

Original Article

Upregulation of long non-coding RNA ATP6V0E2-AS1 predicts a poor prognosis in prostate cancer

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Abstract: Background: Prostate cancer is a common cancer in males. Previous studies had demonstrated more than 90% of the human genome transcribed non-coding RNA. Long non-coding RNAs could regulate many biological processes in human diseases, including epigenetic regulation, RNA splicing, and protein degradation. The present study explored the prognostic value of a novel lncRNA ATP6V0E2-AS1 in prostate cancer (PCa). Method: The TCGA database was used to explore the expression of ATP6V0E2-AS1 in PCa and normal prostate tissues. The expression of ATP6V0E2-AS1 in PCa clinical samples was detected by real-time PCR. Finally, co-expression analysis, together with bioinformatics analysis, was used to research the functions of ATP6V0E2-AS1 in PCa. Results: The present study showed that ATP6V0E2-AS1 was significantly upregulated in PCa tissues. The qRT-PCR assay showed that ATP6V0E2-AS1 was overexpressed in PCa cell lines compared to WPMY-1. Our results also showed that ATP6V0E2-AS1 was upregulated in high pathology stage PCa. Upregulated ATP6V0E2-AS1 was positively correlated with a shorter survival time in PCa patients by analyzing the Kaplan-Meier dataset. Bioinformatics analysis showed that ATP6V0E2-AS1 was involved in protein translation regulation in PCa. Conclusion: We believe this research will provide new ideas for the diagnosis and treatment of prostate cancer.

Keywords: ATP6V0E2-AS1, prostate cancer, TCGA, bioinformatics analysis

Introduction

Non-coding RNAs (ncRNAs), which were regarded as transcription noise, have been validated as key regulators in human disease progression [1-3]. ncRNAs could be divided into several types according to their lengths. Of note, lncRNAs, a type of ncRNAs longer than 200 bps, has been implicated in cancers by regulating many biological processes including epigenetic regulation, RNA splicing, and protein degradation [4, 5].

In the United States, prostate cancer (PCa) was the most commonly diagnosed cancer in males [6]. lncRNA HOTAIR, LOC440040, and PVT1 have been found to be related to PCa. HOTAIR was upregulated in PCa samples, whose down-regulation could inhibit the proliferation of PCa cells [7]. lncRNA XIST was down-regulated and acted as a tumor suppressor in PCa [8]. Several independent groups demonstrated that PCA3 could be detected in blood, urine, and PCa tis-

suess with better sensitivity and specificity than prostate-specific antigen (PSA) [9-13].

We analyzed publically available databases to explore the expression of ATP6V0E2-AS1. Then, the prognostic value of lncRNA ATP6V0E2-AS1 in PCa was also analyzed. Next, a series of bioinformatic analysis was performed to reveal the roles of lncRNA ATP6V0E2-AS1 in PCa. This study can further advance the understanding of lncRNA ATP6V0E2-AS1 in PCa.

Materials and methods

Patients and clinicopathological data

497 PCa patients with clinical data and detailed expression of ATP6V0E2-AS1 were downloaded from The Cancer Genome Atlas (TCGA) data portal. The inclusion criteria were: (1) histologic diagnosis was prostate cancer; (2) data of samples were available on the expression of genes and characteristics of patients. The exclusion criteria were: (1) first histological diagnosis was

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not prostate cancer; (2) patients suffered from other malignant neoplasms except prostate cancer. GSE6919 [14, 15] was contributed by Federico AM, which contained 233 normal and 267 PCa tissues. All the patients were staged according to the 7th edition of the TNM staging standard of 2009.

Patients and tissue samples

The Research Ethics Committee of Jingzhou Central Hospital approved this study. 18 tumor and 6 normal tissue samples were collected from Jingzhou Central Hospital. Written informed consent was obtained from the patients. The patients did not receive any other treatment. LNCaP, WPMY-1, PC-3, 22RV1, and DU145 cells were purchased from Cell Bank of Chinese Academy of Sciences and cultured in 1640-media (10% FBS, Gibco, USA).

Real-time PCR (qRT-PCR) analysis

Total RNAs of samples and cell lines were extracted using TransZol Up Plus RNA Kit (TransGen BioTech, China). Reverse transcript PCR was carried out using BeyoRT First Strand cDNA Synthesis Kit (Beyotime, China). qRT-PCR was performed using SuperReal PreMix Plus (TIANGEN, China) on Roche LightCycler 480. β -actin was selected as a reference. The $2^{-\Delta\Delta Ct}$ method was performed to analyze the data.

Co-expression network construction

The Pearson correlation coefficient of ATP6VOE2-AS1-co-expressed genes pairs was calculated. The absolute value of the Pearson correlation coefficient LightCycler 480. β -actin was selected as Cytoscape software was used to perform the co-expression network.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

MAS3.0 system (<http://mas.capitalbiotech.com/mas3/>) was used to present the GO analysis. DAVID system (<https://david.ncifcrf.gov/home.jsp>) [16, 17] was used to present the KEGG pathway analysis. $P < 0.05$ was considered as statistically significant.

Statistical analysis

The data were shown as the mean \pm SD. The chi-squared test, t-test, Fisher's exact test, or

Mann-Whitney U-test was used to analyze the data. The survival times were analyzed by Kaplan-Meier curves (log-rank test) and were used to analyze the association between lncRNA ATP6VOE2-AS1 and the prognosis of PCa. $P < 0.05$ was selected as a reference.

Results

ATP6VOE2-AS1 is upregulated in PCa

As shown in **Figure 1A**, our analysis showed that ATP6VOE2-AS1 was overexpressed in tumor tissues ($P < 0.001$). Then, the GSE6919 dataset analysis showed that ATP6VOE2-AS1 was overexpressed in primary PCa and metastatic PCa samples (**Figure 1B**, $P < 0.05$).

Furthermore, we observed that ATP6VOE2-AS1 was overexpressed in PCa cell lines LNCaP ($P < 0.05$), PC-3 ($P < 0.001$), DU145 ($P < 0.05$), and 22Rv1 ($P < 0.001$) compared to noncancerous prostatic cells WPMY-1 by using RT-qPCR assay (**Figure 1C**). We evaluated the expression of ATP6VOE2-AS1 in PCa samples to validate our results. Comparing to normal tissues, the expression of ATP6VOE2-AS1 was higher in PCa samples (**Figure 1D**, $P < 0.001$).

Upregulation of ATP6VOE2-AS1 was positively correlated with poor prognosis

We also evaluated the expression levels of ATP6VOE2-AS1 with clinical varies, including Gleason score, T staging, and N staging. Our analysis showed that ATP6VOE2-AS1 was up-regulated in Gleason 8 (Gleason 8 VS Gleason 6, $P < 0.05$; Gleason 8 VS Gleason 7, $P < 0.01$) and Gleason 9 (Gleason 9 VS Gleason 6, $P < 0.01$; Gleason 9 VS Gleason 7, $P < 0.01$) PCa patients compared to Gleason 6 and Gleason 7 PCa samples (**Figure 2A**). The results also showed that ATP6VOE2-AS1 levels were overexpressed in T3/T4 PCa compared to T2 PCa (**Figure 2B**, $P < 0.01$). Furthermore, we found that the ATP6VOE2-AS1 was overexpressed in N1 stage PCa compared to N0 PCa (**Figure 2C**, $P < 0.001$). These results suggested ATP6VOE2-AS1 was involved in PCa prognosis.

As shown in **Figure 3**, the Kaplan-Meier curve method showed that the 5-year BCR-free survival and overall survival rates were higher in ATP6VOE2-AS1-low patients compared to ATP6VOE2-AS1-high patients (**Figure 3A**, $P < 0.001$;

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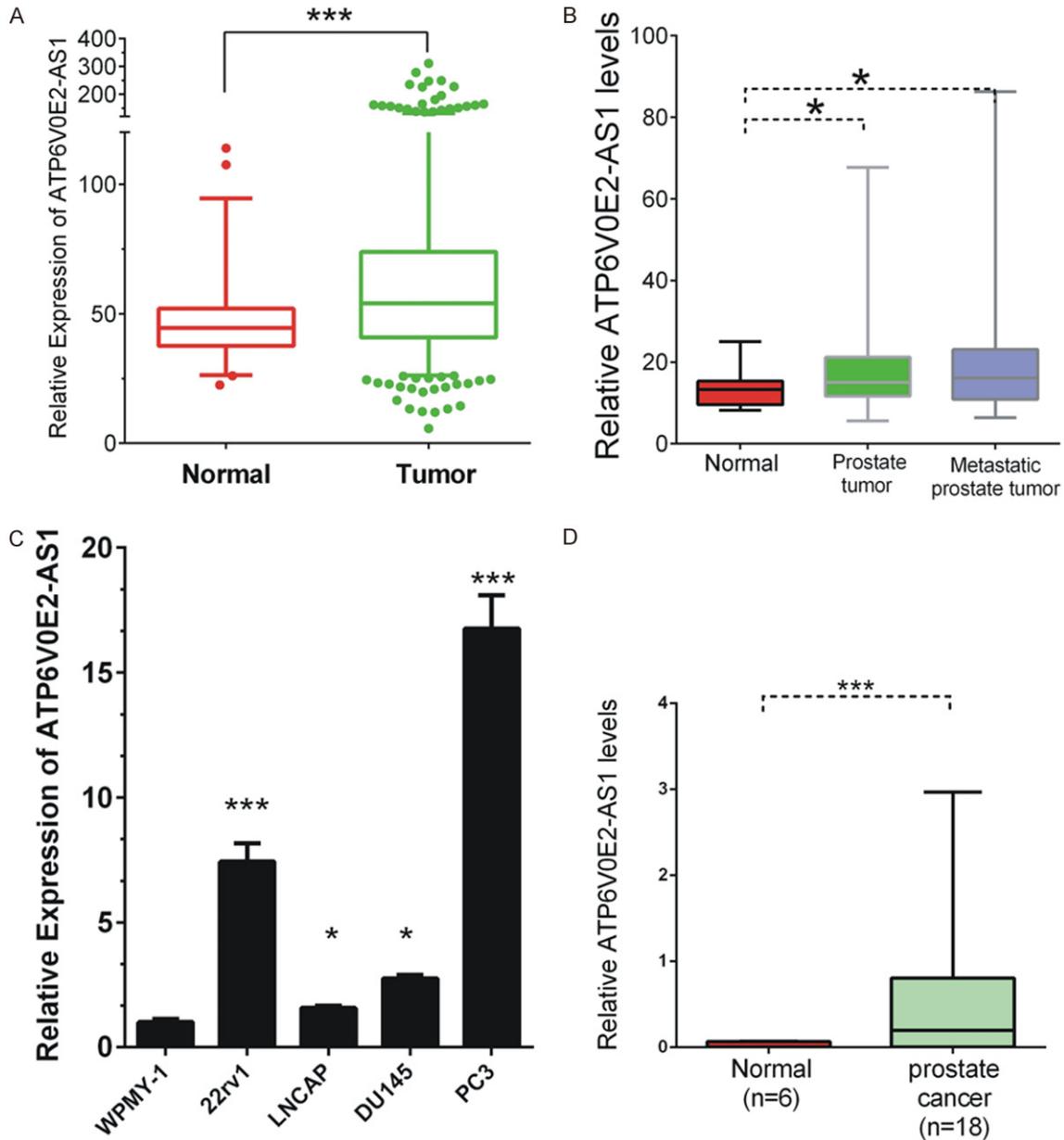


Figure 1. ATP6VOE2-AS1 is upregulated in prostate cancer. A. ATP6VOE2-AS1 was upregulated in tumor tissues compared with normal tissues by analyzing the TCGA dataset. B. ATP6VOE2-AS1 was overexpressed in tumor tissues compared with normal tissues by analyzing the GSE6919 dataset. C. ATP6VOE2-AS1 was significantly overexpressed in PCa cell lines compared to WPMY-1 by using qRT-qPCR assay. D. ATP6VOE2-AS1 was overexpressed in 18 prostate cancer tissues compared with 6 normal tissues. The Mann-Whitney U-test was used to analyze the expression levels of ATP6VOE2-AS1 between normal prostate and PCa samples. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

3B, $P < 0.01$), indicating that ATP6VOE2-AS1 could serve as a biomarker for PCa.

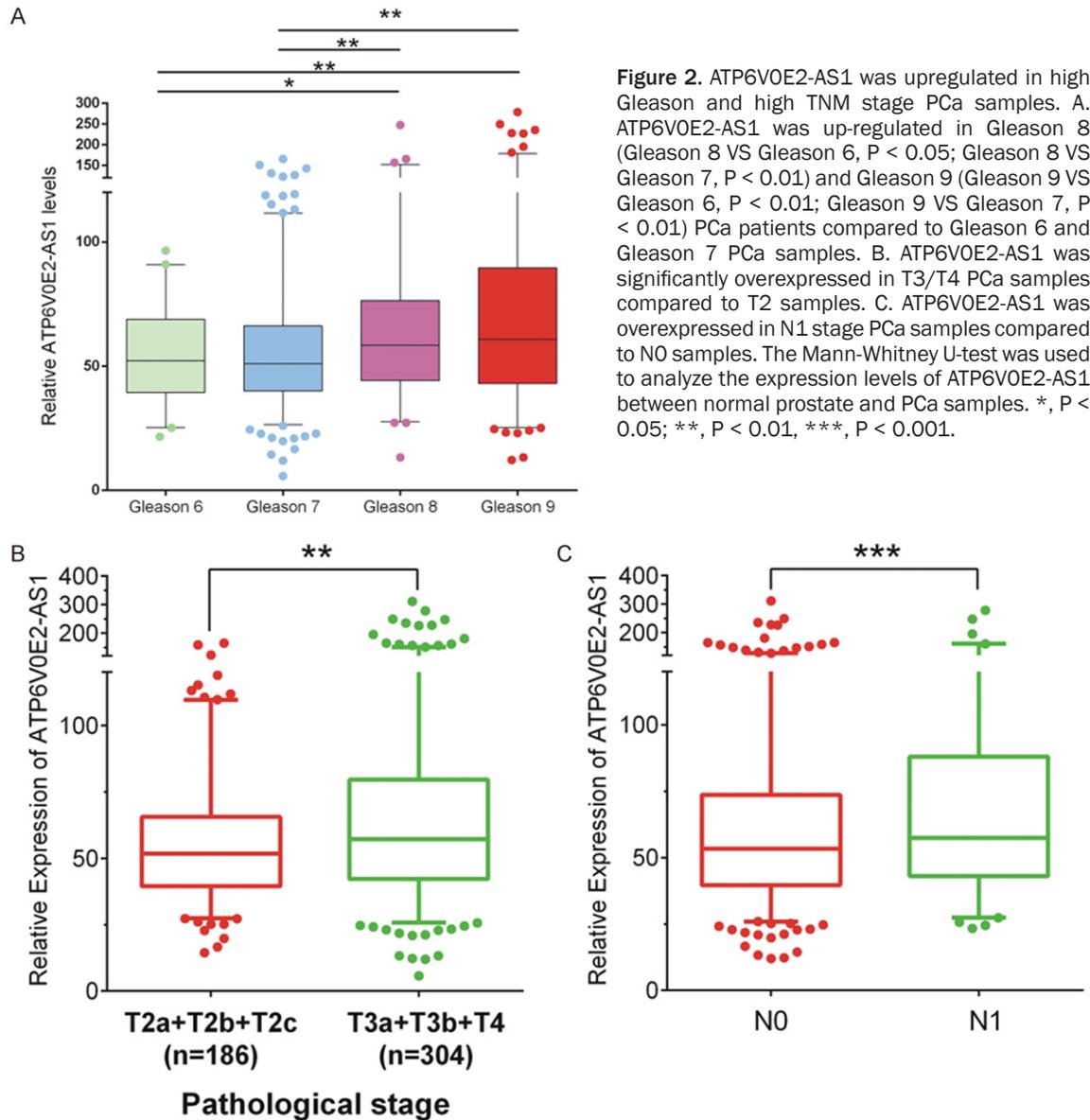
GO and KEGG pathway analysis

The co-expression networks were constructed by analyzing the Pearson correlation coefficient of ATP6VOE2-AS1-targets pairs. The absolute

value of the Pearson correlation coefficient of ATP6VOE2-AS1-targets pairs. The absolute value of the PCa (PCa. A total of 1638 mRNAs were selected to construct a co-expression network.

The DAVID system was used to perform GO analysis based on the co-expressed genes of

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ATP6VOE2-AS1. The analysis revealed that ATP6VOE2-AS1 was significantly associated with translation, translational initiation, rRNA processing, cytoplasmic translation, mRNA splicing, via spliceosome, protein targeting to mitochondrion, mitochondrial translation, DNA repair, ribosomal large subunit biogenesis, aerobic respiration, proteasome-mediated ubiquitin, ribosomal small subunit biogenesis, spliceosomal snRNP assembly, cell-cell adhesion, and tRNA processing (Figure 4A). KEGG analysis showed that ATP6VOE2-AS1 was related to the regulation Spliceosome, Pyrimidine metabolism, Notch signaling pathway, RNA polymerase, Base excision repair, Protein processing in endoplasmic reticulum, VEGF signaling

pathway, DNA replication, and RNA transport (Figure 4B).

Construction of ATP6VOE2-AS1 mediated PPI network in PCa

Furthermore, the protein-protein interacting network (PPI) was constructed to predict the interactions among ATP6VOE2-AS1 co-expressed proteins in PCa (combined score > 0.4) using the STRING database [18, 19]. The Mcode plugin (degree cut-off ≥ 2 and the nodes with edges ≥ 2 -core) was used to module analysis the PPI network. Three key modules were identified. Module 1 included 79 nodes and 2898 edges (Figure 5A), Module 2 included 74

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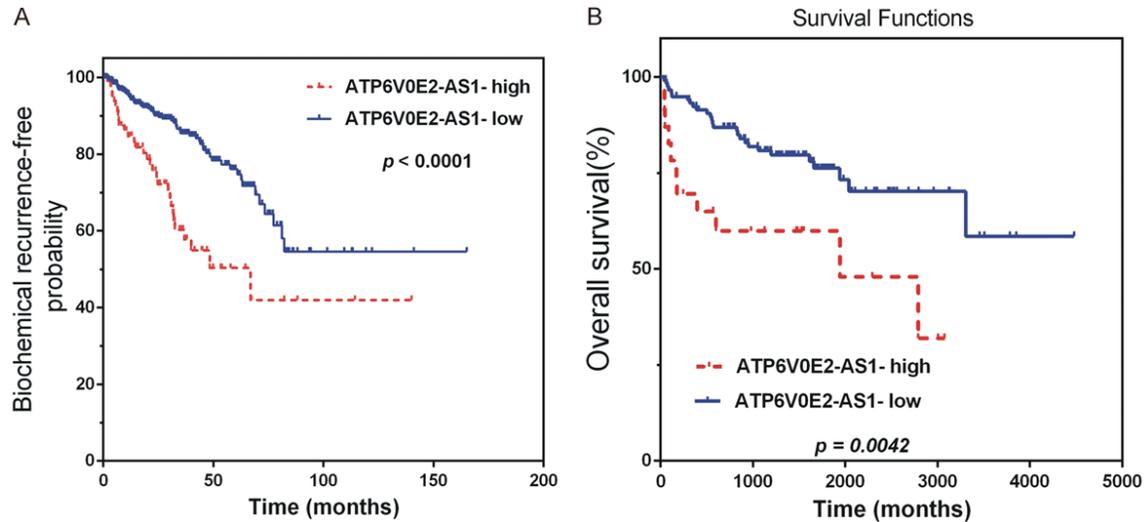


Figure 3. The upregulation of ATP6V0E2-AS1 predicts a poor prognosis in prostate cancer. The rates of 5-year BCR-free survival (A) and overall survival (B) were higher in ATP6V0E2-AS1-low patients compared to ATP6V0E2-AS1-high patients. The log-rank test was used to compare the differences in survival times.

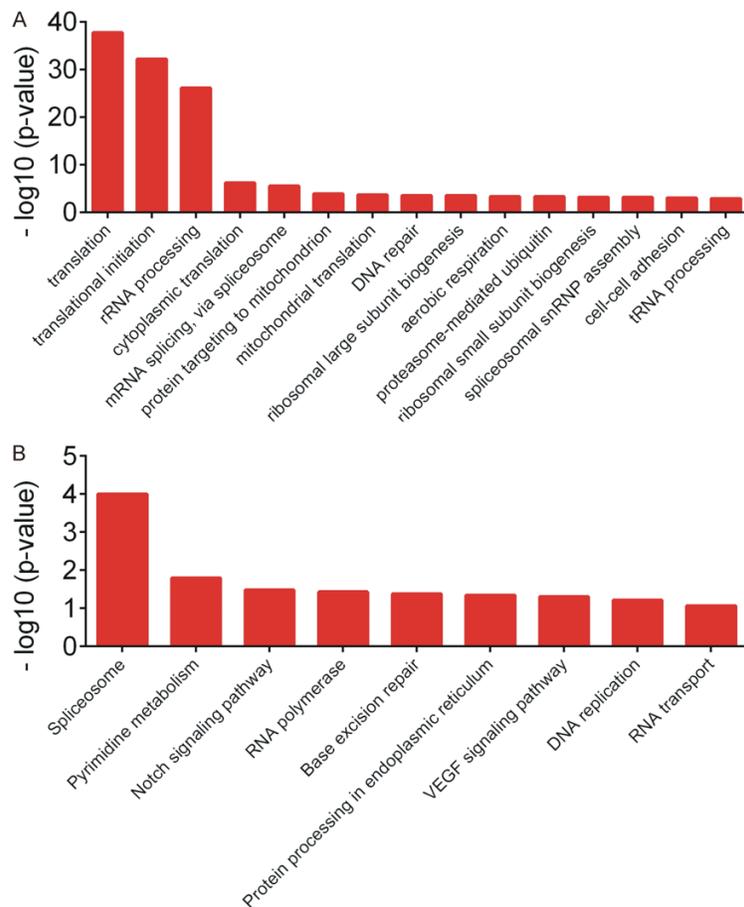


Figure 4. Bioinformatic analysis of ATP6V0E2-AS1 in prostate cancer. A. The Gene Ontologies (GO) categories analysis of ATP6V0E2-AS1 in prostate cancer. B. The KEGG analysis of ATP6V0E2-AS1 in prostate cancer.

nodes and 1226 edges (**Figure 5B**), and Module 3 included 24 nodes and 231 edges (**Figure 5C**).

We performed ClueGO analysis to explore the potential roles of these key modules by using Cytoscape software. We found that Module 1 was associated with translation, negative regulation of protein ubiquitination, and cleavage involved in rRNA processing (**Figure 6A**). Module 2 was associated with protein ubiquitination, mRNA splicing, snRNA metabolic process, DNA-templated transcription, and regulation of mRNA processing (**Figure 6B**). Module 3 was associated with ATP metabolic process, oxidative phosphorylation, hydrogen ion transmembrane transport, and mitochondrial electron transport (**Figure 6C**).

Discussion

Prostate cancer was a common cancer in males worldwide [20]. LncRNAs have been

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A

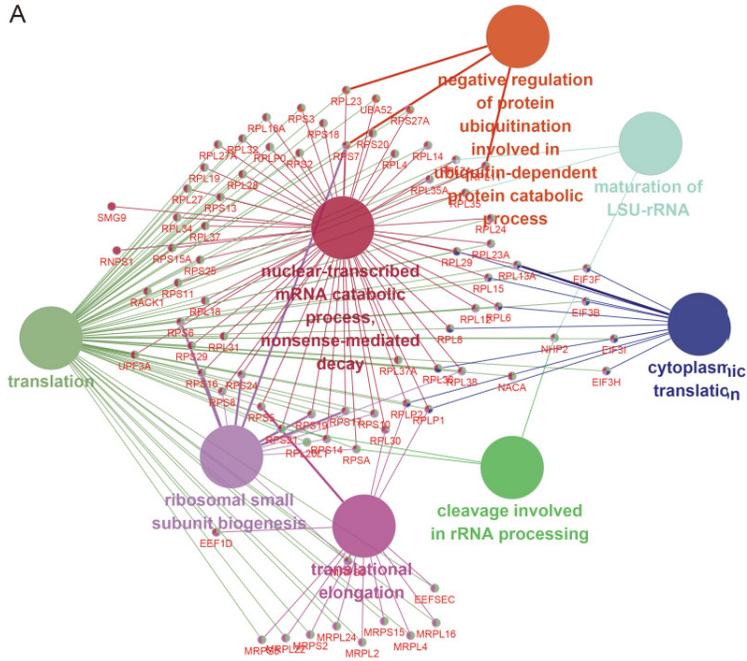
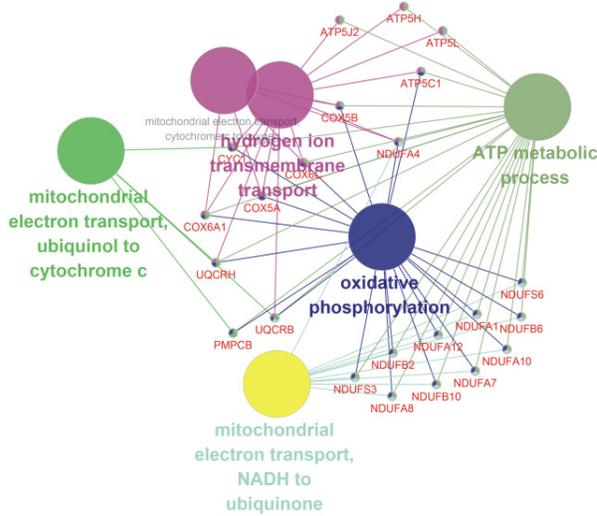
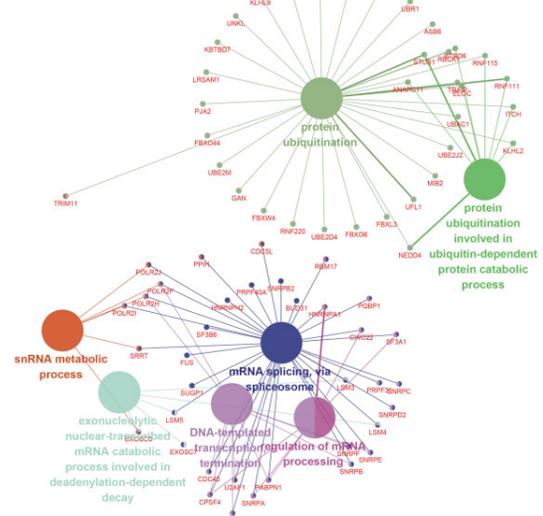


Figure 6. Bioinformatics analysis of 3 key ATP6VOE2-AS1 related protein-protein interaction network. A. Module 1 was associated with translation, negative regulation of protein ubiquitination, cleavage involved in rRNA processing. B. Module 2 was associated with protein ubiquitination, mRNA splicing, snRNA metabolic process, DNA-templated transcription, and regulation of mRNA processing. C. Module 3 was associated with ATP metabolic process, oxidative phosphorylation, hydrogen ion transmembrane transport and mitochondrial electron transport.

B



C



worse survival in patients with PCa. This study revealed that lncRNA ATP6VOE2-AS1 could be a biomarker for PCa. Furthermore, we performed a co-expression analysis for lncRNA ATP6VOE2-AS1. Bioinformatic analysis revealed that ATP6VOE2-AS1 was significantly associated with translation, rRNA processing, mRNA splicing, protein targeting to mitochondrion, mitochondrial translation, DNA repair, cell-cell adhesion, and tRNA processing. Moreover, we constructed an ATP6VOE2-AS1-related PPI network in PCa. Three key modules were identified. Bioinformatics analysis module 1 was associated with translation, and module 2 was associated

with protein ubiquitination, mRNA splicing, while module 3 was associated with ATP metabolic process.

Several limitations should be noted in the present study. First, the present study showed that ATP6VOE2-AS1 played an important role in PCa, mainly based on bioinformatic analysis. Therefore, further validation of our analysis using large-scale clinical samples was still needed. Second, the molecular functions of ATP6VOE2-AS1 in PCa still need more exploring. The loss-of-function assays should be applied to explore the molecular functions of

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ATP6VOE2-AS1 in PCa. Finally, the mechanisms of ATP6VOE2-AS1 in PCa remained to be further investigated.

In conclusion, we for the first time revealed that the potential functional roles of ATP6VOE2-AS1 were involved in PCa. ATP6VOE2-AS1 was up-regulated and involved in protein translation regulation in PCa. We believe this research will advance the diagnosis and treatment of PCa.

Acknowledgements

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Disclosure of conflict of interest

None.

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