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FENDRR reduces tumor invasiveness in prostate cancer PC-3 cells by targeting CSNK1E

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Abstract. – OBJECTIVE: Prostate cancer, one of the most common malignant tumors in urology, now has become a malignant disease that seriously threatens the health of men in China. Although there are a large number of clinical studies on the treatment of patients with prostate cancer, many patients have entered the advanced stage of diagnosis, and little is known about its pathogenesis.

MATERIALS AND METHODS: We identified a series of ncRNA and TF by differential expression analysis, co-expression analysis, enrichment analysis, connectivity analysis, and hypergeometric test strategies for prostate cancer expression genomes.

RESULTS: 53 modules related to prostate cancer PC-3 cells were obtained, involving module focusing of 4448 genes. Based on these modules, we predicted that miR-26a-5p, miR-130a-3p, miR-519d-3p, etc. have important regulatory effects on prostate cancer PC-3 cells. At the same time, a series of transcription factors (relating to RELA, SOX10, TP53, and TWIST2, etc.) were obtained and may play a key regulatory role in prostate cancer PC-3 cell-related modules.

CONCLUSIONS: These results suggest that FENDRR in prostate cancer may reduce tumor invasion in prostate cancer PC-3 cells by targeting CSNK1E, which may have favourable effort to better understand the underlying pathogenesis of prostate cancer and provide a tough theoretical basis for further studying prostate cancer.

Key Words:
Prostate cancer, Malignant tumors, Differential expression analysis, Transcription factors.

Introduction

Prostate cancer, one of the most common causes of cancer-related death in men worldwide, in China, has the highest incidence in male genitourinary tumors[1,2]. Many methods against the diagnosis and treatment of prostate cancer still lack targeting, have large side effects, and have a high probability of recurrence. Therefore, it is crucial to identify specific and effective therapeutic targets to determine appropriate treatment and management. Fortunately, many biologists and medical researchers have devoted themselves to the pathogenesis of prostate cancer and the exploration of its effective targets, and have purchased great results. Among them, Jones et al[3] investigated Trabectedin which reduces the size of bone prostate cancer and the influence of M2 macrophages and found that M2-like macrophages induced *in vitro* were more familiar with phagocytosis (cytoplasmic effect) of dead tumor cells than M1-like macrophages. These findings suggest that M2-like monocytes and macrophages promote PCa bone metastasis and that trabectedin may represent a prostate cancer-specific therapeutic target. Through a simple synthesis of the natural alkaloid evodiamine/evodiamine and thieno[2,3-d]pyrimidinone hybrids, compound 11a was found to show effective cytotoxicity against human prostate cancer cell line PC-3 cell line. These outcomes demonstrate the potential anticancer therapeutic use of thieno[2,3-d]pyrimidinone and quinazolinone hybrids[4]. Yang et al[5] enhanced the anti-tumor effect of IL-24 on prostate cancer by studying tumor-penetrating peptides and the results showed that IL-24-iRGD induced apoptosis and significantly inhibited the growth of PC-3 cells, compared with IL-24 treatment alone. Thus, it can provide an improved strategy for the clinical treatment of prostate cancer. Ba et al[6] found that piperine not only has the potential to regulate voltage-gated K + current, but also has an effect on cell cycle arrest and apoptosis

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of human prostate cancer cells. In addition, its anticancer effect is directly related to the blockade of IK in LNCaP and PC-3 cells. In terms of targeted therapy, studies[7] with respect to the regulation of miR-195 against MAP2K1 applied to inhibit ADM drug resistance in prostate cancer cells, demonstrate that the down-regulation of miR-195 is associated with ADM resistance in prostate cancer cells. The previous report showed that phospholipase A2 receptor 1 (PLA2R1) was ectopically overexpressed in LNCaP with silenced PLA2R1 and was reduced in PC-3 cells with constitutively increased PLA2R1 expression relative to normal prostate epithelial cells. In LNCaP xenografts, PLA2R1-dependent colony formation regulation appears to exceed the receptor's oncogenic gene characteristics, resulting in reduced tumor growth and supporting the tumor suppressive effect of PLA2R1[8]. Based on the proliferation and migration of cancer cells, studies on the synergistic inhibition of proliferation and migration of prostate cancer cells by caffeic acid potassium acetate and coffee alcohol have shown that Kahweol acetate and cafestol synergistically inhibit the progression of prostate cancer. Thus, these coffee compounds are considered to be new therapeutic candidates for prostate cancer[9]. Previous studies[10] have verified that CC chemokine receptor 7 (CCR7) is important for invasion and metastasis in prostate cancer. According to the latest research[11], the upregulation of Notch1 by CCR7 can accelerate the evolution of EMT and promote the invasion and metastasis of prostate cancer cells by activating prostate cancer MAPK and NF-κB signaling pathways, providing new molecular evidence for targeted therapy. Besides, Chi et al[12] reported that silencing of CCR7 inhibits prostate cancer cell growth, invasion, and migration induced by vascular endothelial growth factor C (VEGFC) which is expressed in tumor cell, suggesting that CCR7 may be used as a new gene therapy approach for prostate cancer[13].

Meanwhile, reducing the aggressiveness of cancer cells is also one of the important directions for the treatment of prostate cancer. The expression of FGD4 was also confirmed to be positively correlated with the aggressive phenotype of prostate cancer, and the data demonstrated that FGD4 has the function of promoting tumor growth and cell migration in prostate cancer cells[14]. With the development of nanotechnology, clinically, there are many possibilities for the treatment of prostate cancer. For example, Kommineni et al[11], based on nanomedicine-based prostate cancer combined chemotherapy, showed that cationic lipids encapsulated by both Cabazitaxel and silybin are are applied for CD44 targeted delivery, and the results of this therapy demonstrated that HA-coated CBX and SIL co-loaded liposomes can be potential methods for eradicating prostate cancer[12]. In microRNA studies, miR-93 promotes PC progression and metastasis by inhibiting DAB2 activation of the Akt/ERK1/2 pathway, and elevated DAB2 and inactivation of Akt/ERK1/2 may be potential therapeutic targets for PC[15]. In addition, studies have found that IATL dose-dependently inhibits the growth of cancer cell and induces apoptosis in PC-3 and DU145 cells, suggesting that IATL biological activity may provide a novel anti-cancer candidate for prostate cancer[16]. Argenziano et al[17] have shown that MEB55 and ST362 have anticancer activity as solerolactone (SL) analogue. Glutathione/pH-responsive nanosponge (GSH/pH-NS) is an effective tool for the delivery of SL control to prostate cancer cells and can enhance the therapeutic efficacy of these compounds. In addition, the previous report showed that the overexpression of LRIG3 gene expression may inhibit prostate cancer cell viability, adhesion, invasion and migration, and promote apoptosis, while RAD-treated LRIG3 gene may contribute to the study on treatment of prostate cancer[18]. Although biologists have done various experimental studies on prostate cancer, further exploration is still required for a comprehensive understanding of the detailed pathogenesis and key therapeutic targets of prostate cancer.

We present a new binding method of network-based disease-related dysfunction modules and combine prostate cancer-related data for a comprehensive analysis and explore the possibility of key factors involved in the reduction of prostate cancer PC-3 cells aggressiveness and identify CSNK1E as a candidate biomarker for the diagnosis of cancer. The comprehensive strategy based on this dysfunction module not only contributes to explore the process of prostate cancer, but also provides a new way for biologists to undergo further design experiments.

Materials and Methods

Differential Expression Analysis of Prostate Cancer Genes

Prostate cancer samples were collected from the NCBI Gene Expression Omnibus (GEO) database including 13 gene expression profiles of

prostate cancer samples and 8 normal samples, numbered GSE55945[19]. The chip platform was the Affymetrix Human Genome U133 Plus 2.0 Array. This study used the basic data processing package of R language expression profiling chip (including R.utils, R.oo, R.methodsS3, and hgu133plus-2cdf) to construct disease and normal sample expression profiles for GEO databases, using the R language limma package for calculation[20]. With respect to chip data, the background correct function was first applied for background correction and standardization. The quantile normalization method based on normalizing the Between Array function filtered out control probes and low expression probes for high-quality standardized data. The lmFit and eBayes functions of limma package with default parameters were used to screen the differential expression of disease full expression profile $p<0.05$ with 4448 differentially expressed genes obtained.

Co-Expression Analysis

To explore the synergistic expression of these potential pathogenic genes in prostate cancer, weighted gene co-expression network analysis was performed (WGCNA)[21] to analyse the differential expression matrix of the prostate, searching for gene modules for synergistic expression. Unlike general clustering methods, the WGCNA clustering criteria are biologically significant. It is based on the correlation coefficient of the inter-gene expression level to the nth power, enabling the distribution of the correlation coefficient values to gradually conform to the scale-free distribution. Based on cohesion, a hierarchical clustering tree is established by correlation coefficients between genes. Genes with similar patterns are classified as the same branch. Diverse branches of the clustering tree represent diverse gene modules, and various colors represent various modules. Therefore, the results obtained by this method are more reliable. This study established a disease expression profile of 4448 differential genes at $p<0.05$ for co-expression analysis and got 53 modules, 4448 module genes, and the core genes of each module.

Function and Pathway Enrichment

Exploring the function and signal pathway of genes is often a valid mean to investigate the molecular mechanism of diseases while those of the module gene can characterize the dysfunction mechanism of the module in the process of disease occurrence. Therefore, we conducted the analysis of Go function (p-value Cutoff = 0.01, q-value Cutoff = 0.01) and the KEGG pathway (p-value Cutoff = 0.05, q-value Cutoff = 0.2) enrichment for the genes of 53 functional modules using the R language Clusterprofiler package, respectively[22]. Outcomes showed that these signaling pathways are inextricably linked to the occurrence and development of prostate cancer.

Exploring the Interaction of Modular Genes to Identify Internal Drive Genes

The potential pathogenic genes of prostate cancer are clustered into functional modules based on synergistic expression relationships, but there are target interactions among genes in addition to co-expression. Therefore, observing the target interactions of genes within the module is helpful to understand the core molecules that drive the function and dysfunction of the module. Based on the String database, a protein interaction subnet for each module was established[23]. Then, Cytoscape is used for display and network analysis (including connectivity calculation)[24] screening the genes with the greatest connectivity which is considered as the core molecule to regulate the progress of the module and identified as the intrinsic gene. The internal drive genes may characterize pathogenic molecules in prostate cancer.

Prediction of the Multifactorial Regulation of Modules

Data on transcriptional and post-transcriptional target regulation relationships are included in the TRRUST v2.0 database and the RAID v2.0 database[25,26]. Among them, we downloaded and used all human transcription factor target data in the TRRUST v2 database, involving 2492 transcription factors and 9396 TF-Module interaction pairs. 431937 ncRNA-protein interactions of 5431 ncRNAs against humans were screened in a RAID 2.0 database. These regulatory factors often mediate the development of the disease. To explore the driving force of co-expression modules of genes related to prostate cancer, we conduct pivotal analysis based on these interaction data. Pivot analysis refers to searching for at least two interacting drivers with the module in the target pair and calculating the significance of the interaction between the driver and the module according to the hypergeometric test, Pivot analysis (find the control module) in the context of TF_Pivot data, p-value < 0.01, gained a total of 40 transcription factors, 44 pairs of TF-Module pairs. Finally, via statistical analysis on pivots, ones that

have a regulatory effect on more dysfunctional modules are identified as core pivots. The ncRNA and TF-based target data were predicted as background sets, and the pivotal regulator of the regulatory dysfunction module was obtained.

Results

Differential Expression of Prostate Cancer-Related Genes

Through differential screening of prostate cancer expression profiles, we obtained 4448 (p<0.05) differentially expressed genes **(Table S1)**. Among them, LINC00844 and CRISP3 have higher variation ratios, and these differentially expressed genes are all associated with prostate cancer, so they may contribute to the development of cancer.

Related Genes with Synergistic Expression Behaviour in the Pathogenesis of Prostate Cancer

To systematically study the action mechanism of prostate cancer-related genes in patient samples, we performed extensive analytical studies. First, expression profiles were conducted in 4448 prostate cancer-related genes in the samples of the patient. In light of co-expression network analysis, we obtained the expression of 53 groups of prostate cancer modules (Figures 1A, 1B, 1C), and then found that 53 groups of modules have a synergistic expression in the group. These function modules may participate in different functions and pathways, symbolizing that different regulatory mechanisms mediate the occurrence and progression of prostate cancer.

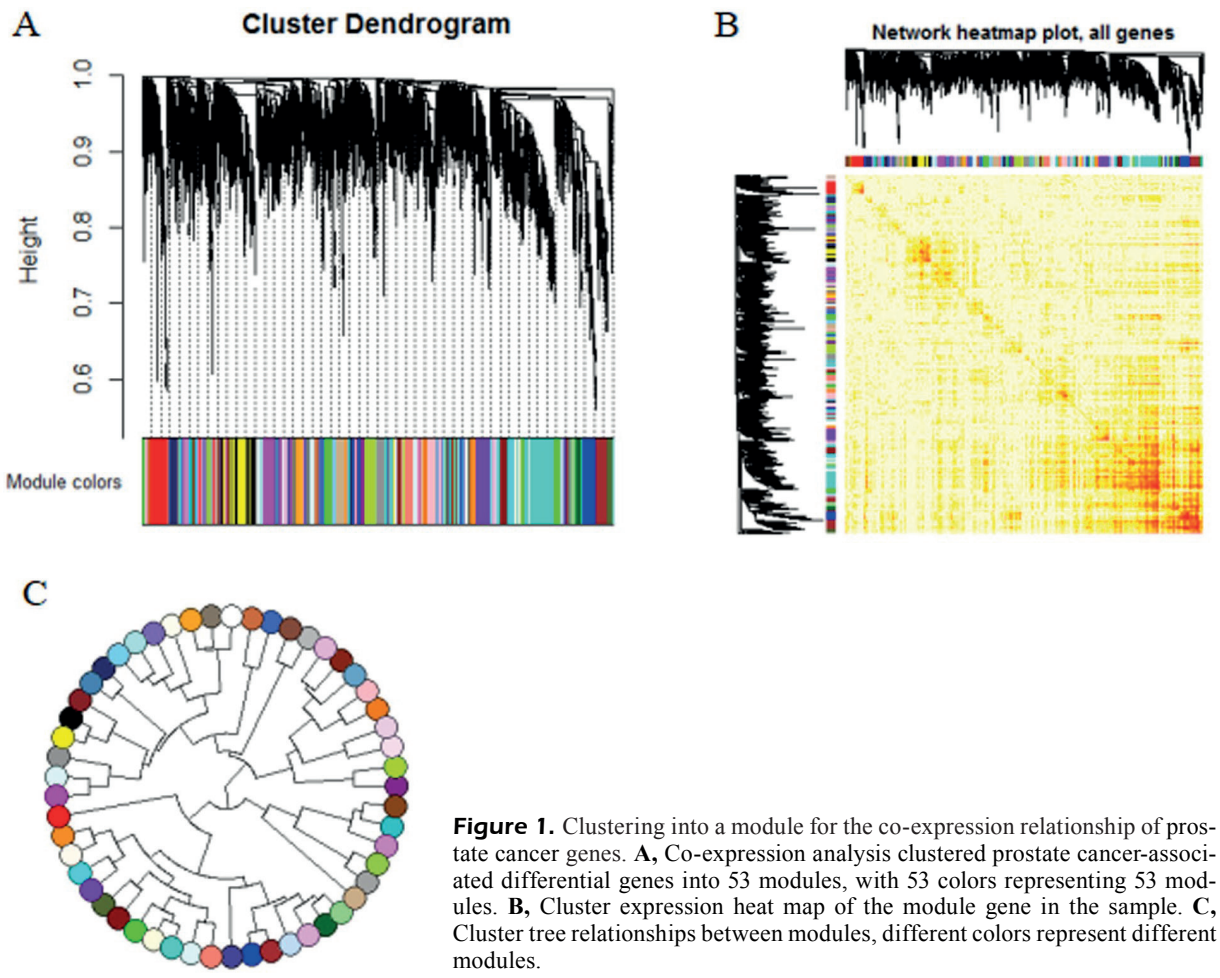

Figure 1. Clustering into a module for the co-expression relationship of prostate cancer genes. **A,** Co-expression analysis clustered prostate cancer-associated differential genes into 53 modules, with 53 colors representing 53 modules. **B,** Cluster expression heat map of the module gene in the sample. **C,** Cluster tree relationships between modules, different colors represent different modules.

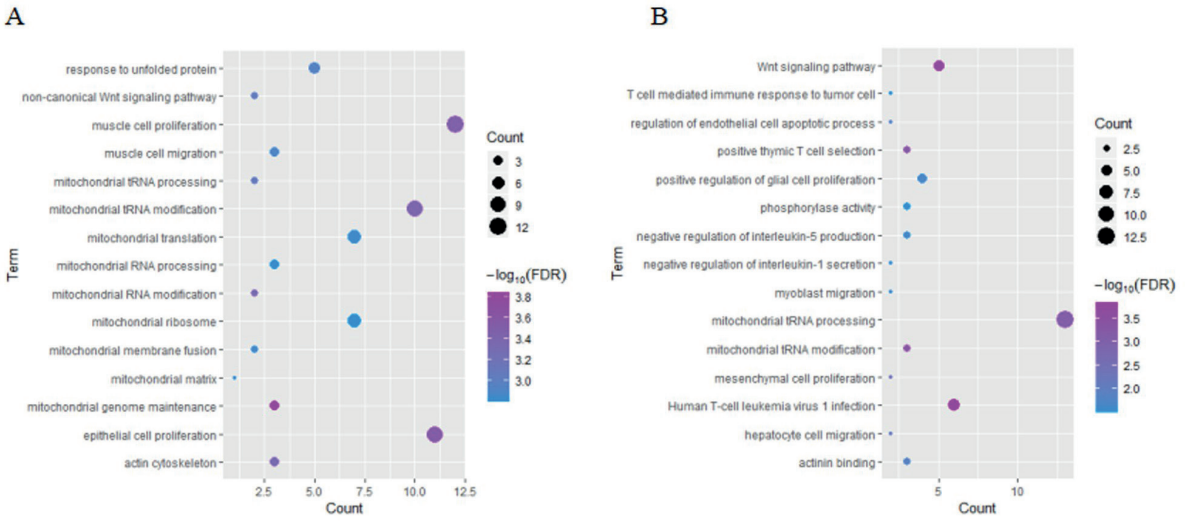

Figure 2. Modules involved in the function and pathway identification of the prostate cancer dysfunction module. ***A,*** Module gene GO function enrichment analysis excerpt. The deeper the color, the stronger the enrichment. The larger the circle, the more significant the proportion of the gene in the module that accounts for the GO function. ***B,*** Module gene KEGG pathway enrichment analysis excerpt. The deeper the color, the stronger the enrichment. The larger the circle, the more significant the proportion of the gene in the KEGG pathway entry.

Functional Dysfunction Module Characterizing the Pathogenesis of Prostate Cancer

Studying the function and pathway of gene involvement is an important mean to identify the mediated pathogenesis. To study the possible dysfunction caused by module gene disorder, we respectively analysed the enrichment of function and pathway of each module. The analysis of GO function and KEGG pathway enrichment of 53 modules showed that there were 1688 cell component entries, 3247 molecular function terms, and 13794 biological processes (Figure 2A). Based on functional analysis, it was observed that related functional modules tend to enrich multiple disease-related functions, including cell proliferation and apoptosis, regulation of interleukin-2 production, and positive regulation of cell migration. The results of the KEGG analysis indicated (Figure 2B) that these modular genes involved 475 KEGG pathway enrichment. They are mainly involved in the TGF-beta signaling pathway, Wnt signaling pathway and NF-kappa B signaling pathway, which are all related to the occurrence and development of prostate cancer. Given that the functional and pathway results of modular gene enrichment are closely related to prostate cancer, we identified these 53 modules as dysfunction modules.

The Dysfunction Module's Internal Drive Genes Characterizing Pathogenic Molecules in Prostate Cancer

According to our study, the prostate cancer dysfunction module consists of numerous potential pathogenic genes for prostate cancer and clusters into modules based on co-expression relationships. The study found that these genes not only have a synergistic expression relationship, but also a mutual control relationship between the targets. We investigated the interactions of genes within the module and calculated their connectivity (Figure 3). The genes with the highest connectivity of each module were screened, regarded as an active regulatory role in the module, and were thus identified as an internal drive gene for the prostate cancer dysfunction module. Then, the screening score was greater than 500, with a total of 1632 pairs. The most interconnected gene in each of these modules is considered to be the most active and significant endogenous gene. Therefore, 16 driver genes were obtained via analysis **(Table S2)**, including VCL, ACTB, and ACTA2. Among them, VCL, ACTB and ACTA2 drive module 1 altogether while VCL and ACTB connectivity is up to 34, while ACTA2 connectivity is 32. These driving genes play an important regulatory role in the module, affecting the formation and development of prostate cancer.

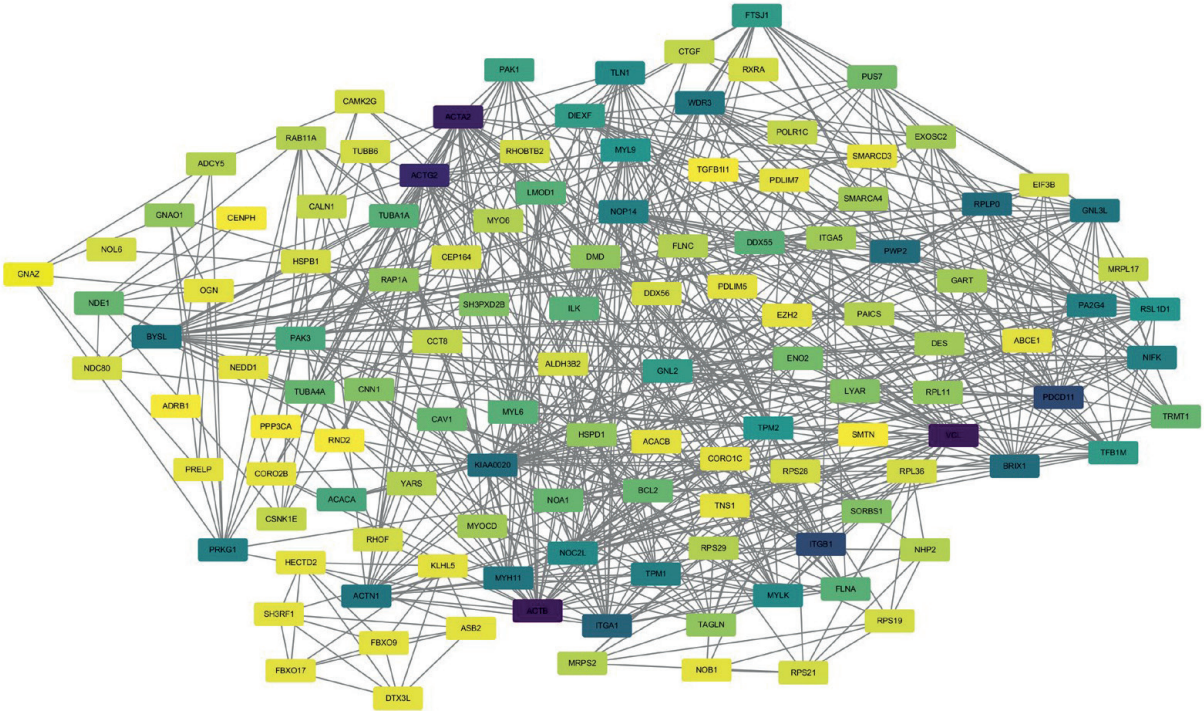

Figure 3. From yellow to purple, the deeper the purple, the greater the connectivity which is regarded greater regulating ability.

The NcRNA Mediating Dysfunction Module

The transcription and post-transcriptional regulation of genes are recognized as a key factor regulating the occurrence and progression of diseases, and ncRNA are considered to be salient regulators[27]. Scientific prediction of ncRNA regulating dysfunction module genes has favourable effect on exploring the transcriptional regulation mechanism of prostate cancer. Thus, pivot analysis, based on the targeting relationship between ncRNA and gene, was performed to explore ncRNA regulators causing module dysfunction. The predicted results (**Table S3**, Figure 4A) show that 1028 ncRNAs significantly regulate modules, involving 1650 Pivot-Module interaction pairs. In addition, from statistical analysis of the results, it was found that TUG1 has a significant regulatory relationship with 9 dysfunction modules and plays a large role in the dysfunction of the module. FENDRR, miR-301b-3p, miR-340-5p, and miR-590-3p have been identified with a valid effect on seven dysfunction modules, which may become potential pathogenic factors for prostate cancer. Other ncRNAs also show significant regulation of dysfunction modules and make a difference to the pathogenesis of prostate cancer. In summary (Figure 4B), we believe that FENDRR in prostate cancer may participate in the Wnt signaling pathway to attenuate tumor aggressiveness in prostate cancer PC-3 cells by targeting CSNK1E-mediated module 1.

The Key Regulatory Role of Transcription Factors in Dysfunction Modules

Transcription factors are important with respect to the regulation of gene transcription and many studies have shown that disordered expression of transcription factors may cause various diseases. The occurrence of prostate cancer is also closely related to the disorder of transcription factors in terms of the regulation of transcription factors on dysfunction modules. Accordingly, pivot analysis was performed to predict the module according to the regulation of gene by transcription factors. The results (**Table S4**, Figure 5) indicated that a total of 40 transcription factors have significant transcriptional regulation of the prostate cancer dysfunction module, involving 44 TF-Module regulatory pairs. Statistical analysis of these transcription factor regulatory pairs revealed that the

tumor suppressor genes RELA, SOX10, TP53, and TWIST2 significantly regulate two dysfunction modules, which have potential effects on the regulation of prostate cancer. These data suggest that transcription factors probably make a difference to the mechanism of prostate cancer and they have significant regulatory effects on multiple dysfunction modules, identified as the core transcription factors of prostate cancer.

Discussion

Prostate cancer, as one of the most commonly diagnosed cancers among men worldwide, is still one of the leading causes of cancer-related death in men[28,29]. Identifying biomarkers in prostate cancer can effectively diagnose treatment and design better drugs. In recent years, scientists explore prostate cancer, mainly focusing on certain genes or proteins, as well as related signaling pathways. However, the pathogenesis of these genes, proteins and signaling pathways in prostate cancer remains unclear without better targets to eradicate prostate cancer. With the accumulation of "omics" data, especially gene expression data, we provide a more efficient way to detect biomarkers. Therefore, we combined a series of analytical methods to explore its pathogenesis and specific targets. First, we constructed a total expression profile of prostate cancer samples for differential analysis and screened 4448 potential pathogenic genes. Related studies have shown that the novel lncRNA LINC00844 regulates prostate cancer cell migration and invasion through androgen receptor (AR) signaling. Compared with clinical specimens of malignant and metastatic prostate cancer, the expression of LINC00844 is higher in normal prostate and incidence of poor prognosis with low expression poor and biochemical recurrence gets higher clearly, indicating that LINC00844 plays an important role in inhibiting tumor progression and metastasis[29]. At the same time, based on the role of AR in prostate cancer, we also found that the expression of cysteine-rich secreted protein 3 (CRISP3) in prostate cancer cells is androgen-dependent, and its overexpression can also lead to poor prognosis of prostate cancer[30,31]. These results demonstrated that LINC00844 and CRISP3 are central to the development and migration of prostate cancer. Subsequently, with the co-expression network, 53 functional modules were identified in conjunction with the cohesiveness of the functional cluster. In light of the results of enrichment analysis, the module genes are mainly involved in the TGF-beta signaling pathway, Wnt signaling pathway and NF-kappa B signaling pathway, which are critical for the initiation of prostate cancer. Some studies[32] have shown that TGF-β up-regulates a series of genes involved in cancer invasion and bone metastasis in prostate cancer cells, and the TGFBR1 inhibitor SD 208 effectively attenuates prostate cancer bone metastasis. Compared with normal prostate cells, Capn4 is highly regulated in prostate cancer cell lines regulated by microRNA-520b and plays a carcinogenic role in prostate cancer cells by promoting Wnt/β-catenin signal-

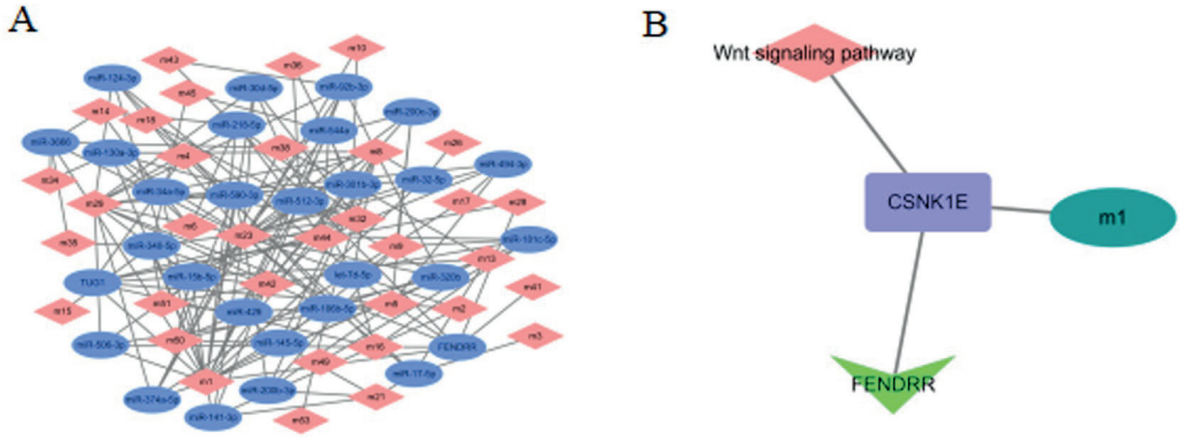

Figure 4. A regulatory network diagram of prostate cancer. ***A***, ncRNA for prostate cancer related modules. ***B***, FENDRR in prostate cancer may participate in the Wnt signaling pathway to attenuate tumor aggressiveness in prostate cancer PC-3 cells by targeting CSNK1E-mediated module 1.

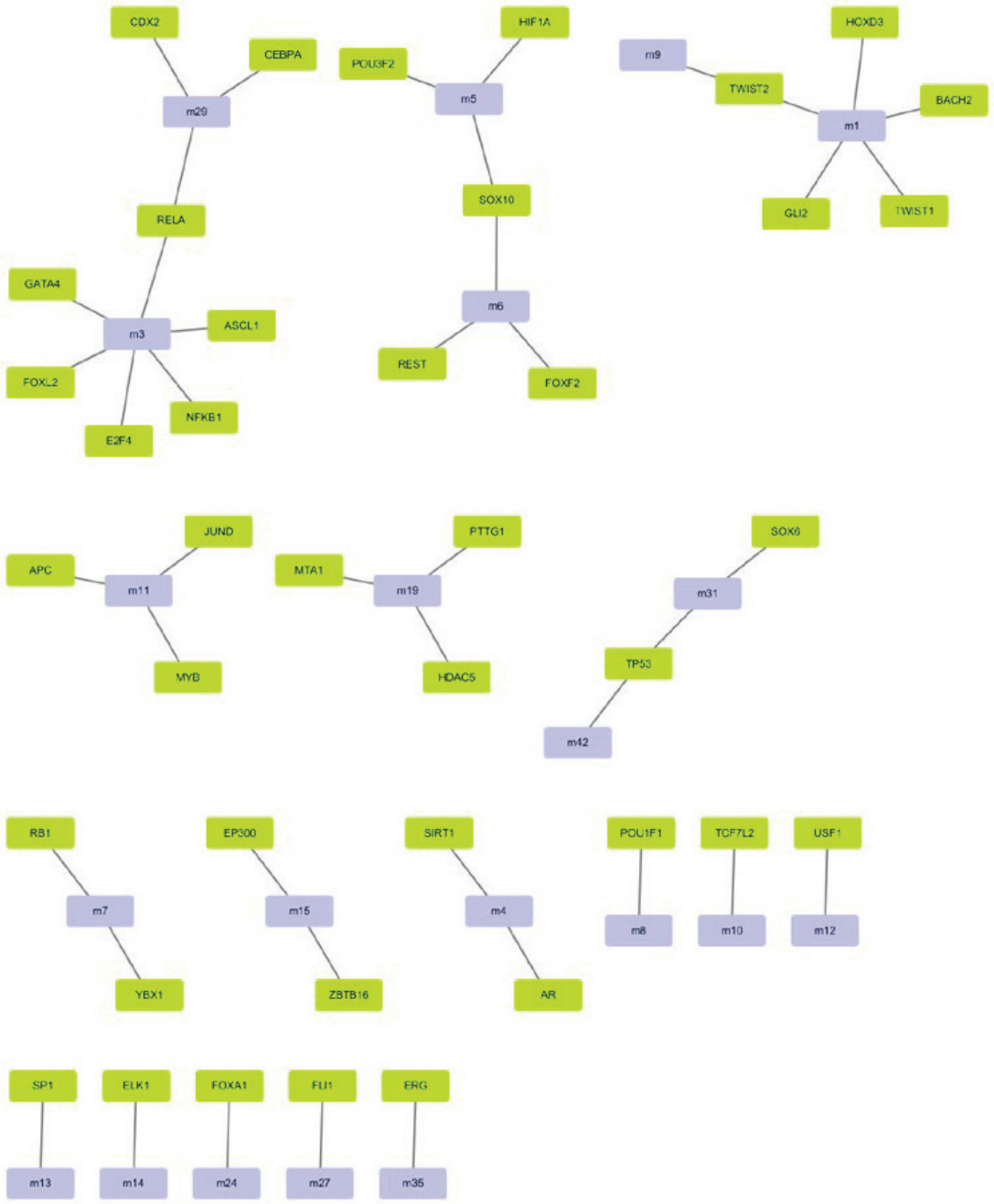

Figure 5. Network diagram of transcription factor regulation of prostate cancer related functional modules.

ing[33]. In addition, Guo et al[34] suggest that interleukin-8 (IL-8) which is significantly increased in prostate cancer induces proliferation of prostate cancer cells, invasion and attenuates of apoptosis by signal transduction and transcriptional activator 3/protein kinase B/NF-κB signaling pathways. Ectodermal fibroblast growth factor receptor 1 (FGFR 1) enhances inflammation and the occurrence and progression of prostate cancer by promoting NF-κB signaling in prostate cancer cells[35]. Therefore, discovering these key genes involved in the signaling pathway in prostate cancer cells is of great significance for effective diagnosis and treatment. Recently, increasing evidence indicated that proteins play a key regulatory role in the development of prostate cancer. In particular, VCL was identified as a highly expressed protein in PC3 cells, and its DNA and mRNA were am-

plified and up-regulated in some PCa patients, and the expression of KCL significantly inhibited prostate cancer cell migration after knock-out[36]. The report of Zhu et al[37] pointed out that VCL has different expression in benign prostatic hyperplasia (BPH) and prostate cancer (PCa), and can be used as an indicator for differential diagnosis of benign and malignant prostate diseases. With the development of PCa, the expression of VCL gradually decreasing can assess PCa progression and prognosis. Of note, Zhao et al[38] demonstrated that ACTB may be the most suitable reference gene across all cell lines in dihydrotestosterone (DHT) regulatory genes by Western blotting, which also provides a new understanding of reference gene validation for diverse DHT treatments in prostate cancer cells.

In recent years, IncRNAs have received increasing attention from scientists, and they play an important role in the occurrence and development of tumors. For instance, FENDRR, as a long non-coding RNA, may be related to poor prognosis in prostate cancer patients[39]. We established a pathway interaction network to consider the functional dependence between pathways and obtained FENDRR up-regulation to inhibit cell proliferation, increase apoptosis, and reduce invasion and migration. The results indicate that FENDRR may be a potential therapeutic target and biomarker for PCA[40]. In addition, we have certified that miR-301b-3p is up-regulated in tumor and normal prostate tissues, as well as in metastatic and primary sites by other studies, and is therefore considered to be a candidate for carcinogenesis in prostate cancer[41]. Transcription factors that regulate these dysfunction modules were identified, including RELA, SOX10, TP53, and TWIST2. Among them, RELA, as an important component of the NF-KB signaling pathway, is involved in the oncogenic signaling of proinflammatory mediator activation and regulates prostate cancer-associated inflammatory signaling pathways, which in turn affects disease progression[42]. We also found that SOX10 may promote prostate cancer progression by accelerating the proliferation and invasion of prostate cancer cells, so SOX10 may be a potentially effective therapeutic target for prostate cancer[43]. The concept that human cancer is essentially a genetic disease driven by genetic mutations has been established. As a tumor protein, TP53 losing function may promote castration resistance in prostate cancer cells by transiently enhancing androgen-independent cell growth and promoting the occurrence of genomic instability, so TP53 may be a potential for prostate cancer. Biomarkers are used to reduce their invasiveness[44]. TWIST2 is an important transcription factor. In light of other studies, we found that the induction level of epithelial to mesenchymal transition (EMT) is related to the expression level of S18-2 in PCa cell line. When S18-2 overexpress, cells may obtain an increase in migration ability with increased surface expression of CXCR4 cells. These data indicated that S18-2 protein induces EMT via TWIST2/E-cadherin signaling and mediates PCa cell migration via CXCR4[45]. In summary, these regulatory factors significantly regulate the dysfunction module, as well as the development of prostate cancer.

Conclusions

Based on the analysis results of this study, we obtained a more detailed pathogenic module of prostate cancer. These modules provide a number of proven prostate cancer-associated genes and signaling pathways, which underlies a theoretical basis for further research into prostate cancer. Through a systematic study, we believe that FENDRR in prostate cancer may participate in the Wnt signaling pathway to attenuate tumor aggressiveness in prostate cancer PC-3 cells by targeting CSNK1E-mediated module 1. Therefore, a comprehensive strategy based on this dysfunction module not only provides new insights into the study of disease mechanisms, but also proposes new alternatives for developing more effective drugs.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

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