

# Identification of novel biomarkers correlated with prostate cancer progression by an integrated bioinformatic analysis

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## Abstract

Prostate cancer (PCa) is a highly aggressive malignant tumor and the biological mechanisms underlying its progression remain unclear.

We performed weighted gene co-expression network analysis in PCa dataset from the Cancer Genome Atlas database to identify the key module and key genes related to the progression of PCa. Furthermore, another independent datasets were used to validate our findings.

A total of 744 differentially expressed genes were screened out and 5 modules were identified for PCa samples from the Cancer Genome Atlas database. We found the brown module was the key module and related to tumor grade ( $R^2=0.52$ ) and tumor invasion depth ( $R^2=0.39$ ). Besides, 24 candidate hub genes were screened out and 2 genes (BIRC5 and DEPDC1B) were identified and validated as real hub genes that associated with the progression and prognosis of PCa. Moreover, the biological roles of BIRC5 were related to G-protein coupled receptor signal pathway, and the functions of DEPDC1B were related to the G-protein coupled receptor signal pathway and retinol metabolism in PCa.

Taken together, we identified 1 module, 24 candidate hub genes and 2 real hub genes, which were prominently associated with PCa progression. With more experiments and clinical trials, these genes may provide a promising future for PCa treatment.

**Abbreviations:** BP = biological process, DAVID = Database for Annotation, Visualization and Integrated Discovery, DEGs = differentially expressed genes, GEPIA = Gene Expression Profiling Interactive Analysis, GO = gene ontology, GS = gene significance, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function, MM = module membership, PCa = prostate cancer, TCGA = the Cancer Genome Atlas, TOM = topological overlap matrix, WGCNA = weighted gene co-expression network analysis.

**Keywords:** hub genes, key module, prostate cancer, therapeutic targets, weighted gene co-expression network analysis

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ZM and JW contributed equally to this work and are the co-first authors.

The data that support the findings of this study are openly available in TCGA database (<https://cancergenome.nih.gov/>) and Oncomine database (<http://www.oncomine.org>).

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## 1. Introduction

Prostate cancer (PCa) is one of the urologic malignant tumors, which caused eighth leading human cancer associated mortality globally.<sup>[1]</sup> According to the statistics, about 1.3 million new cases of prostate cancer were identified globally in 2018.<sup>[1]</sup> Although a rather indolent clinical course of most primary PCa, patients with aggressive forms of PCa still meet poor prognosis and little treatment options.<sup>[2]</sup> Thus, a reliable method to distinguish the disease progression and prognosis of PCa is highly desirable to make the right therapeutic decisions. Currently, several clinical features, such as Gleason score, prostate biopsies, and clinical stage have been established to diagnosis and predict the disease outcomes.<sup>[3,4]</sup> However, these methodologies are statistically powerful but not sufficient to confirm diagnosis and effective outcomes prediction for PCa patients.<sup>[5,6]</sup> Therefore, to figure out the disease biological mechanism and identify new biomarkers for the management of PCa are in desperate need.

In recent years, high-throughput sequencing and gene-detecting technology afford an unprecedented opportunity to identify specific genes related to PCa progression.<sup>[7-9]</sup> However, most of the previous studies merely follow to single or several essential genes, but seldom pay attention to functional networks and interconnection between genes in PCa. Currently, weighted gene co-expression network analysis (WGCNA) was applied to investigate the relationship between gene mRNA level and

phenotypes to reveal the key genes for progression of many diseases, especially cancer.<sup>[10–13]</sup> Besides, WGCNA has been widely used in several cancers to identify key genes and potential biomarkers for predicting clinical outcomes of patients.<sup>[14–17]</sup>

In this study, WGCNA was applied to analyze RNASeq data of PCa samples and pairs normal samples from the Cancer Genome Atlas (TCGA), and key gene module associated with tumor progression of PCa were identified. Twenty-four candidate hub genes, especially 2 real hub genes, were identified as the key factors in the progression of PCa. Our study provides several novel biomarkers, which might be promising therapeutic targets for PCa treatment.

## 2. Methods

### 2.1. Data obtain and pre-processing

The flow chart of our work was present in Supplemental file 1, <http://links.lww.com/MD/E510> (see Supplemental Digital Content, which illustrates the flow chart of our work). RNA sequencing data and patients' clinic traits of PCa were obtained from TCGA database (<https://cancergenome.nih.gov/>). Fragments per kilobase per million value of each gene was used for subsequent analysis, and 0.5 was set as the threshold of mean expression value to filter low expression genes. To find key genes in PCa, differentially expressed genes (DEGs) with  $|\log_2\text{FoldChange}| > 0.585$  and  $\text{padj} < 0.05$  were selected and used in the following WGCNA analysis. Besides, several vital clinical data of 498 PCa patients was selected which was shown in Table 1. Ethics approval and patient consent for publication are not applicable for this study.

### 2.2. Construction of WGCNA

WGCNA was performed using the DEGs of PCa from TCGA, and the cluster analysis of PCa genes was also performed.<sup>[10,18]</sup> Outlier samples were screened out (cut height=100), and the appropriate soft threshold power (soft power=5) with fitting index on 0.9.<sup>[19]</sup> Then, topological overlap matrix (TOM) was calculated, and TOM dissimilarity (1-TOM) with over 30 genes was applied to identify gene modules. At last, a cutline was chosen to merge some modules to make results more reliable.

### 2.3. Identification of key gene modules in PCa

Next, we used 2 parameters, including gene significance (GS) and module membership (MM), to identify clinical significant modules for PCa patients. The value of GS represents the degree

of correlation between genes expression profile and clinical features, and negative values represent negative correlations, positive values represent positive correlations. And the value of MM represents the correlation between genes and each module.<sup>[20]</sup> Generally, the module with highest correlation coefficient was considered as the key gene module associated with clinical features.

### 2.4. Function annotation of genes in the key module

After obtaining the key module, genes in the key module became the focus of our research, and the gene function was further explored with gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.<sup>[21,22]</sup> Adjusted  $P$  value  $\leq 0.05$  was set as the threshold value for results extraction, and annotations with top ranking illustrate the main function of all genes in the key module.

### 2.5. Hub genes identification and validation

Generally, hub genes were defined as genes that have high degree of correlation with other genes or clinical traits, which tend to be essential in the functional network.<sup>[23,24]</sup> In present study, candidate hub genes were considered with high MM value ( $|\text{MM}| > 0.8$ ) and high GS value ( $|\text{GS}| > 0.2$ ).<sup>[25]</sup> Next, we used Gene Expression Profiling Interactive Analysis database (<http://gepia.cancer-pku.cn>) to identify the real hub genes by analyzing relationship between candidate hub genes and patients' prognosis.<sup>[26]</sup> Moreover, to validate the significance of the real hub genes in PCa, we applied the Oncomine database (<http://www.oncomine.org>).

### 2.6. Statistical analysis

All the statistics were performed using R software, and 2-tailed  $t$ -test was conducted to compare the difference between each group. For categorical variable, we performed the Chi-squared analysis to analyze the difference of the rate among each group. DEGs were screened out with threshold of  $|\log_2\text{FoldChange}| > 0.585$ ,  $\text{padj} < 0.05$ . In all statistics,  $P$  value  $< 0.05$  was set as the threshold value for positive statistical significance.

## 3. Results

### 3.1. Data preprocessing

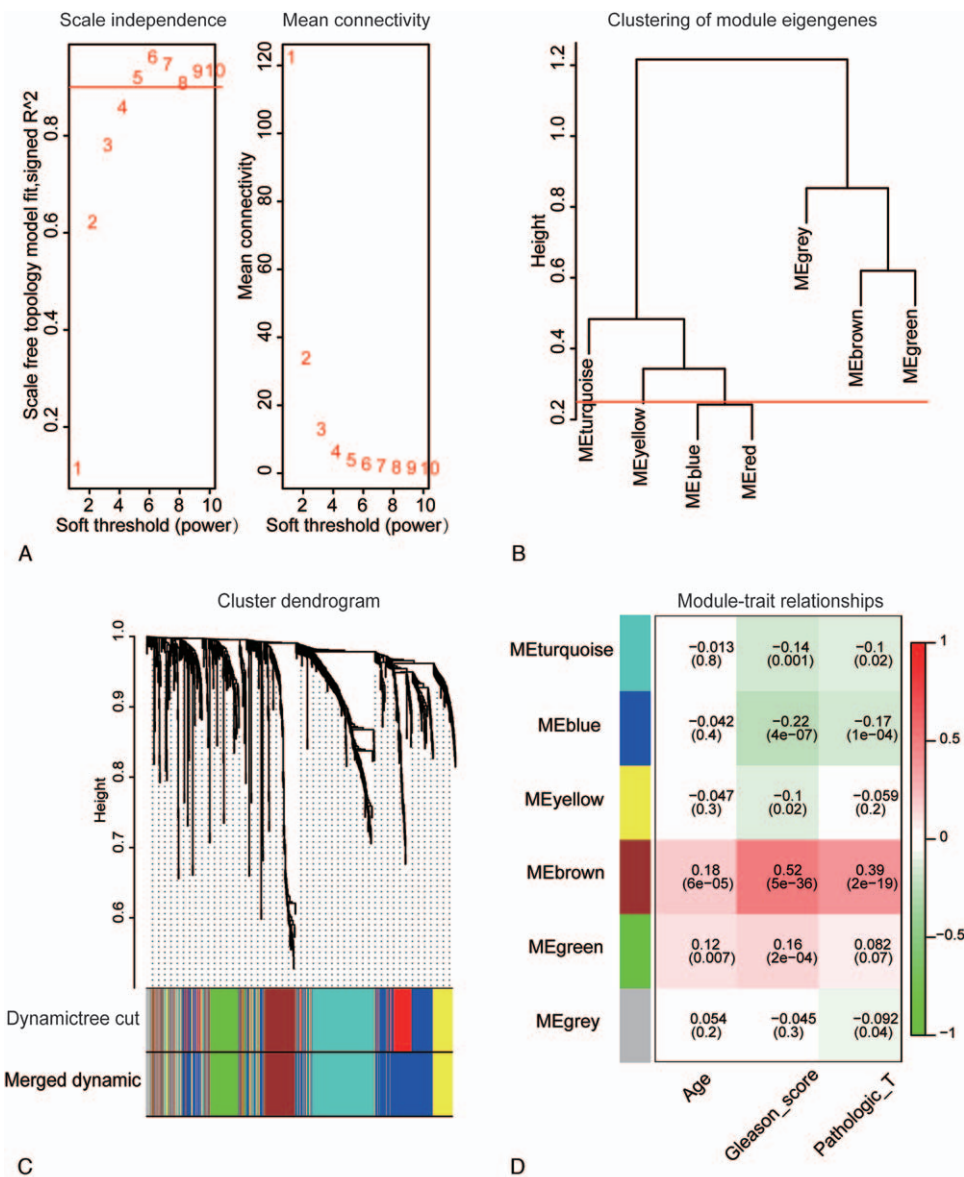
To prepare for constructing the WGCNA, PCa RNASeq data were search and obtained from TCGA. After data preprocessing, 744 DEGs between PCa tumor and pairs normal tissue samples were obtained, and the expression level of fragments per kilobase per million  $< 0.5$  cases were removed. Then, all the DEGs were chosen for WGCNA.

### 3.2. Gene co-expression network construction in PCa

DEGs and 3 clinical traits (age, Gleason score, and pathologic T stage) were selected to conduct a clustering tree, and each brunch of the tree corresponded to a gene module (see Supplemental file 2, <http://links.lww.com/MD/E510>, which illustrates the result of conducting a clustering tree). We first screened the threshold power value of the WGCNA by network topology analysis, and power value  $\beta=5$ , which was the most appropriate power value with independent degree up to 0.9, was chosen as the cutoff value for relatively higher average connectivity (Fig. 1A). Ultimately,

**Table 1**  
Information for 498 prostate cancer patients from the Cancer Genome Atlas Datasets.

Variables	Case number
Age	
< 60	202
> =60	296
Gleason score	
< 8	292
> =8	206
Pathologic T stage	
< 3	187
> =3	304



**Figure 1.** Weighted gene co-expression network analysis in prostate cancer tissues from the Cancer Genome Atlas. (A) Identification of the soft-thresholding power according to the standard of the scale-free network. (B) Clustering of module eigengenes. The red line represented merging threshold. (C) Clustering dendrogram of differentially expressed genes based on topological overlap matrix dissimilarity (1-topological overlap matrix). Each branch of cluster dendrogram represents 1 gene, and every color below represents 1 co-expression gene module. (D) Heatmap of the module-clinical traits relationships. The clinical traits include age, Gleason score, pathologic T stage.

there are 5 distinct gene modules were generated, and genes not belong to any module were put into the gray module (Fig. 1B and C). Moreover, the relationships among all the gene modules were further investigated by topological overlap and hierarchical average linkage clustering analysis, and results showed that yellow and blue gene modules have a higher topological overlap, which means these 2 modules have a close connection with each other (see Supplemental file 3, <http://links.lww.com/MD/E510> and Supplemental file 4, <http://links.lww.com/MD/E510>, which illustrate the relationships among all the gene modules).

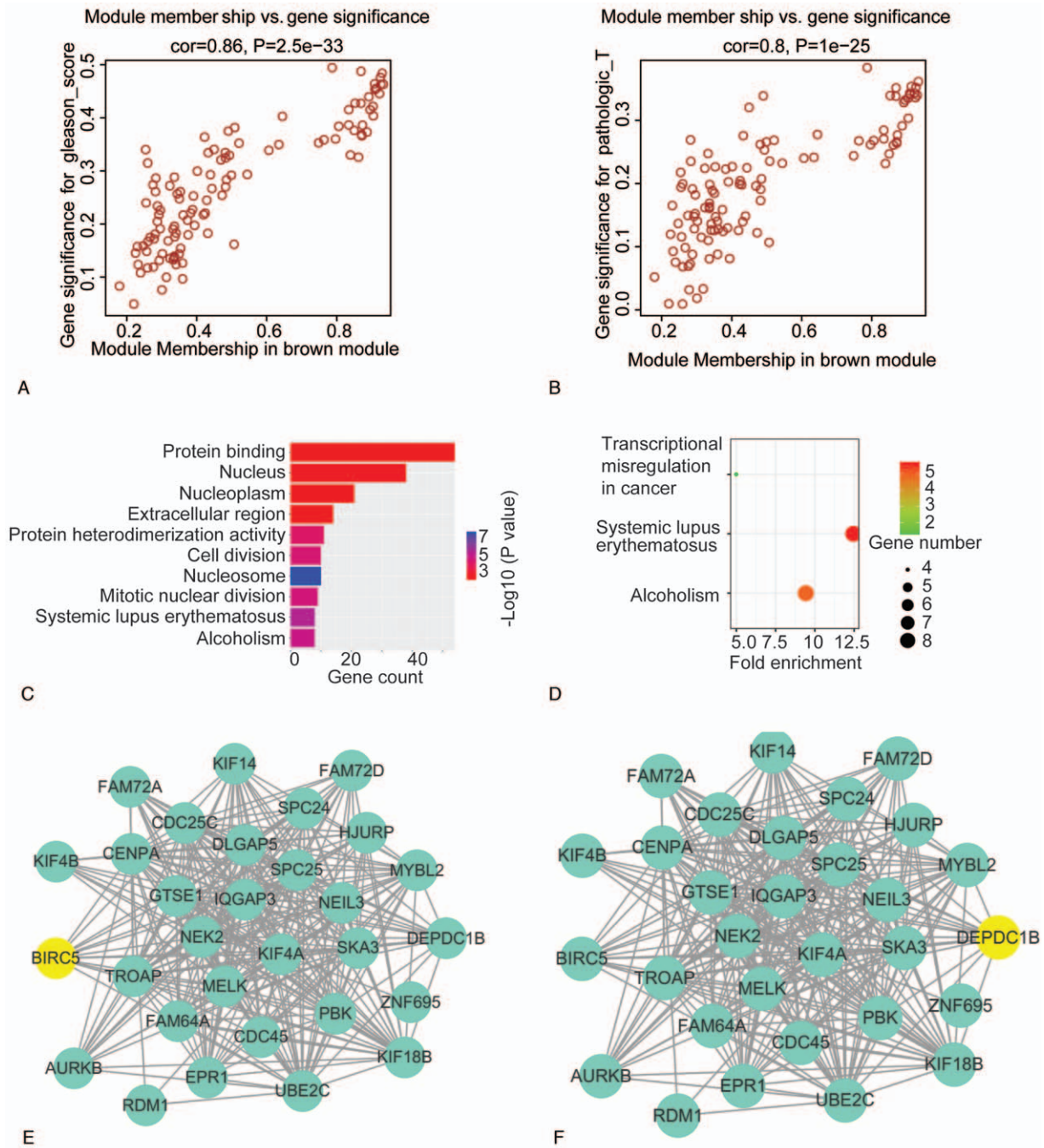
### 3.3. Key module identification in PCa

The clinic traits dataset of PCa was downloaded from TCGA database and useless information for this study was removed. To

analyze the correlations between gene modules and clinical features, we calculated ME value of each gene module with each clinical feature, and the higher of ME indicates a higher of relationship. As shown in Figure 1D, MEbrown shows the highest ME value with 3 clinical features ( $R^2 = 0.52$ ,  $P = 5e-36$  with Gleason score;  $R^2 = 0.39$ ,  $P = 2e-19$  with pathologic T stage;  $R^2 = 0.18$ ,  $P = 6e-05$  with age), which indicates the brown module has significant correlation with PCa progression and was selected as the key module in PCa. Besides, Figure 2A and B also show a positive correlation between MM in brown module and GS for PCa progression.

### 3.4. GO and KEGG pathway analysis

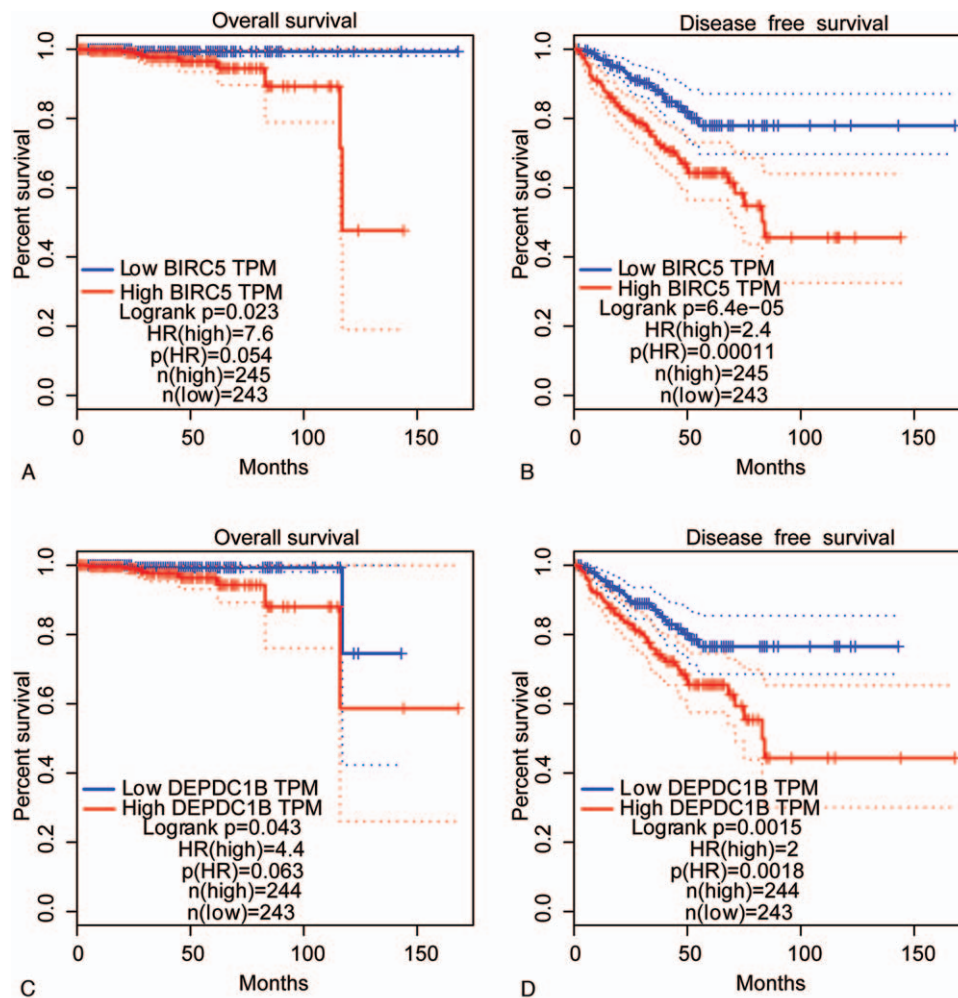
To investigate the potential function of genes, all genes in the key module were subjected to Database for Annotation, Visualization



**Figure 2.** Module visualization and enrichment analysis of brown module genes. (A) Scatter plot of module membership for Gleason score in brown module. (B) Scatter plot of module membership for pathologic T stage in brown module. (C) Gene ontology functional annotation for genes in brown module. (D) Kyoto Encyclopedia of Genes pathway analysis for genes in brown module. (E-F) Hub genes in the brown module. Nodes represent genes, and the yellow nodes represent the real hub genes.

and Integrated Discovery for GO and KEGG pathway analysis. After GO analysis, genes were divided into 3 groups: biological process group, molecular function group, and CC group. Genes in biological process group were concerned with cell division (GO: 0051301,  $P=6.234438e-05$ ) and mitotic nuclear division (GO: 0007067,  $P=3.350178e-05$ ); the molecular function group genes were mostly focus on protein binding (GO: 0005515,  $P=7.532630e-02$ ) and protein heterodimerization activity (GO: 0046982,  $P=1.509493e-04$ ); genes enriched in CC group were

relate to nucleus (GO:0005634,  $P=2.000676e-02$ ), nucleoplasm (GO:0005654,  $P=6.159064e-02$ ), extracellular region (GO:0005576,  $P=6.059723e-02$ ) and nucleosome (GO:0000786,  $P=1.068531e-09$ ) (Fig. 2C). Moreover, the results of KEGG pathway analysis indicated that genes in the key module mainly participate in transcriptional misregulation in cancer ( $P=.04166371$ ), systemic lupus erythematosus ( $P=.00000204$ ) and alcoholism ( $P=.00001300$ ) (Fig. 2D). All the results of functional analysis indicated that genes in the key module were



**Figure 3.** Survival analysis of BIRC5 and DEPDC1B in prostate cancer based on the dataset in gene expression profiling interactive analysis. (A–D) Two real hub (BIRC5 and DEPDC1B) genes in the brown module with significant results of survival analysis ( $P < 0.05$  was regarded as significant).

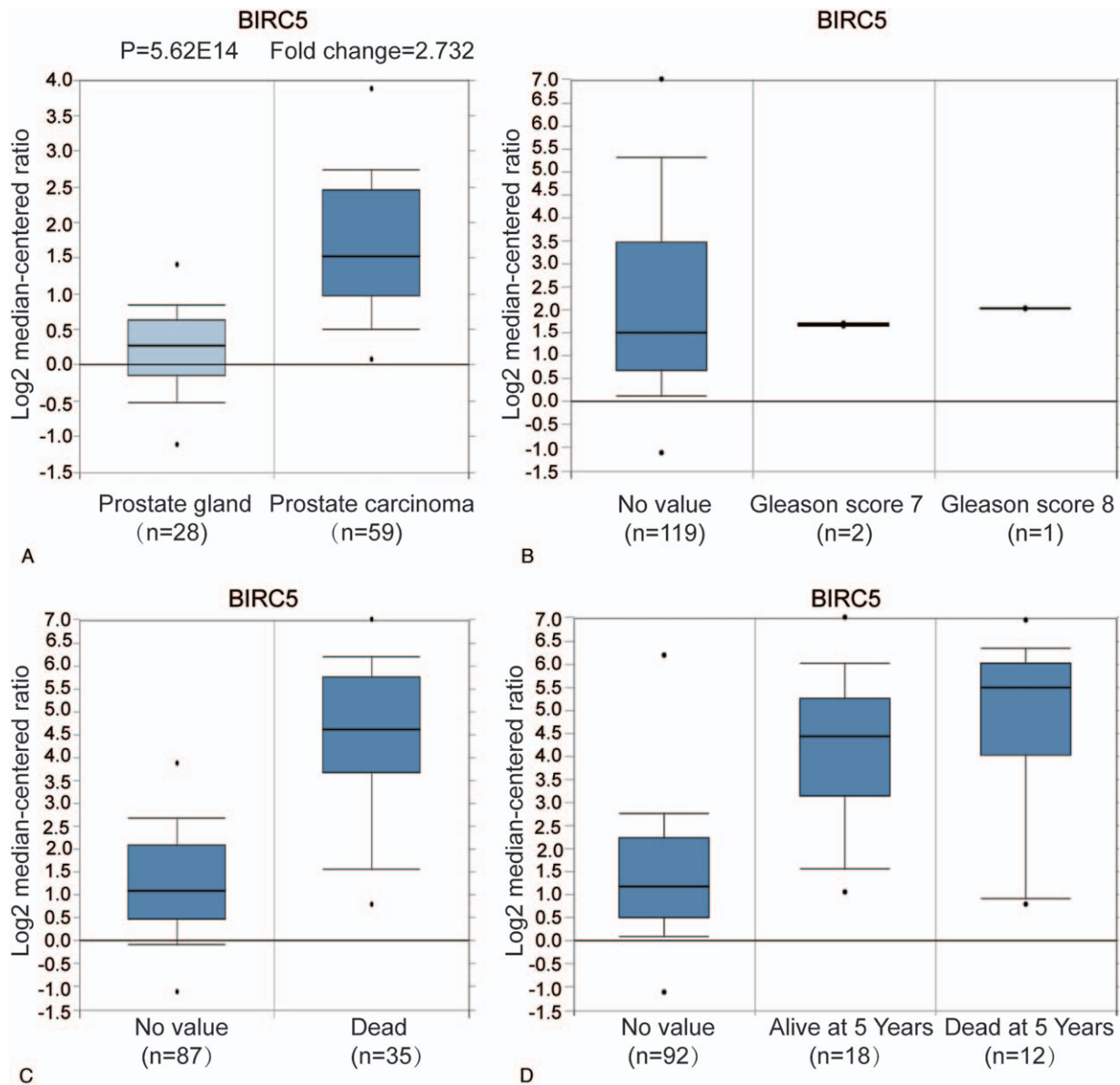
associated with cell mitotic process, which is a vital biological process for cells.

### 3.5. Real hub gene identification and validation

To identify the real hub genes, we first screened out the candidate hub genes in the key module. Results showed 24 genes that with high connectivity were considered as candidate hub genes (Fig. 2E and F). Then, Gene Expression Profiling Interactive Analysis database analysis indicated 2 genes (BIRC5 and DEPDC1B) were identified as the real hub genes, which shown significant correlations with OS and DFS of PCa patiens (Fig. 3A–D). Moreover, we used Oncomine database to further validate the significance of these 2 genes in PCa. As shown in the figure, the expression of BIRC5 was upregulated in PCa tumors than in pairs normal samples ( $P = 5.62E-14$ ; Fig. 4A). Besides, the expression of BIRC5 was in accordance with the tumor grade (Fig. 4B) and disease status (Fig. 4C–D). DEPDC1B was also validated by the Oncomine database (Fig. 5A–D). These results indicated 24 candidate hub genes, especially the 2 real hub genes, play important roles in PCa progression, and may be novel biomarkers and therapeutic targets for PCa diagnosis and treatment.

### 3.6. Biological roles of BIRC5 and DEPDC1B in PCa

To investigate the biological role of 2 real hub genes, median value of the real hub gene expression was used to divide patients into high and low groups in TCGA. Volcano plots analysis illustrated 173 up-regulated and 75 down-regulated genes were identified between BIRC5 high and BIRC5 low expression groups ( $\text{padj} < 0.05$  and  $\log_2\text{FoldChange} > 0.5$  or  $\log_2\text{FoldChange} < -0.5$ , Fig. 6A). Further GO analysis indicated that the DEGs between high and low expression groups mainly belong to plasma membrane (GO: 0005886,  $P = 0.002881696$ ), which primarily participate in the G-protein coupled receptor signaling pathway in PCa (GO: 0007186,  $P = 0.000000000000476$ , Fig. 6B). Besides, KEGG pathway analysis showed that DEGs may associate with the olfactory transduction and neuroactive ligand-receptor interaction pathways (Fig. 6C). As for DEPDC1B, 157 up-regulated and 94 down-regulated genes were identified between high and low expression groups (Fig. 6D). GO and KEGG analysis illustrate that DEGs are associated with the G-protein coupled receptor signaling pathway (GO: 0007186,  $P = 0.00000000263$ ) and retinol metabolism (Fig. 6E–F). Taking together, those findings possibly provided rational interpretations for the biological roles of BIRC5 or DEPDC1B expression in



**Figure 4.** Validation of BIRC5 in Grasso prostate dataset from the Oncomine database. (A) BIRC5 was overexpressed in prostate cancer (PCa) tissues compared with that in paired normal tissues. (B) BIRC5 mRNA level was related to Gleason score of PCa. (C-D) BIRC5 was upregulated in dead PCa patients compared with that in alive PCa patients.

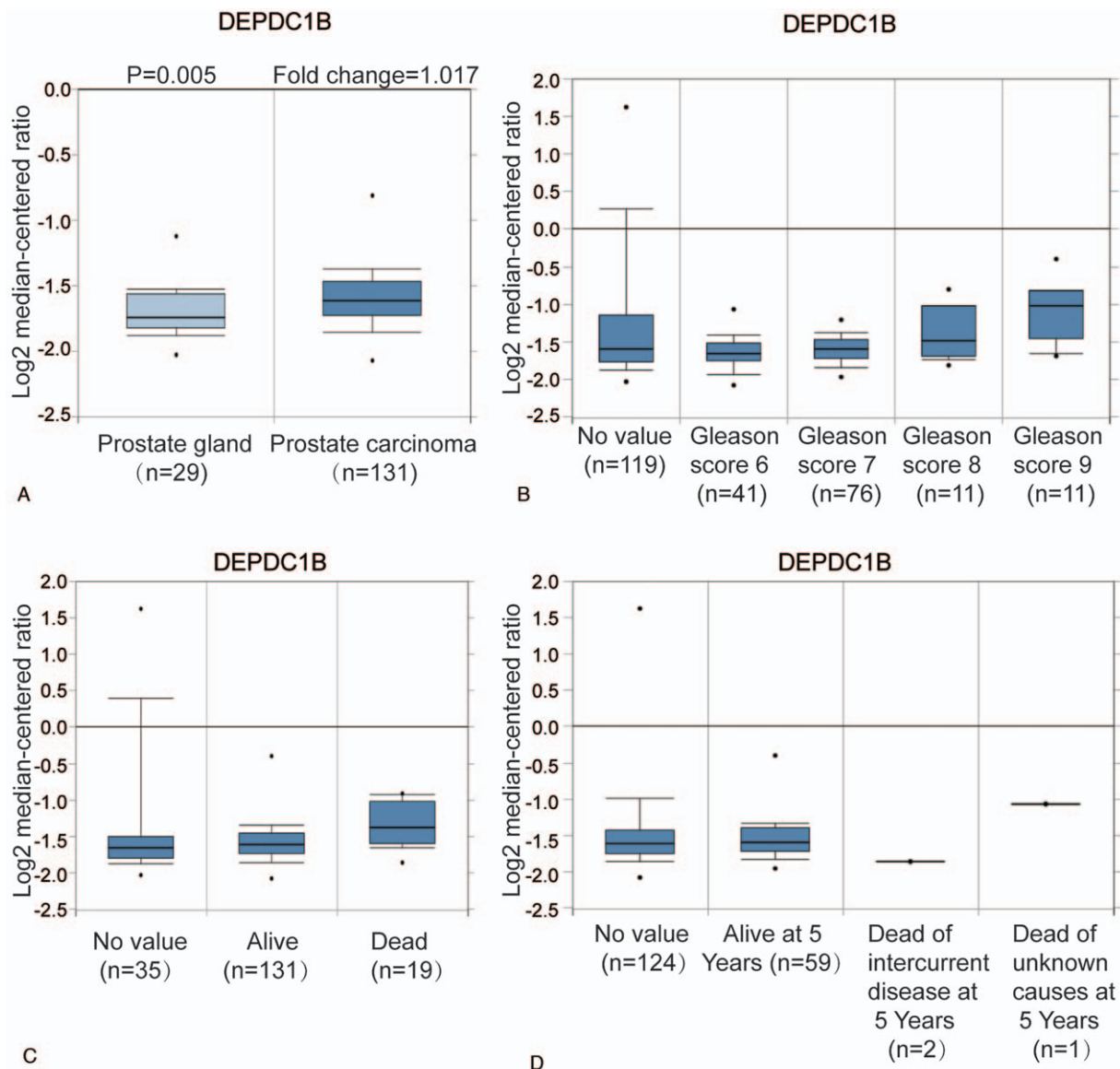
PCa, which may give a hit for the investigation of these 2 real hub genes in PCa in the future.

#### 4. Discussion

PCa is one of the malignant tumors that have a relatively poor prognosis.<sup>[27]</sup> Although the managements of PCa have made a great progress currently, promising and effective treatments are still on the way due to the unclear of biological mechanisms underlying its genesis.<sup>[28]</sup> Currently, several risk factors are considered to predict the progression of PCa.<sup>[29]</sup> However, there is no factor that can precisely predict the disease progression and perfectly regard as tumor treatment targets for PCa. Thus, it is necessary to figure out the molecular mechanisms associated in the genesis and progression of PCa. Furthermore, novel molecular markers are useful for determin-

ing pathological subtypes, identifying individualized treatments and predicting tumor prognosis. In this study, we used WGCNA to identify the key module that significantly associated with the PCa tumor progression. Besides, function annotation of genes in the key module was analyzed, and a total of 24 candidate hub genes were identified. Then, 2 real hub genes were further validated in another datasets from Oncomine database to confirm the reliability of them in PCa. Our findings provide a novel insight of molecular mechanism of PCa, and 24 candidate hub genes, especially the 2 real hub genes, may be novel biomarkers and therapeutic targets for PCa diagnosis and treatment.

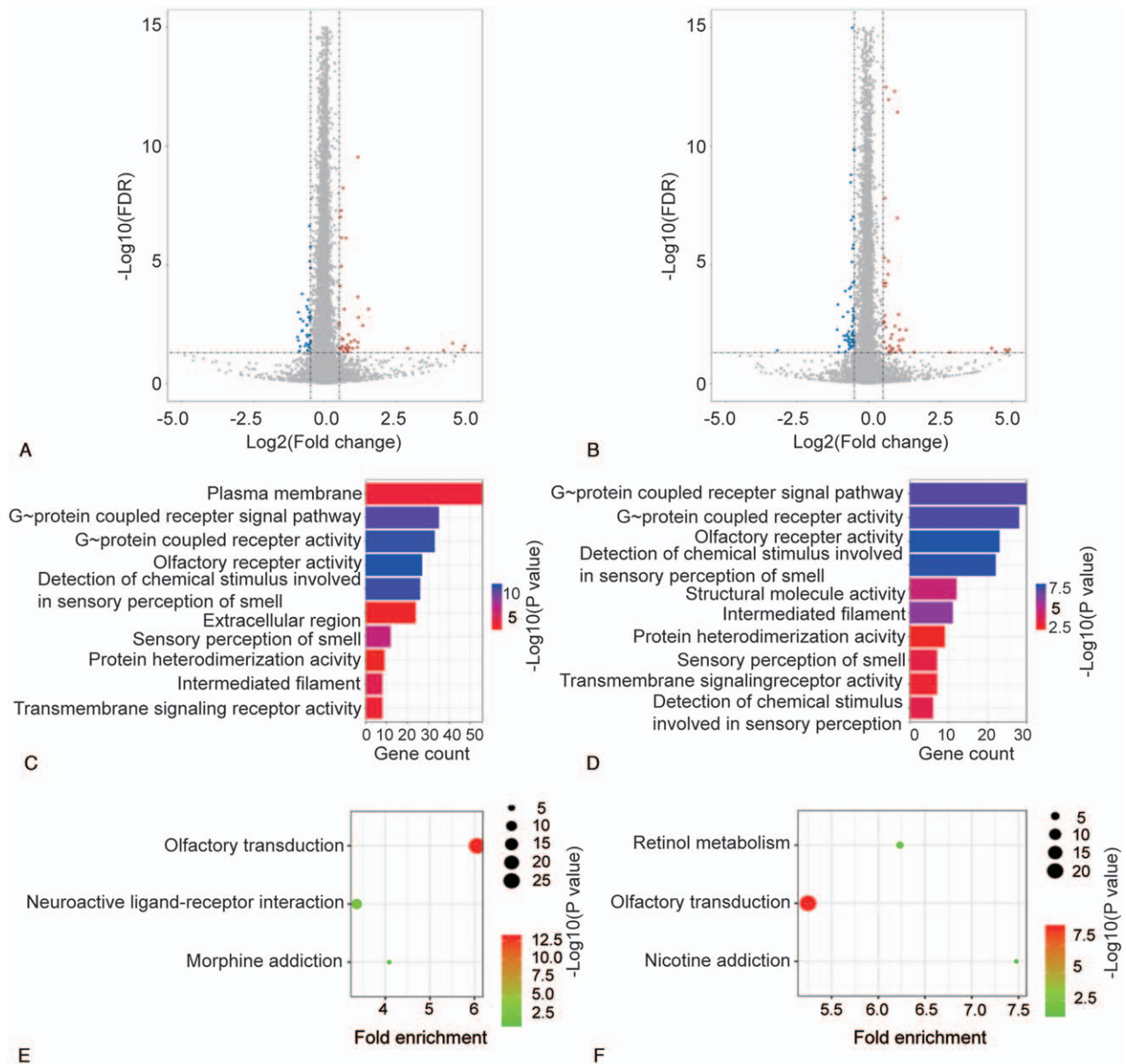
Recently, high throughput sequencing technology and multi-dimensional bioinformatics analysis have made tremendous progress, which may put a deep insight for disease occurrence in the molecular level.<sup>[30,31]</sup> WGCNA, a systems genetics approach



**Figure 5.** Validation of DEPDC1B in Taylor prostate 3 dataset from the Oncomine database. (A) DEPDC1B was overexpressed in prostate cancer (PCa) tissues compared with that in paired normal tissues. (B) DEPDC1B mRNA level was related to Gleason score of PCa. (C-D) DEPDC1B was upregulated in dead PCa patients compared with that in alive PCa patients.

that mainly tries to investigate the correlation between gene clusters and phenotypes, which could identify the physiologically relevant gene clusters for many diseases.<sup>[13]</sup> It constructs a gene co-expression network and provides a global interpretation of gene expression pattern for diseases.<sup>[10]</sup> Currently, WGCNA algorithm has been utilized for identifying the key genes and therapeutic targets for many diseases.<sup>[32–35]</sup> To date, WGCNA algorithm has also been applied to identify the key genes of prostate cancer from GEO database, and several key genes were identified in PCa progression.<sup>[36,37]</sup> Due to the insufficient sample sizes, more tumor samples must be collected and investigated to find the real potential targets for PCa treatment. Thus, we are the used WGCNA to analyze the RNAseq data from TCGA and found 24 candidate hub genes and 2 real hub genes, which have investigated this problem in another perspective and provided a new insight of tumor progression of PCa.

BIRC5 (baculoviral IAP repeat containing 5) is a mitotic spindle checkpoint gene that encodes survivin protein, which is important for regulating cell apoptosis.<sup>[38,39]</sup> To our knowledge, the main function of survivin protein is to prevent apoptotic cell death and regulate cell cycle progression.<sup>[40]</sup> Recently, many studies indicated that aberrant BIRC5 function involves in the progression and confers resistance to several therapeutic strategies in various types of cancers.<sup>[41,42]</sup> Dysfunction of BIRC5 was reported in several cancers, including lung cancer,<sup>[43]</sup> breast cancer,<sup>[44]</sup> colon cancer,<sup>[45]</sup> and pancreas cancer,<sup>[46]</sup> while normal tissues from these caners did not detect the expression of BIRC5. Although the dysfunction of BIRC5 shows evident correlation with cancer progression, the exact molecular mechanism remains unknown. BIRC5 has been reported to be upregulated in PCa and its expression is associated with tumor prognosis, which is in consistent with our study.<sup>[37,47–51]</sup>



**Figure 6.** Biological roles of the real hub genes in prostate cancer. (A) Volcano plot of differential gene profiles between BIRC5 high and BIRC5 low groups. (B) Volcano plot of differential gene profiles between DEPDC1B high and DEPDC1B low groups. (C) Gene ontology functional annotation differentially expressed genes (DEGs) between BIRC5 high and BIRC5 low groups. (D) Gene ontology functional annotation DEGs between DEPDC1B high and DEPDC1B low groups. (E) Kyoto Encyclopedia of Genes pathway analysis for DEGs between BIRC5 high and BIRC5 low groups. (F) Kyoto Encyclopedia of Genes pathway analysis for DEGs between DEPDC1B high and DEPDC1B low groups.

However, most of previous studies are mainly focused on the single gene in PCa and insufficient in exploring its functional roles in the genesis of PCa. We performed a new method (WGCNA) to investigate the key module and gene expression network in the progression of PCa by analyzing data from TCGA. We revealed that BIRC5 expression level was significant correlated with the OS and DFS time of PCa patients. Additionally, further analysis shows the functional roles of BIRC5 may involve in plasma membrane and relate to the olfactory transduction and neuroactive ligand-receptor interaction signaling pathway in PCa. Our study revealed the role of BIRC5 from the perspective of gene expression network in PCa, which is somewhat broaden the understanding of underline mechanism of PCa genesis.

Because of its differential expression in cancers compared with corresponding normal tissues and its potential function in cancers, BIRC5 is considered as an attractive therapeutic target for cancers, especially for prostate cancer treatments.

DEP domain-containing protein 1B (DEPDC1B) is a new identified gene, which encodes a protein containing 2 conserved domains: DEP domain and RhoGAP domain. Studies show that the function of DEP domain main facilitates proteins to interact with G protein coupled receptors, and the RhoGAP domain is related with the Rho GTPase signaling.<sup>[52–54]</sup> However, the exact molecular role of DEPDC1B is still unclear. It is reported that DEPDC1B is a cell cycle related gene, which regulates the interplay between de-adhesion and cell cycle in the mitotic



process.<sup>[55]</sup> Moreover, some studies indicated DEPDC1B participate in cancers progression may due to its important function in regulating cellular activities. Besides, upregulated DEPDC1B has been detected in many cancers, such as metastatic soft tissue sarcoma,<sup>[56]</sup> oral cancer,<sup>[57,58]</sup> and lung cancer.<sup>[59]</sup> Moreover, 1 study has already reported that DEPDC1B was overexpressed and is associated with the biochemical recurrence-free survival time of patients with PCa.<sup>[54]</sup> However, the functional role of DEPDC1B in the progression and OS survival of PCa is unclear. Our study revealed that DEPDC1B expression is upregulated in PCa tumors and is a poor predictor for OS and DFS of PCa. Furthermore, we demonstrated that the functions of DEPDC1B may relate to the G-protein coupled receptor signaling pathway and retinol metabolism, which may give a hit for investigating the role of DEPDC1B in PCa in the future. Thus, with more research in future, DEPDC1B may be an effective therapeutic target for PCa treatment.

We have to acknowledge that there are some limitations in our study. First of all, this study mainly focused on the high-throughput sequencing RNASeq data from TCGA and our findings were not validated by further experiments. Besides, the results of WGCNA may be changed when set the different parameters or cutoff, and we only validated our findings with 2 Oncomine datasets and more datasets should be used to confirm our results. More amounts of samples with more complete and precise clinical information should be investigated to confirm these findings.

## 5. Conclusions

In summary, we used WGCNA to reveal a tumor progression associated module (brown module) in PCa from TCGA. After functional analyzed for genes in brown module, 24 candidate hub genes, especially 2 real hub genes, were found with a significant meaning for PCa progression and prognosis. Although the molecular mechanism of PCa needs to be further investigated, these hub genes, especially the real hub genes, might be considered as novel therapeutics for PCa patients in the future.

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## Author contributions

ZM designed and instructed this research. JW analyzed the data and wrote this manuscript. LD and YC revised the manuscript.

**Conceptualization:** Yujun Chen.

**Data curation:** Lingyan Ding.

**Writing – original draft:** Jianming Wang.

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