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Research article

Identification of 10 differently expressed lncRNAs as prognostic biomarkers for prostate adenocarcinoma

Ziyu Wu[1], Sugui Wang[1], Qiang Li[1], Qingsong Zhao[2] and Mingming Shao[3,*]

[1] Department of Urology, Huai'an Hospital Affiliated of Xuzhou Medical University, 62 South Huaihai Road, Huai'an 223002, China
[2] Department of Urology, Jining NO.1 People's hospital, 6 Jiankang Road, Jining 272000, China
[3] Department of Urology, Jining NO.1 People's hospital, 99 Shixian Road, Jining High-tech Zone, Jining 272000, China

* **Correspondence:** Email: gouxiaji2gq@163.com.

Abstract: Prostate adenocarcinoma (PRAD) is one of the most frequently diagnosed cancer in males. Previous studies had demonstrated long non-coding RNAs (lncRNAs) played crucial roles in human cancers. In present study, we reported ten disease-free survival time related lncRNAs in PRAD, including RP11-468E2.5, GS1-393G12.13, CTD-2228K2.7, RP11-783K16.13, RP11-631N16.4, CTC-435M10.12, RP11-1109F11.5, RP11-228B15.4, RP11-496I9.1, and RP11-95O2.5. Higher expression of these lncRNAs significantly correlates to shorter DFS time in patients with PRAD. We next constructed lncRNAs regulating PPI networks in PRAD. Bioinformatics analysis revealed these DFS-related lncRNAs were associated with the regulation of cell cycle, glucose metabolic process, histone modification, and RNA splicing. AR and SPOP were identified to be involved in regulating these lncRNAs expression in PRAD. The prognostic value and molecular functions of these lnRNAs in human diseases remained largely unknown. We thought this study for the first time demonstrated that they could act as novel potential biomarkers for PRAD.

Keywords: biomarker; key lncRNA; prostate cancer; PPI network; co-expression networks

Abbreviations: PRAD: Prostate Adenocarcinoma; Lncrnas: Long Non-coding RNAs; PSA: Prostate-Specific Antigen; DRE: Digital Rectal Examination; TCGA: The Cancer Genome Atlas Project; ANOVA: Analysis of Variance; DFS: Disease-free Survival; AR: Androgen-receptor

1. Introduction

Prostate adenocarcinoma (PRAD) is one of the most frequently diagnosed cancer in males [1]. Prostate-specific antigen (PSA) measurements and the digital rectal examination (DRE) is the most widely used for the screening and diagnosis of PRAD [2]. However, the specificity of both methods was low. There was still an urgent need to identify novel and better biomarkers for PRAD. In the past decades, several targets were found to be dysregulated and associated the progression of PRAD, including long non-coding RNA PCA3 [3]. A recent study indicated the hypermethylation of ST6GALNAC3 and ZNF660 promoter [4] could serve as the potential biomarker for PRAD tissues and liquid biopsies.

Long non-coding RNAs (lncRNAs) were a class of non-coding RNAs longer than 200 bps [5]. Emerging studies demonstrated that lncRNAs played crucial roles in PRAD tumorigenesis and progression. For instance, lncRNA TTTY15 was found to promote PRAD progression by sponging let-7 [6]. LncRNA NEAT1, a target of estrogen receptor alpha was identified as a key regulator in PRAD [7]. Interestingly, recent studies showed lncRNAs could serve as biomarkers for PRAD. For example, multi-institutional analysis showed low expression of PCAT-14 correlated to poor prognosis [8,9]. Wan et al. found AR-regulated lncRNAs could serve as new diagnostic and prognostic markers for PRAD [10].

Disease-free survival (DFS) was defined as the length of time after primary treatment for a cancer ends that the patient survives without any signs or symptoms of that cancer. Measuring the disease-free survival is one way to see how well a new treatment works. The current study identifies differentially expressed lncRNAs in PRAD using the Cancer Genome Atlas Project (TCGA) database [11]. Ten lncRNAs were found to be correlated to the disease-free survival time in patients with PRAD. Moreover, co-expression analysis and KEGG pathway analysis [12] was used to predict the potential functions of ten novel lncRNAs in PRAD. Of note, we evaluated the potential up-stream regulators of ten lncRNAs in PRAD. We thought this study could provide new insights into the identifying effective biomarkers and mechanisms of PRAD.

2. Materials and methods

2.1. Data preparation and processing

The gene list of differently expressed lncRNAs in PRAD were downloaded from GEPIA dataset (http://gepia.cancer-pku.cn/) [13]. Only lncRNAs with $\log2|FC| > 2.0$ and $P < 0.001$ were defined as significantly expressed lncRNAs. Moreover, the lncRNAs associated with disease-free survival time in PRAD patients were also downloaded from GEPIA dataset. The median expression of target lncRNA were considered as cutoff to divide PRAD samples as high- and low- group.

2.2. Co-expression network construction and analysis

In this study, the Pearson correlation coefficient was calculated according to the expression value between lncRNA-mRNA pair. The co-expressed DEG-lncRNA pairs with the absolute value of Pearson correlation coefficient ≥ 0.75 were selected for the construction of co-expression network using cytoscape software (Version 3.4.0, available online: http://www.cytoscape.org/).

2.3. Statistical analysis

Statistical analyses were conducted using SPSS version 21.0 software, and graphs were generated using GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA). All values are expressed as the mean ± SD. The two groups were compared using Students' t-test. The differences between multiple groups were analyzed with one‐way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Identification of 10 disease-free survival time related lncRNAs in PRAD

In present study, we analyzed GEPIA dataset to identify disease-free survival (DFS) time related lncRNAs. The top 100 genes (including mRNAs and lncRNAs) were listed in Supplementary Table 1. RP11-468E2.5, GS1-393G12.13, CTD-2228K2.7, RP11-783K16.13, RP11-631N16.4, CTC-435M10.12, RP11-1109F11.5, RP11-228B15.4, RP11-496I9.1 and RP11-95O2.5 were the most significantly correlated to DFS time in PRAD patients.

As shown in Figure 1A–J, we found higher expression of RP11-468E2.5, GS1-393G12.13, CTD-2228K2.7, RP11-783K16.13, RP11-631N16.4, CTC-435M10.12, RP11-1109F11.5, RP11-228B15.4, RP11-496I9.1 and RP11-95O2.5 were significantly associated with shorter DFS time in PRAD.

3.2. Disease-free survival time related lncRNAs were up-regulated in PRAD

Next, we evaluated the expression levels of these disease-free survival time related lncRNAs in PRAD. Our results showed RP11-468E2.5, GS1-393G12.13, CTD-2228K2.7, RP11-783K16.13, RP11-631N16.4, CTC-435M10.12, RP11-1109F11.5, RP11-228B15.4, RP11-496I9.1, and RP11-95O2.5 were significantly overexpressed in PRAD compared to normal samples (Figure 2A–J).

3.3. Construction of DFS related lncRNAs affecting PPI network in PRAD

Furthermore, we calculated the Pearson correlation coefficients of lncRNA-mRNA pairs using GEPIA database. The top 200 correlated genes were selected as the potential targets of DFS related lncRNAs. Then, PPI networks were constructed to reveal the relationship among the targets of each lncRNA. The PPI networks related to RP11-468E2.5 (Figure 3A), GS1-393G12.13 (Figure 3B), CTD-2228K2.7 (Figure 3C), RP11-631N16.4 (Figure 3D), CTC-435M10.12 (Figure 3E), RP11-1109F11.5 (Figure 3F), RP11-228B15.4 (Figure 3G), RP11-496I9.1 (Figure 3H), RP11-95O2.5 (Figure 3I) and RP11-783K16.13 (Figure 3J) were shown in Figure 3.

In order to explore the molecular functions of each lncRNA in PRAD, we conducted clue-go analysis for each of these lncRNAs by using their co-expressing genes in PPI networks. Our analysis revealed that RP11-468E2.5 may affect small nuclear ribonucleoprotein complex through DDX39B and SNRNP70, and affect formation of the spliceosomal b complex through PRPF3, SNRNP70, SRRM2, RBM5, SRRT, and HNRNPU (Figure 3K).

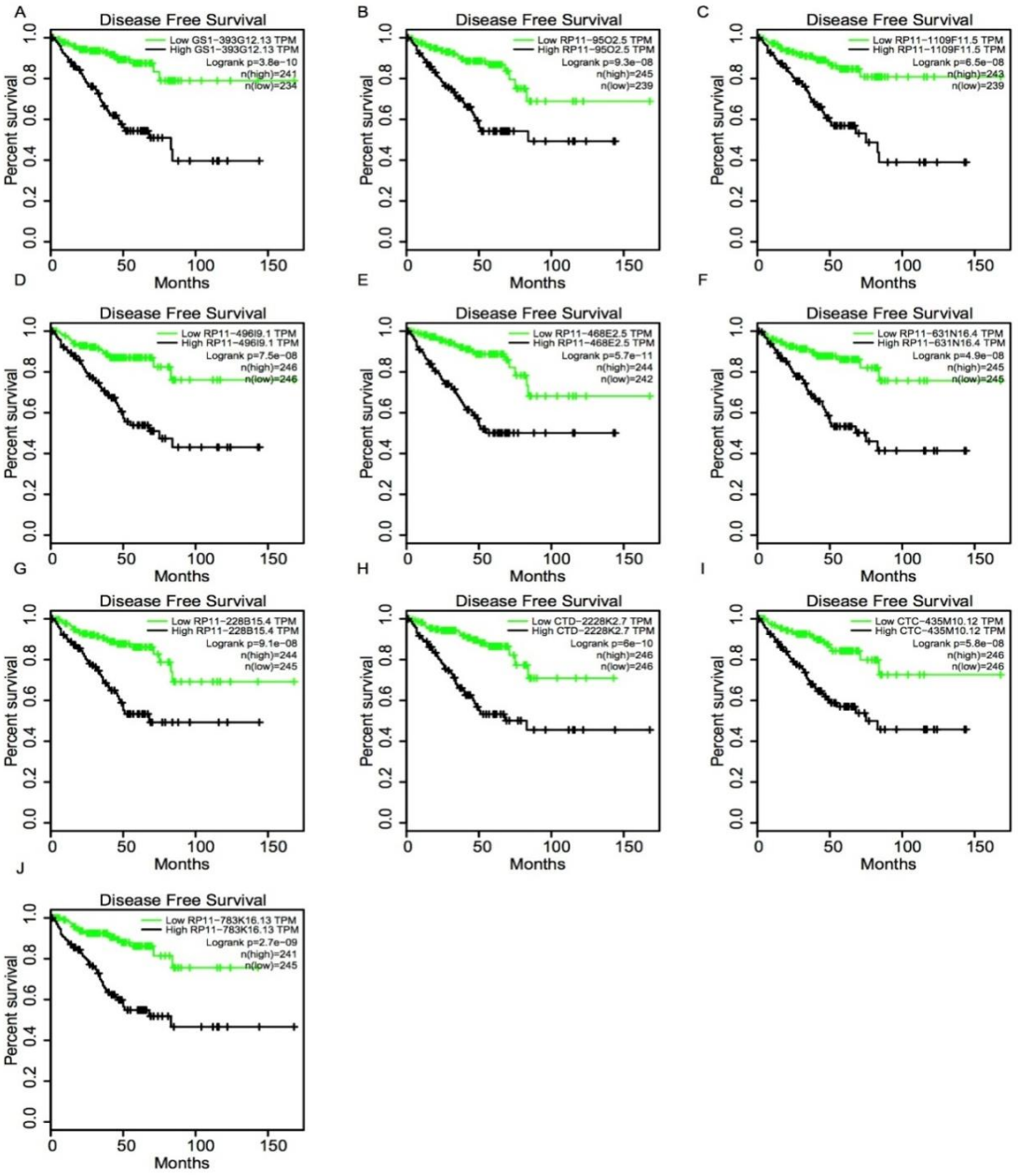

Figure 1. Identification of 10 disease-free survival time related lncRNAs in TC. A–J: Higher expression levels of (A) GS1-393G12.13, (B) RP11-95O2.5, (C) RP11-1109F11.5, (D) RP11-496I9.1, (E) RP11-468E2.5, (F) RP11-631N16.4, (G) RP11-228B15.4, (H) CTD-2228K2.7, (I) CTC-435M10.12 and (J) RP11-783K16.13 were significantly correlated to shorter disease-free survival time in patients with PRAD.

3.4. Bioinformatics analysis of differently expressed lncRNAs in PRAD

Furthermore, we performed bioinformatics analysis for differentially expressed lncRNAs in PRAD using MCODE plugin in Cytoscape software (Figure 4). Our results showed 10 differently expressed lncRNAs might be related to some pathways: Base-excision repair, protein export fromnucleus, termination of RNApolymerase II transcription, intrinsic apoptotic signaling pathway

in response to DNA damage, cell cyclecheck point, DNA recombination, regulation of G2/Mtransition of mitoticcell cycle, DNA-dependent DNA replication, DNA metabolic process, DNA replication initiation, regulation of DNA replication, telomere maintenance, microtubule cyto skeleton organization, glucose metabolic process, histone modification, RNA splicing, aerobic respiration, histone lysine methylation, tRNA metabolic process, regulation of cyclin-dependent protein serine/threonine kinase activity, regulation of extent of cell growth, dicarboxylic acid transport, response toionizing radiation, spliceosome complex assembly.

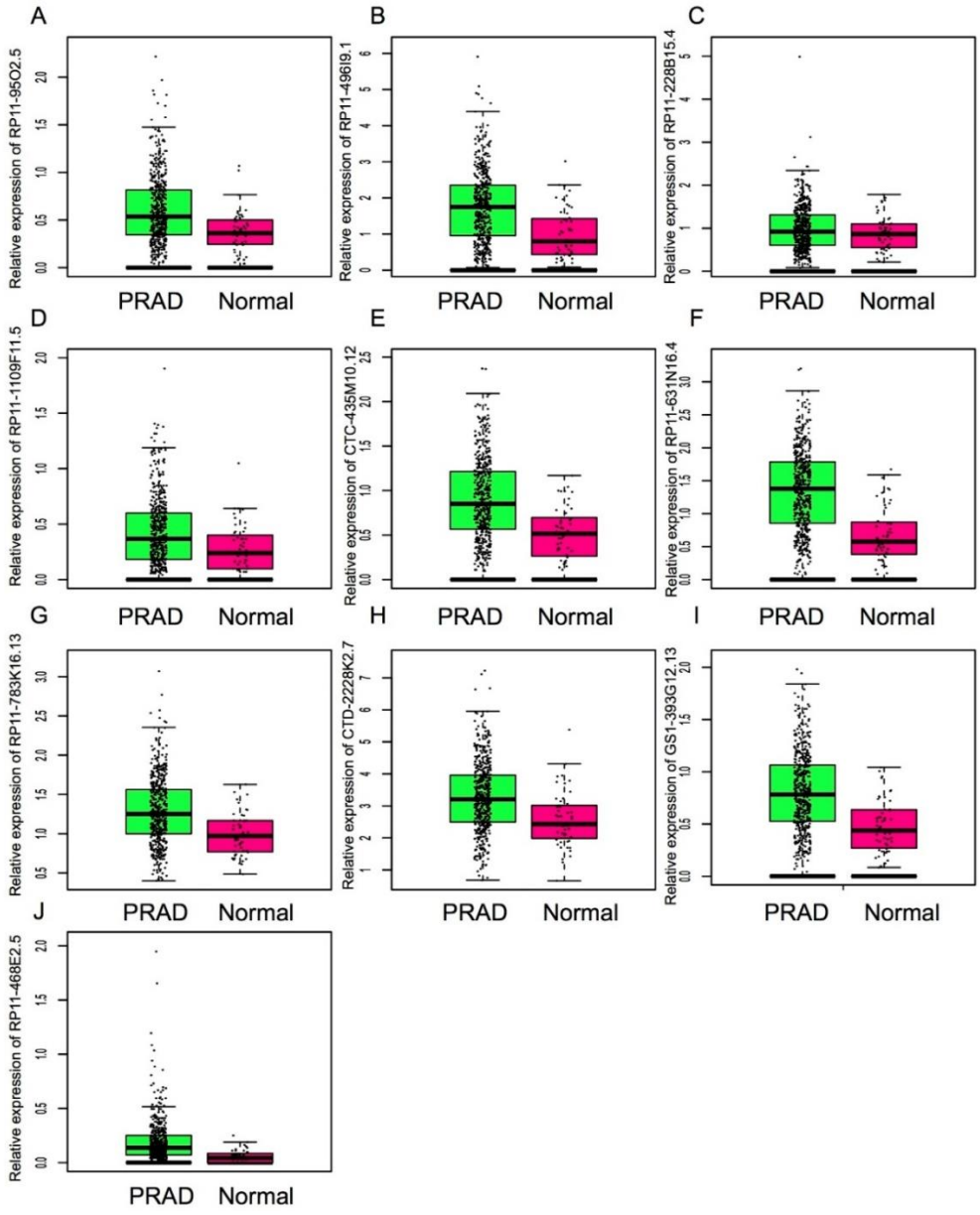

Figure 2. DFS-related lncRNAs were up-regulated lncRNAs in PRAD. (A–J): The expression levels of (A) RP11-95O2.5, (B) RP11-496I9.1, (C) RP11-228B15.4, (D) RP11-1109F11.5, (E) CTC-435M10.12, (F) RP11-631N16.4, (G) RP11-783K16.13, (H) CTD-2228K2.7, (I) GS1-393G12.13 and (J) RP11-468E2.5 were up-regulated in PRAD samples compared to normal tissues.

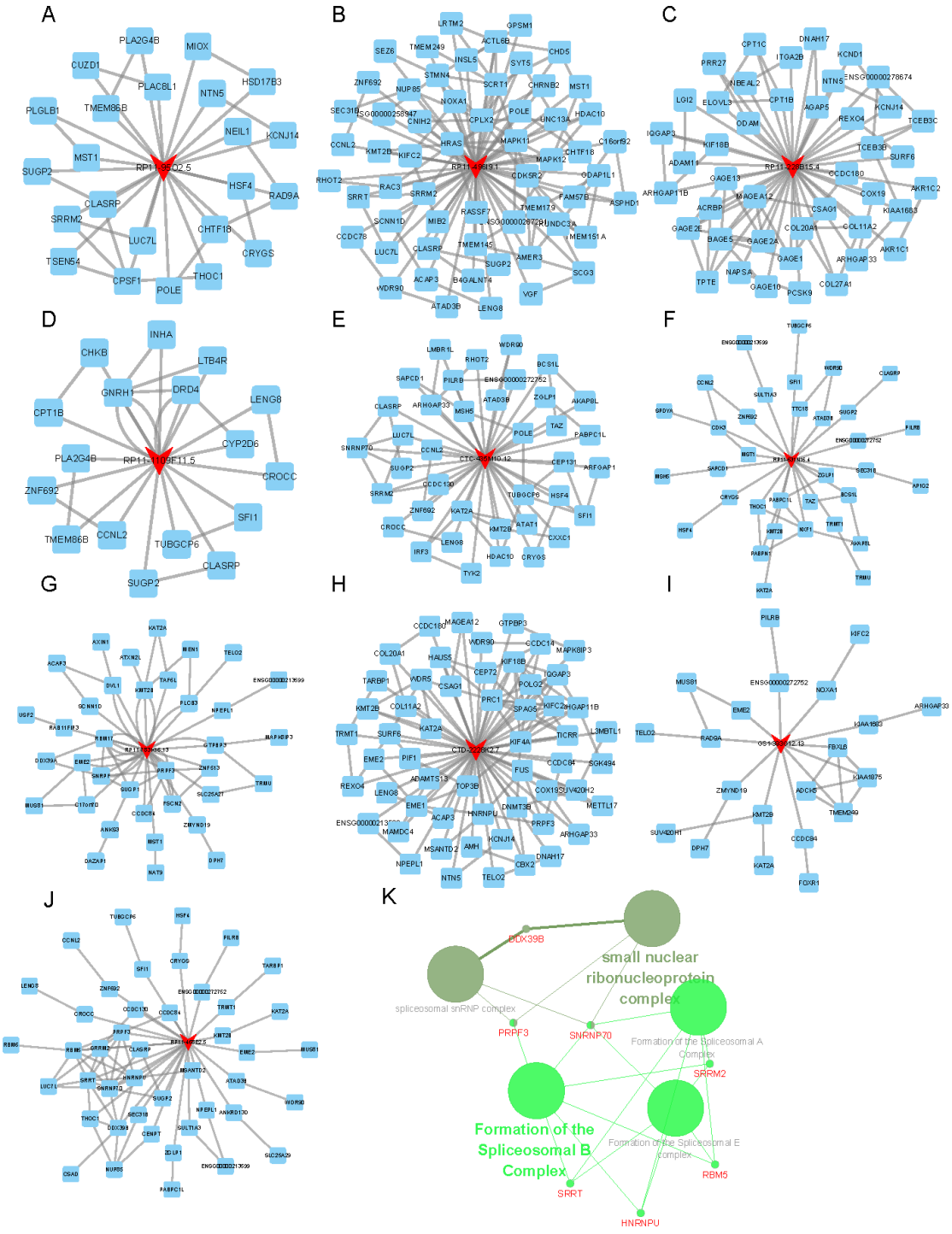

Figure 3. Construction of DFS-related lncRNAs regulating PPI network in PRAD. Construction of PPI network related to (A) RP11-95O2.5, (B) RP11-496I9.1, (C) RP11-228B15.4, (D) RP11-1109F11.5, (E) CTC-435M10.12, (F) RP11-631N16.4, (G) RP11-783K16.13, (H) CTD-2228K2.7, (I) GS1-393G12.13 and (J) RP11-468E2.5 in PRAD. Red nodes, lncRNA; green nodes, mRNA. (K) Bioinformatics analysis revealed that RP11-468E2.5 may affect small nuclear ribonucleoprotein complex and formation of the spliceosomal b complex.

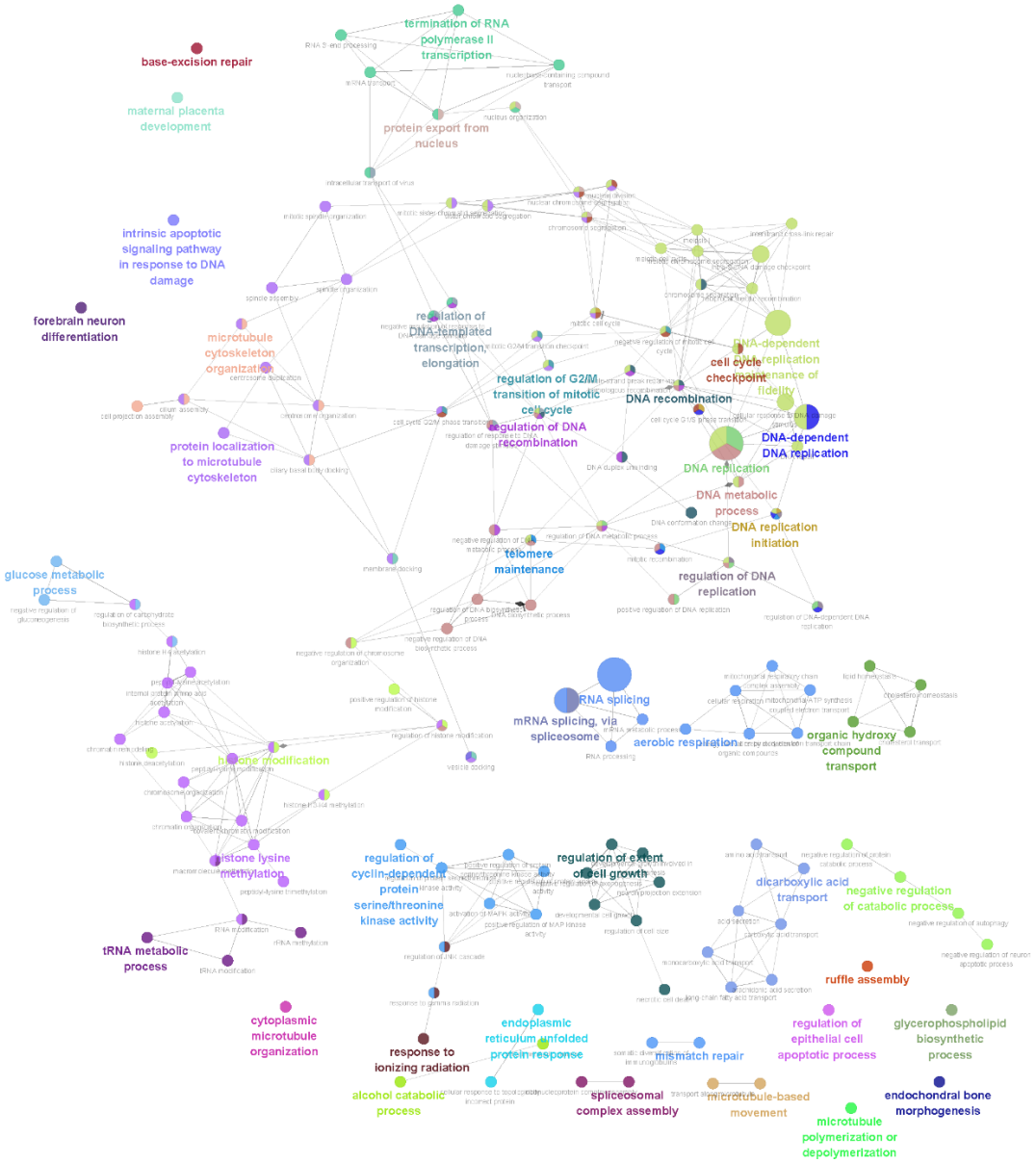

Figure 4. Bioinformatics analysis of differently expressed lncRNAs. MCODE plugin in Cytoscape software was used to predict the potential functions in PRAD.

3.5. AR and SPOP were involved in regulating differently expressed lncRNAs in PRAD

The up-stream regulators of these differently expressed lncRNAs in PRAD remained elusive. Androgen-receptor (AR) and SPOP is the most important regulators of PRAD progression. Therefore, we explore whether AR and SPOP were involved in regulating differently expressed lncRNAs in PRAD. As shown in Figure 5A–C, our results showed that AR was positively correlated to the expression of RP11-783K16.13, RP11-228B15.4 and CTD-2228K2.7. However, our results (Figure 5D–K) showed SPOP was significantly negatively correlated to the expression of RP11-468E2.5, GS1-393G12.13, RP11-783K16.13, RP11-631N16.4, CTC-435M10.12, RP11-1109F11.5, RP11-496I9.1 and RP11-95O2.5.

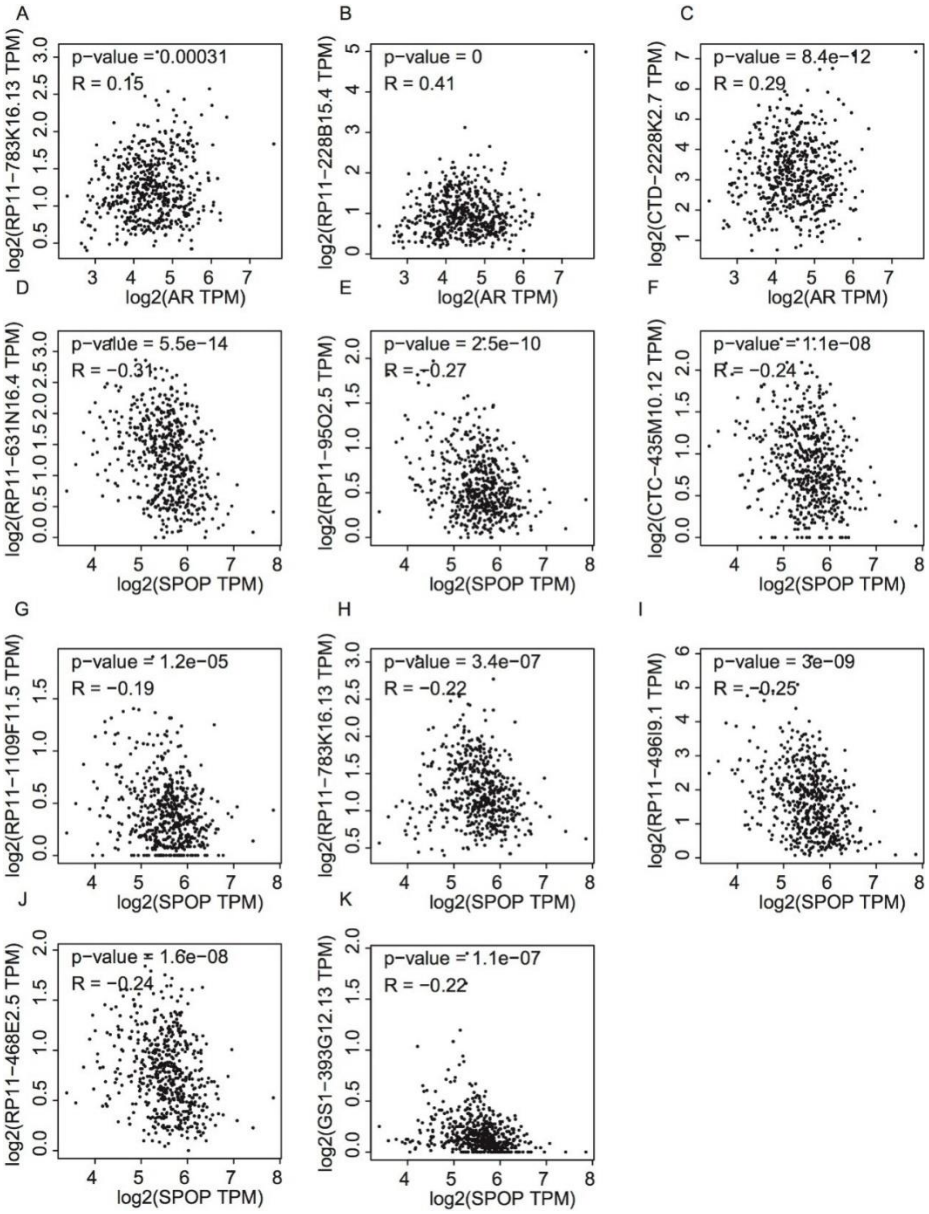

Figure 5. AR and SPOP were involved in regulating differently expressed lncRNAs in PRAD. (A–C) AR was positively correlated to the expression of (A) RP11-783K16.13, (B) RP11-228B15.4 and (C) CTD-2228K2.7. (D–K) SPOP was significantly negatively correlated to the expression of RP11-631N16.4, RP11-95O2.5, CTC-435M10.12, RP11-1109F11.5, RP11-783K16.13, RP11-496I9.1, RP11-468E2.5 and GS1-393G12.13. The x lab and y lab in All sub-figure is lncRNAs' TPM value, which is transformed to log2 value.

4. Discussion

Emerging studies had demonstrated lncRNAs played crucial roles in human cancers, including PRAD. LncRNAs were involved in regulating cancer cell growth, apoptosis, metastasis and autophagy. In PRAD, a few lncRNAs were reported to be associated the cancer progression. For example, the longer transcripts of PCAT19 promoted PRAD cell cycle, growth and metastasis

though interacting with HNRNPAB [14]. LncRNA ARLNC1 promoted PRAD progression though activating global AR signaling [15]. Moreover, lncRNAs were found to be associated the diagnosis and prognosis of PRAD. For example, PCA3 was reported to be a better biomarker than PSA in PRAD [16]. LncRNA PVT1 predicted the prognosis of PRAD [17]. Exploring the functional roles of lncRNAs in PRAD could provide novel biomarkers for PRAD. The present study screened disease-free survival (DFS) time related lncRNAs using GEPIA dataset. Higher expression of RP11-468E2.5, GS1-393G12.13, CTD-2228K2.7, RP11-783K16.13, RP11-631N16.4, CTC-435M10.12, RP11-1109F11.5, RP11-228B15.4 and RP11-496I9.1 were significantly associated with shorter DFS time in PRAD.

In order to explore the potential roles of these lncRNAs in PRAD, we constructed lncRNAs regulating PPI networks [18]. Bioinformatics analysis showed 10 differently expressed lncRNAs were significantly related to multiple biological processes in cancer, including cell cycle checkpoint, DNA recombination, regulation of G2/M transition of mitotic cell cycle, DNA-dependent DNA replication, regulation of cyclin-dependent protein serine/threonine kinase activity, regulation of extent of cell growth and intrinsic apoptotic signaling pathway. Moreover, we found these lncRNAs were involved in regulating RNA splicing and histone modification. RNA spicing had been demonstrated as a key regulator of PRAD progression. For example, RNA Splicing of the BHC80 gene regulates neuroendocrine PRAD Progression [19]. A recent study showed alternative RNA splicing of the GIT1 gene is associated with the progression of neuroendocrine PRAD [20].

AR signaling is the most important pathway in the regulation of PRAD [21]. AR signaling involved in regulating multiple pathways in PRAD, including cell apoptosis, cell cycle and metastasis. Recent studies demonstrated Androgen-responsive lncRNAs were involved in the progression of PRAD. For instance, CTBP1-AS promotes both hormone-dependent and castration-resistant PRAD growth though binding to PSF [22]. Higher expression of AR-regulated lncRNA TMPO-AS1 was associated with tumor progression and poor prognosis in PRAD [23]. In this study, we found AR was positively correlated to the expression of RP11-783K16.13, RP11-228B15.4 and CTD-2228K2.7, suggested that AR is a positive regulator of these lncRNAs. SPOP, a E3 ubiquitin ligase, is the most widely mutated gene in PRAD and plays a critical role in PRAD [24]. SPOP acts as a tumor suppressor via destabilizing downstream oncoproteins in PRAD, including FASN. However, the regulatory roles of SPOP in lncRNAs remained unclear. This study showed SPOP was significantly negatively correlated to the expression of RP11-468E2.5, GS1-393G12.13, RP11-783K16.13, RP11-631N16.4, CTC-435M10.12, RP11-1109F11.5, RP11-496I9.1, and RP11-95O2.5, suggested that SPOP may serve as a negative regulator of these lncRNAs.

5. Conclusion

In conclusion, we reported ten disease-free survival time related lncRNAs in PRAD, including RP11-468E2.5, GS1-393G12.13, CTD-2228K2.7, RP11-783K16.13, RP11-631N16.4, CTC-435M10.12, RP11-1109F11.5, RP11-228B15.4, RP11-496I9.1, and RP11-95O2.5. Higher expression of these lncRNAs significantly correlates to shorter DFS time in patients with PRAD. We next constructed lncRNAs regulating PPI networks in PRAD. Bioinformatics analysis revealed these DFS-related lncRNAs was related to cell cycle, glucose metabolic process, histone modification, and RNA splicing. AR and SPOP were identified to be involved in regulating these lncRNAs expression

in PRAD. The prognostic value and molecular functions of these lnRNAs in human diseases remained largely unknown. We thought this study for the first time demonstrated that they could act as novel potential biomarkers for PRAD.

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Conflict of interest

The authors declare there is no conflict of interest.

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