, Dai F, Lu Y, Dai L, et al. Circular RNA circCORO1C promotes laryngeal squamous cell carcinoma progression by modulating the let-7c-5p/PBX3 axis. Molecular Cancer. 2020;19(1):99-117.

34. Hong X, Liu N, Liang Y, He Q, Yang X, Lei Y, et al. Circular RNA CRIM1 functions as a ceRNA to promote nasopharyngeal carcinoma metastasis and docetaxel chemoresistance through upregulating FOXQ1. Molecular Cancer. 2020;19(1):33-49.

35. Wang W, Hu W, Wang Y, An Y, Song L, Shang P, et al. Long non-coding RNA UCA1 promotes malignant phenotypes of renal cancer cells by modulating the miR-182-5p/DLL4 axis as a ceRNA. Molecular Cancer. 2020;19(1):18-35.

36. Pan J, Fang S, Tian H, Zhou C, Zhao X, Tian H, et al. IncRNA JPX/miR-33a-5p/Twist1 axis regulates tumorigenesis and metastasis of lung cancer by activating Wnt/beta-catenin signaling. Molecular
37. Peng L, Sang H, Wei S, Li Y, Jin D, Zhu X, et al. circCUL2 regulates gastric cancer malignant transformation and cisplatin resistance by modulating autophagy activation via miR-142-3p/ROCK2. Molecular Cancer. 2020;19(1):156-175.

38. Yu Y, Gao F, He Q, Li G, Ding G. IncRNA UCA1 Functions as a ceRNA to Promote Prostate Cancer Progression via Sponging miR143. Molecular Therapy-Nucleic Acids. 2020;19:751-758.

39. Wu X, Xiao Y, Zhou Y, Zhou Z, Yan W. LncRNA FOXP4-AS1 is activated by PAX5 and promotes the growth of prostate cancer by sequestering miR-3184-5p to upregulate FOXP4. Cell Death & Disease. 2019;10(7):472-486.

40. Liao B, Chen S, Li Y, Yang Z, Yang Y, Deng X, et al. LncRNA BLACAT1 Promotes Proliferation, Migration and Invasion of Prostate Cancer Cells via Regulating miR-29a-3p/DVL3 Axis. Technology in Cancer Research & Treatment. 2021;20.

41. Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Research. 2014;42(D1):D92-D97.

42. Dweep H, Gretz N. miRWalk2.0: a comprehensive atlas of microRNA-target interactions. Nature Methods. 2015;12(8):697.

43. Dweep H, Sticht C, Pandey P, Gretz N. miRWalk - Database: Prediction of possible miRNA binding sites by “walking” the genes of three genomes. Journal of Biomedical Informatics. 2011;44(5):839-847.

44. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120(1):15-20.

45. Friedman RC, Farh KK-H, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Research. 2009;19(1):92-105.

46. Shin C, Nam JW, Farh KK-H, Chiang HR, Shkumatava A, Bartel DP. Expanding the MicroRNA Targeting Code: Functional Sites with Centered Pairing. Molecular Cell. 2010;38(6):789-802.

47. Lee YH, Bae SC, Song GG. Meta-analysis of gene expression profiles to predict response to biologic agents in rheumatoid arthritis. Clinical Rheumatology. 2014;33(6):775-782.

48. Jia J, Absmeier E, Holton N, Pietrzyk-Brzezinska AJ, Hackert P, Bohnsack KE, et al. The interaction of DNA repair factors ASCC2 and ASCC3 is affected by somatic cancer mutations. Nature Communications. 2020;11(1):5535-5548.

Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures
Figure 1

LncRNA expression profile in PCa. a Robustanoids A structure. b Box Plot presents the normalized intensity value in LncRNA expression between control and B group samples. c The scatter plot was used for assessing the variation in LncRNA expression between control and Robustanoids A (+) group samples. d The volcano plot was constructed using fold-change values and P-values. e The cluster heat map showed the differentially expressed LncRNAs over 2.5-fold change. f TCONS_00027385 expression
was detected in PCa tissue and adjacent normal tissue by qRT-PCR. g-h Associations between TCONS_00027385 expression and tumor size or clinical stage were detected by qRT-PCR. i Kaplan-Meier analysis was used to assess the relation between TCONS_00027385 expression level and overall survival in PCa patients. *p<0.05, **p<0.01, ***p<0.001. All experiments were repeated at least for three times and mean ± SD was used to represent the final result. j qRT-PCR was applied to confirm the expression level of TCONS_00027385 in PCa cell lines and human normal prostate stromal immortalized cell line.

Figure 2

TCONS_00027385 promoted PCa cells proliferation and inhibited cells apoptosis in vitro and in vivo. a The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells after transfection with sh-NC or sh-TCONS_00027385 were detected by RT-PCR. b The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR. c The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR. d The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR. e The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR. f The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR. g The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR. h The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR. i The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR. j The expression levels of TCONS_00027385 in 22RV1 and DU-145 cells were detected by RT-PCR.
and DU-145 cells after transfection with si-NC or si-TCONS_00027385 were detected by RT-PCR. c Transfection efficiency of TCONS_00027385 overexpression Lentivirus in 22RV1 and DU-145 cells were evaluated by qRT-PCR. d Colony formation assays. e-f The effects of TCONS_00027385 knockdown on the proliferation of 22RV1 and DU-145 cells were examined by MTT assay. g Flow cytometry was performed to determine the effect of TCONS_00027385 on apoptosis by flow cytometry analysis. h EdU assays were used to detect the proliferation rate of 22RV1 and DU-145 cells after TCONS_00027385 knockdown and overexpression. i TUNEL assays were used to detect the apoptosis rate of 22RV1 and DU-145 cells after TCONS_00027385 knockdown and overexpression.
Antibody array were performed to measure the expression of apoptosis markers in PCa cells. & Animal experiments. a-b The scatter and volcano plots showed the variation of proteins expression between two groups. c-d The PCA and heatmap are conducted on all DEPs between two groups. e-h Protein function annotation GO and KEGG pathway analysis in the TCONS_00027385 (sh-1) group versus normal group. GO analysis includes: biological process (Fig. 3e), molecular function (Fig. 3f) and cellular component
KEGG is systematic analysis of gene functions, linking genomic information with higher order functional information (Fig. 3h). i Zebrafish assays were used to detect the proliferation rate of DU-145 cells after TCONS_00027385 knockdown and overexpression. Tumor burden was quantified at 1 dpi (days post injection). Green = PCa cells. j-k The tumor volumes and weights of sh-TCONS_00027385 group compared with NC group were quantified. Tumor volumes were analyzed by ANOVA.
TCONS_00027385 was a sponge for miR-28-5p. a The expression level of TCONS_00027385 in the subcellular fractions of 22RV1 and DU-145 cells were detected by qRT-PCR. U6 and GAPDH were used as nuclear and cytoplasmic markers, respectively. b The location of TCONS_00027385 (green) in 22RV1 and DU-145 cells were determined by RNA FISH assay. DAPI-stained nuclei are blue. c ceRNA analysis for TCONS_00027385. Cytoscape was used to visualize lncRNA TCONS_00027385-miRNA-target gene interactions. d Schematic diagram representing the predicted binding sites for miR-874-5p in TCONS_00027385. e-g Luciferase reporter assay and RIP assay was performed to demonstrate that miR-874-5p was a downstream target of TCONS_00027385. h-i The miR-874-5p expression levels under TCONS_00027385 silencing and TCONS_00027385 overexpression were evaluated in vitro. j The expression of miR-874-5p in PCa tissue and normal tissue were detected by qRT-PCR. k Schematic diagram representing the predicted binding sites for ASCC2 in miR-874-5p. l Luciferase reporter assay was performed to determine the association between miR-874-5p and ASCC2. m-n The ASCC2 mRNA expression levels under TCONS_00027385 silencing and TCONS_00027385 overexpression were evaluated in vitro. o-p After miR-874-5p mimics, the mRNA expression of ASCC2 was evaluated by RT-PCR and western blot in 22RV1 and DU-145 cells. (*P < 0.05, **P < 0.01, and ***P < 0.001)
ASCC2 was a downstream target of miR-874-5p in PCa. a-b The correlation between ASCC2 and TCONS_00027385 as well as the correlation between ASCC2 and miR-874-5p were analyzed by Spearman's rank correlation test. c Relative ASCC2 expression in tumor tissue and normal tissue were detected by qRT-PCR. d The expression levels of ASCC2 in 22RV1 and DU-145 cells after transfection with si-NC or si-ASCC2 were detected by RT-PCR. e The expression levels of ASCC2 in 22RV1 and DU-145 cells...
after transfection with si-NC or si-ASCC2 were detected by western blot. f The TCONS_00027385 expression levels under ASCC2 silencing were evaluated in vitro. g Flow cytometry was performed to determine the effect of ASCC2 on apoptosis by flow cytometry analysis. h-i MTT assay was performed to detect proliferation of cells transfected with ASCC2 siRNA and cells co-transfected with ASCC2 siRNA and miR-874-5p mimics. j The schematic diagram of the oncogenic role of TCONS_00027385 in PCa cells. TCONS_00027385 functions as a miRNA sponge to positively regulate ASCC2 expression through sponging miR-874-5p and subsequently promotes malignant phenotypes of PCa cells, thus playing an oncogenic role in prostate cancer pathogenesis.

**Supplementary Files**

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- flowchart.tif