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Original Article

Analysis of differentially expressed genes, clinical value and biological pathways in prostate cancer

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Abstract: The present study aimed to investigate the gene expression changes in prostate cancer (PC) and screen the hub genes and associated pathways of PC progression. The authors employed integrated analysis of GSE46602 downloaded from the Gene Expression Omnibus and The Cancer Genome Atlas databases to identify 484 consensual differentially expressed genes (DEGs) in PC, when compared with adjacent normal tissue samples. Functional annotation and pathway analysis were performed. The protein-protein interaction (PPI) networks and module were constructed. RT-qPCR was used to validate the results in clinical PC samples. Survival analysis of hub genes was performed to explore their clinical value. GO analysis results revealed that DEGs were significantly enriched in negative regulation of nitrobenzene metabolic process, extracellular space and protein homodimerization activity. KEGG pathway analysis results revealed that DEGs were most significantly enriched in focal adhesion. The top 10 hub genes were identified to be hub genes from the PPI network, and the model revealed that these genes were enriched in various pathways, including neuroactive ligand-receptor interaction, p53 and glutathione metabolism signaling pathways. RT-qPCR results validated that expression levels of eight genes (PIK3R1, BIRC5, ITGB4, RRM2, TOP2A, ANXA1, LPAR1 and ITGB8) were consistent with the bioinformatics analysis. ITGB4 and RRM2 with genetic alterations exhibited association with a poorer survival rate, compared with those without alterations. These results revealed that PC-related genes and pathways have an important role in tumor expansion, metastasis and prognosis. In summary, these hub genes and related pathways may act as biomarkers or therapeutic targets for PC.

Keywords: Prostate cancer, TCGA, GEO, bioinformatic analysis, differentially expressed genes

Introduction

Among the diagnosed American male cancer patients, prostate cancer is the most common cancer except skin cancer [1]. In 2016, there were 181,000 newly diagnosed cases and 26,000 cases of mortality in the United States [2]. With the development of clinical and experimental research, progress has been made regarding the treatment and understanding of the fundamental biology underlying PC. In terms of detection, prostate-specific antigen (PSA) is still the commonly used marker to identify increased risk [3]. Various studies have shown that certain genes have an important role in the development and progression of PC, such as MXI1, BRCA1 and BRCA2. Regarding associated signaling pathways, it has been demonstrated that glioma and integrated breast cancer pathways, in addition to notch signaling and androgen receptor (AR) pathways, are associated with PC [4]. Furthermore, numerous novel biomarkers aid with profitable prognostic information, which may have vital therapeutic implications. This information may be used as selection criteria for patients eligible for active surveillance or candidates for radiotherapy/surgery [5].

However, identification of a valid biomarker to complement PSA for screening, molecular stratification methods and treatment of metastatic disease is of primary concern. The excavation of disease-related genes or biomarkers associated with the pathogenesis and molecular mechanism of PC is of great significance in the diagnosis and treatment of patients [6].

With the rapid development of molecular biology and bioinformatics, chip and sequencing technology is widely used, and research in this area is continuous. The Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) database have accumulated abundant genomic and gene expression profiles for different diseases during the past decade. Through the analysis of these data, various key genes and signaling pathways related to the disease may be identified, which will result in a better understanding of the occurrence and development mechanism of the disease.

In the present study, large-scale gene data sets regarding PC were downloaded from the GEO and TCGA databases. GEO2R and The R Programming Language (R) was utilized for preprocessing and analysis of these data to obtain the differentially expressed genes (DEGs). For these DEGs, the Database for Annotation Visualization and Integrated Discovery (DAVID) database was used to facilitate the functional annotation and pathway analysis, and the STRING database was used to construct the protein-protein interaction (PPI) network and modules selection. Then, RT-PCR was used to validate the hub genes in clinical PC samples. Finally, a survival analysis of the hub genes was conducted to explore their clinical value. The present study aimed to identify critical genes involved in PC, which may be helpful for the development of novel targets for therapeutic intervention.

Materials and methods

Microarray data

The gene expression profile GSE46602 was downloaded from the GEO database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/geo). Then, the probe-level information was converted into the corresponding gene symbol according to the explanation data downloaded from platform GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array), and 54,675 probes were used to detect levels of gene transcription. The genome expression dataset consisted of 36 tumor sample specimens from patients with prostate cancer and 14 control samples from patients with benign prostate glands adjacent to cancer or benign prostate glands. In the present study, the dataset including the 14 control and 35 PC samples was selected. The genomic data and clinical data of PC from TCGA (https://cancergenome.nih.gov/) were also downloaded. These RNA sequencing (RNA-seq) data from Illumina HiSeq RNASeq platform included 498 tumor sample specimens from patients with prostate cancer and 52 control samples from patients with benign prostate glands adjacent to cancerous glands.

Data preprocessing and DEGs screening

GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) is an interactive web tool which was applied to detect DEGs by comparing two or more groups of samples in a GEO series [7]. GEOquery and Limma R package in GEO2R was applied to identify the DEGs between PC samples and control samples. The Benjamini-Hochberg (BH) method [8] was introduced to adjust the raw *P*-values into a false discovery rate to avoid the multi-test problem, which might produce too many false positive results. The adjust *P* value <0.05 and |log2 fold change (FC)| ≥1 were set as the thresholds for identifying DEGs.

The RNA-Seq data of PC samples and control samples were downloaded from TCGA in September 2017. The edgeR package in R was subsequently used for the calculation of DEGs by comparing PC samples and control samples. The adjust *P* value <0.05 and I $\log_2$ fold change (FC) I ≥1 were set as the cut-off criteria. The genes that presented in both GEO and TCGA analysis results were selected as the final DEGs.

Functional and pathway enrichment analysis of DEGs

The DAVID (https://david.ncifcrf.gov/) database is a biological database regularly used to facilitate functional annotation and pathway analysis. In order to better understand the biological functions and characteristics, the present study performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses in the DAVID database to identify DEGs. The human genome was selected as the background list parameter, and *P* value <0.05 and count ≥2 were chosen as the thresholds to indicate a statistically significant difference.

Table 1. Primer sequences for RT-qPCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
PIK3R1	ACCACTACCGGAATGAATCTCT	GGGATGTGCGGGTATATTCTTC
BIRC5	AGGACCACCGCATCTCTACAT	AAGTCTGGCTCGTTCTCAGTG
ITGB4	GCAGCTTCCAAATCACAGAGG	CCAGATCATCGGACATGGAGTT
RRM2	GTGGAGCGATTTAGCCAAGAA	CACAAGGCATCGTTTCAATGG
TOP2A	ACCATTGCAGCCTGTAAATGA	GGGCGGAGCAAAATATGTTCC
ANXA1	CTAAGCGAAACAATGCACAGC	CCTCCTCAAGGTGACCTGTAA
LPAR1	CTTTGCTGGGTTGGCCTACTT	GCCATGTGCTAACAGTCAGTCT
ITGB8	ACCAGGAGAAGTGTCTATCCAG	CCAAGACGAAAGTCACGGGA
GAPDH	GAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATTT

PPI network construction and modules selection

The present study used the STRING database (http://string-db.org/) to construct a PPI network. PPI analysis provides novel insights into protein function and may help to reveal the generic organization principles of functional cell systems and aid in the discovery of functional associations between proteins on a genome-wide scale. All the DEGs were imported into Cytoscape plugin to create network visualizations. Then, the resulting PPI network was subjected to module analysis with the Plugin Molecular Complex Detection (MCODE) with the default parameters (degree cutoff ≥2, node score cutoff ≥2, K-core ≥2, and maximum depth =100).

Validation based on clinical samples of PC

The aforementioned section described the creation of the PPI network using STRING and Cytoscape software. The hub genes were screened out according to the degree. To further verify the data of the hub genes, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was conducted to detect the expression levels of the hub genes within clinical PC samples (n=12) obtained from the First Affiliated Hospital of Guangzhou Medical University. All of the individuals participating in the project gave informed consent, and the study was approved by the human study ethics committee of the First Affiliated Hospital of Guangzhou Medical University. Total RNA was extracted from tissues using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). A total of 1 μg total RNA was reverse transcribed to cDNA, which was amplified by PCR within a 10 μl reaction system using PrimeScript™ RT Reagent Kit (Takara Bio, Inc., Otsu, Japan). RT-qPCR procedures were performed using SYBR Premix Ex Taq™ GC (Takara Bio, Inc.) on a BIO-RAD system, according to the manufacturer's instructions. Relative expression values were calculated using the $2^{-\Delta CT}$ method [9]. The primers were synthesized by Generay Biotech Co., Ltd. (Shanghai, China), and sequences are listed in **Table 1**. All values were normalized against GAPDH expression levels. A paired-sample t-test was performed to compare the hub genes between PC and para-cancerous prostate tissues, using SPSS software, version 22.0 (IBM SPSS, Armonk, NY, USA).

Survival analysis

In order to reveal the genetic alterations and Kaplan-Meier curves, cBioportal (http://www.cbioportal.org/) [10, 11] was applied to analyze the hub genes. The hub genes were imported to cBioPortal to investigate gene expression changes in PC with mRNA expression data (n=499) from the TCGA Prostate Project dataset, as compared with normal prostate samples. The aberrant mRNA expression threshold was defined as z-score ±2.0.

Results

Data preprocessing and DEG screening

The gene data were downloaded from GEO and TCGA. Based on the GEO2R and R analysis, a total of 3,714 DEGs were identified in PC compared with the control samples in GEO, and 1,415 DEGs were identified in PC compared with the control samples in TCGA. A total of 484 DEGs presented in both the GEO and TCGA analysis results. These genes included 168 upregulated and 316 downregulated genes. Two volcano plots of DEGs and one Venn diagram of the DEG screening are presented in **Figure 1**.

Functional and pathway enrichment analysis of DEGs

Three GO category results are presented, through the use of DAVID, including biological processes, cellular components and molecu-

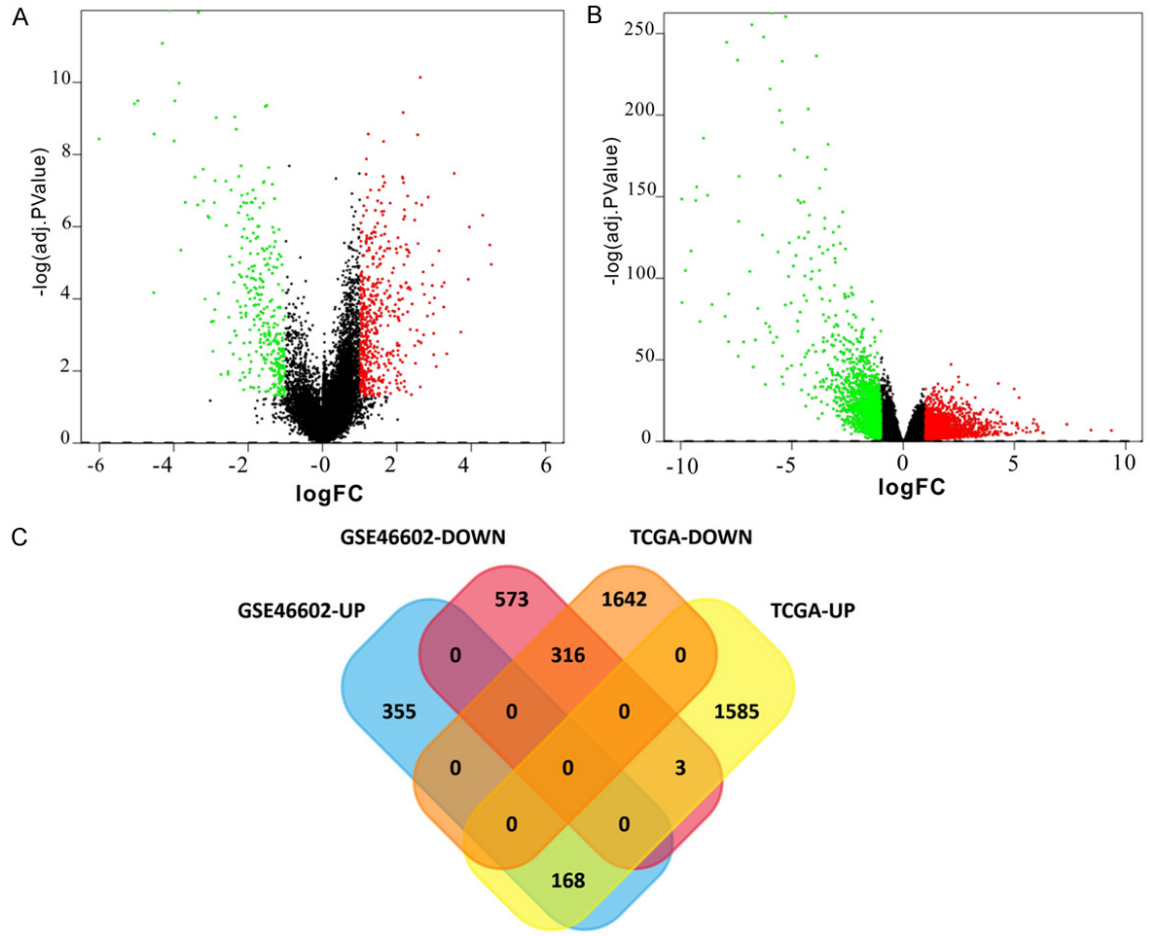

Figure 1. Two volcano plots of DEGs and one Venn diagram of the DEGs screening. For the volcano, the volcano plot on the left (A) is the result of the GEO database and the volcano plot on the right (B) represents the result of the TCGA database. The abscissa is logFC and the ordinate is -log10 (adj. *P* Value). The red and green spots represent DEGs. The black dots represent genes that are not differentially expressed between PC and control samples. Red: upregulated; green: downregulated. The Venn diagram (C) indicates the number of DEGs in four different datasets and the crossing area indicates the cross-DEGs in different datasets. 168 upregulated and 316 downregulated genes were identified from the data obtained from the TCGA and GEO databases. GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; PC, prostate cancer; DEG, differentially expressed gene.

lar functions. The biological process results revealed that DEGs were primarily enriched in nitrobenzene metabolic processes, glutathione derivative biosynthetic process and cochlea development. The cellular component results indicated that DEGs were mainly enriched in extracellular space, plasma membrane and proteinaceous extracellular matrix. The molecular function results showed that DEGs were mainly enriched in protein homodimerization activity, calcium ion binding and glutathione binding (**Figure 2A-C**; **Table 2A**). To investigate pathway enrichment, KEGG signaling pathway analysis was used to identify the top five pathways, which included 'focal adhesion', 'glutathione metabolism', and 'chemical carcinogenesis' (**Figure 2D**; **Table 2B**).

PPI network construction and module selection

All DEGs were analyzed using the STRING online database and Cytoscape software. 'Confidence score ≥0.7' was set as the cut-off criterion. A total of 145 DEGs of the 484 commonly altered DEGs were filtered into the DEG PPI network complex, including 145 nodes and 288 edges (**Figure 3A**). Of the 145 DGEs, 14 hub genes were identified with the criteria of degree >10. The top 10 node degree genes

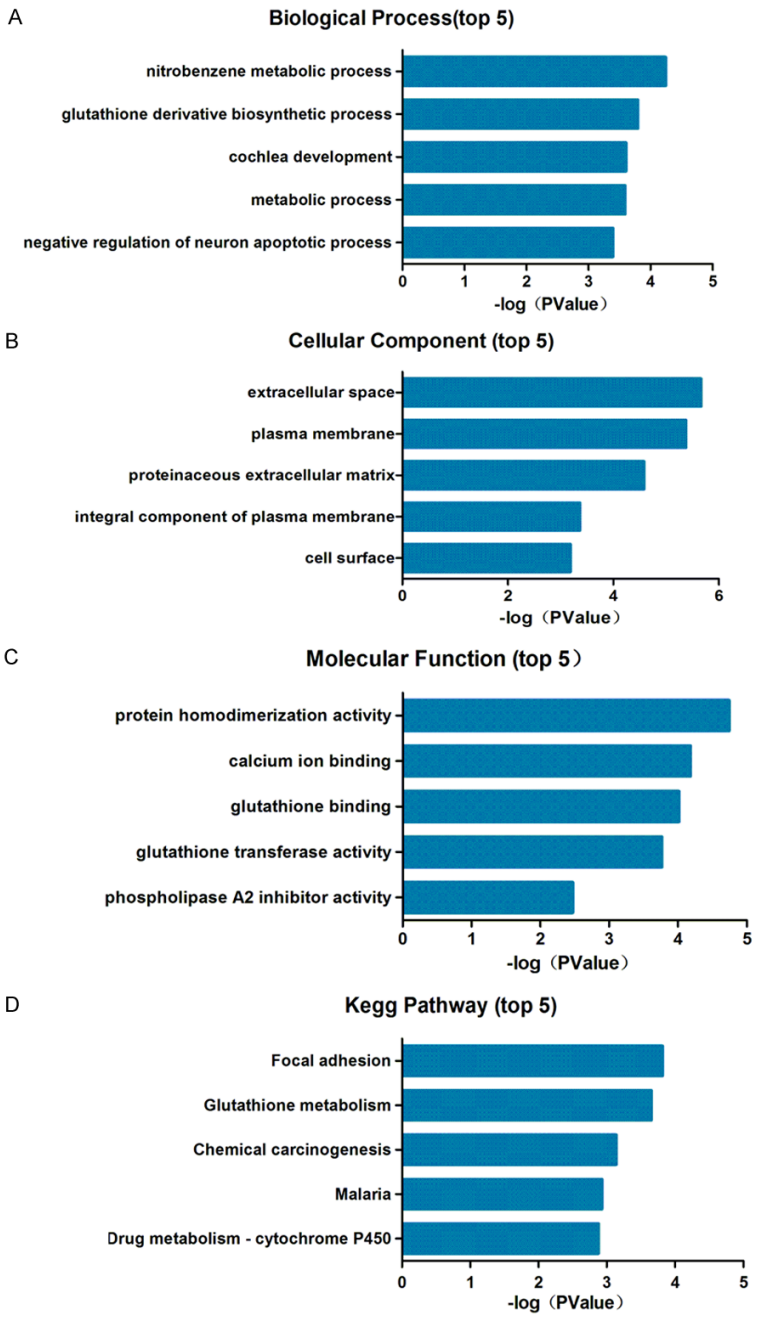

Figure 2. Top five Gene Ontology enrichment analysis and KEGG pathways. (A) Biological processes, (B) cellular components, (C) molecular functions and (D) KEGG pathway analysis. KEGG, Kyoto Encyclopedia of Genes and Genome.

were PIK3R1, BIRC5, ITGB4, AGTR1, RRM2, TOP2A, ANXA1, LPAR1, CCNB2 and ITGB8.

Furthermore, the top 3 significant modules from the PPI network were identified using MCODE plugin in Cytoscape (**Figure 3B-D**). The DEGs in the top 3 modules were selected to perform pathway enrichment analysis. The pathway enrichment analysis results revealed that the genes in module 1 and module 3 were predominantly associated with neuroactive ligand-receptor interaction, p53 signaling pathway and glutathione metabolism (**Table 3**).

Validation based on clinical samples of PC

To validate the findings in the integrated analysis, eight hub genes were selected for RT-qPCR in 12 tissues obtained from PC patients, compared with matched para-cancerous tissue. According to the experimental results, the expression pattern of selected genes in PC and matched para-cancerous tissue was similar to that observed in the integrated analysis (**Figure 4**). The expression levels of BIRC5, RRM2 and TOP2A were up-regulated in PC compared with matched para-cancerous tissue, whereas the expression levels of PIK3R1, ITGB4 and ANXA1, LPAR1 and ITGB8 were down-regulated.

Survival analysis

Finally, two genes (ITGB4 and RRM2) were screened from cBioPortal. The Oncoprint from cBioPortal revealed that a total of 12% of cases with genetic alterations could be obtained. A total of two genes (ITGB4 and RRM2) had genetic alterations which included amplification, deep deletion, mRNA upregulation, truncating mutation (putative passenger) and missense mutation (putative passenger) (**Figure 5A**). It was demonstrated that the cases with genetic alterations in these genes (ITGB4, P=0.00158; RRM2, P=0.00771) exhibited a poorer survival rate

Table 2A. The top 15 enriched Gene Ontology terms of differentially expressed genes

Category	Term	Count	*P* Value
BP	Nitrobenzene metabolic process	4	5.72E-05
BP	Glutathione derivative biosynthetic process	6	1.60E-04
BP	Cochlea development	6	2.48E-04
BP	Metabolic process	14	2.57E-04
BP	Negative regulation of neuron apoptotic process	12	4.04E-04
CC	Extracellular space	61	2.16E-06
CC	Plasma membrane	140	4.21E-06
CC	Proteinaceous extracellular matrix	20	2.60E-05
CC	Integral component of plasma membrane	55	4.32E-04
CC	Cell surface	27	6.50E-04
MF	Protein homodimerization activity	38	1.81E-05
MF	Calcium ion binding	36	6.59E-05
MF	Glutathione binding	5	9.70E-05
MF	Glutathione transferase activity	7	1.72E-04
MF	Phospholipase A2 inhibitor activity	3	3.39E-03

BP: biological process; CC: cellular component; MF: molecular function.

Table 2B. The top five enriched pathways of differentially expressed genes

Category	Term	Count	*P* Value
KEGG_PATHWAY	Focal adhesion	16	1.54E-04
KEGG_PATHWAY	Glutathione metabolism	8	2.24E-04
KEGG_PATHWAY	Chemical carcinogenesis	9	7.31E-04
KEGG_PATHWAY	Malaria	7	1.18E-03
KEGG_PATHWAY	Drug metabolism-cytochrome P450	8	1.32E-03

compared with cases without alterations (**Figure 5B**).

Discussion

PC has become one of the most common non-skin malignancies among men with an incidence of approximately 0.01% worldwide [12]. Efficient progress has been made in genetics and molecular pathogenesis, however the detection of PC and treatment of the localized disease remains of primary concern, and requires further investigation [6].

In the present study, GEO2R and R was used to analyze the gene data downloaded from GEO and TCGA databases. A total of 484 DEGs in PC compared with control samples were identified, which included 168 upregulated and 316 downregulated genes. DEGs were mainly enriched in 15 GO terms, including negative regulation of nitrobenzene metabolic process, extracellular space and protein homodimerization activity. The KEGG pathway enrichment analysis result showed that the DEGs were related to focal adhesion, glutathione metabolism and chemical carcinogenesis. The focal adhesion pathway has great significance in the transfer and treatment of prostate cancer. In this pathway, talin1, a focal adhesion complex protein, enhances prostate cancer cell adhesion, migration and invasion [13]. Previous studies indicate that the overexpression of bone sialoprotein (BSP) in PC is correlated with tumor progression [14-16]. Gordon et al. demonstrated that BSP stimulates focal adhesion kinase and focal adhesion-related signaling pathways [17]. Therefore, monitoring of this signaling pathway may be beneficial to understanding the mechanism of carcinogenesis and researching treatment of prostate cancer.

Furthermore, the present study constructed PPI networks to investigate the critical DEGs, and 10 hub genes were identified. Furthermore,

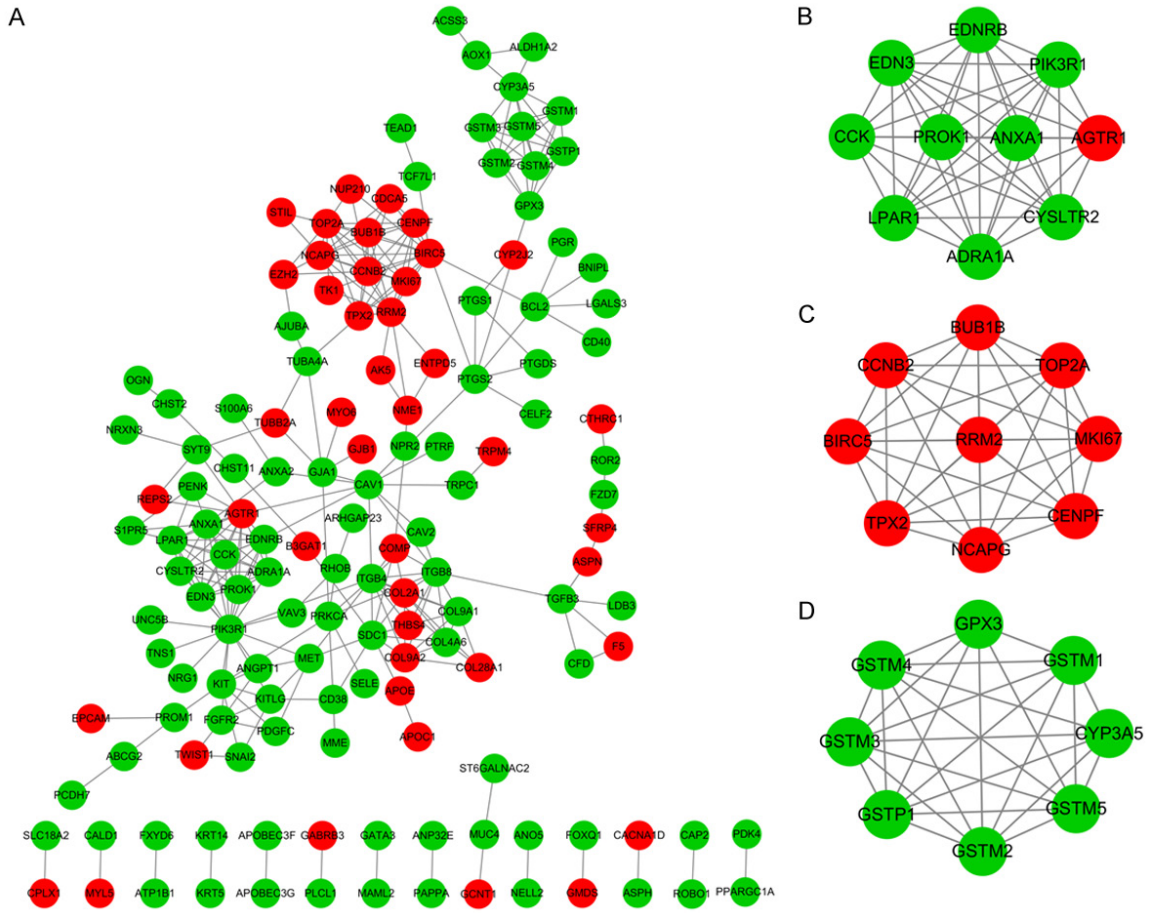

Figure 3. PPI network of the DEGs and modular analysis. (A) DEG PPI network complex, (B) module 1 of DEGs from PPI network, (C) module 2 of DEGs from PPI network and (D) module 3 of DEGs from PPI network. Red nodes represent the upregulated DEGs and green nodes represent the downregulated DEGs. Increased node interaction suggests a greater biological significance. PPI, protein-protein interaction; DEG, differentially expressed gene.

Table 3. Top three significant modules selected from the protein-protein interaction network

Module	Name	Count	*P* value	Genes
Module 1	Neuroactive ligand-receptor interaction	5	1.22E-05	EDNRB, AGTR1, CYSLTR2, ADRA1A, LPAR1
	cGMP-PKG signaling pathway	4	1.31E-04	EDNRB, AGTR1, ADRA1A, PIK3R1
	Calcium signaling pathway	4	1.65E-04	EDNRB, AGTR1, CYSLTR2, ADRA1A
	Pathways in cancer	4	1.68E-03	EDNRB, AGTR1, LPAR1, PIK3R1
	Adrenergic signaling in cardiomyocytes	3	4.25E-03	AGTR1, ADRA1A, PIK3R1
Module 2	p53 signaling pathway	2	2.8E-02	CCNB2, RRM2
Module 3	Glutathione metabolism	7	8.31E-13	GSTM1, GSTM2, GSTM3, GSTM4, GPX3, GSTM5, GSTP1
	Drug metabolism-cytochrome P450	7	5.04E-12	GSTM1, GSTM2, CYP3A5, GSTM3, GSTM4, GSTM5, GSTP1
	Metabolism of xenobiotics by cytochrome P450	7	8.52E-12	GSTM1, GSTM2, CYP3A5, GSTM3, GSTM4, GSTM5, GSTP1
	Chemical carcinogenesis	7	1.38E-11	GSTM1, GSTM2, CYP3A5, GSTM3, GSTM4, GSTM5, GSTP1

the differential expression of eight of these genes (PIK3R1, BIRC5, ITGB4, RRM2, TOP2A, ANXA1, LPAR1 and ITGB8) was verified in 12 tissues of PC patients, compared with matched paracancerous tissues, via RT-qPCR. The phosphoinositide 3-kinase (PI3K) signaling pathway and AR signaling may mediate prostate cancer survival signals and androgen inhibits PIK3R1 in prostate cancer cells [18]. Therefore, PIK3R1 may be a target for the treatment of PC, however this hypothesis requires further investigation. Survivin (encoded by the gene BIRC5) is an anti-apoptotic protein that is overexpressed in many cancer

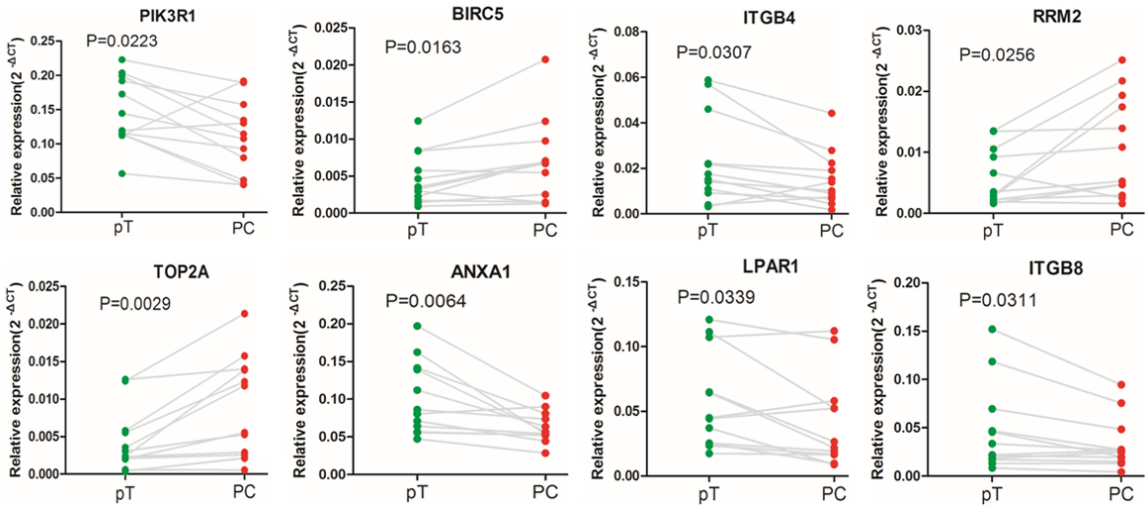

Figure 4. The expression levels of eight hub genes were detected in 12 tissues of PC patients and their matched para-cancerous tissue, using reverse transcription-quantitative polymerase chain reaction. GAPDH was used as an internal reference gene for normalization.

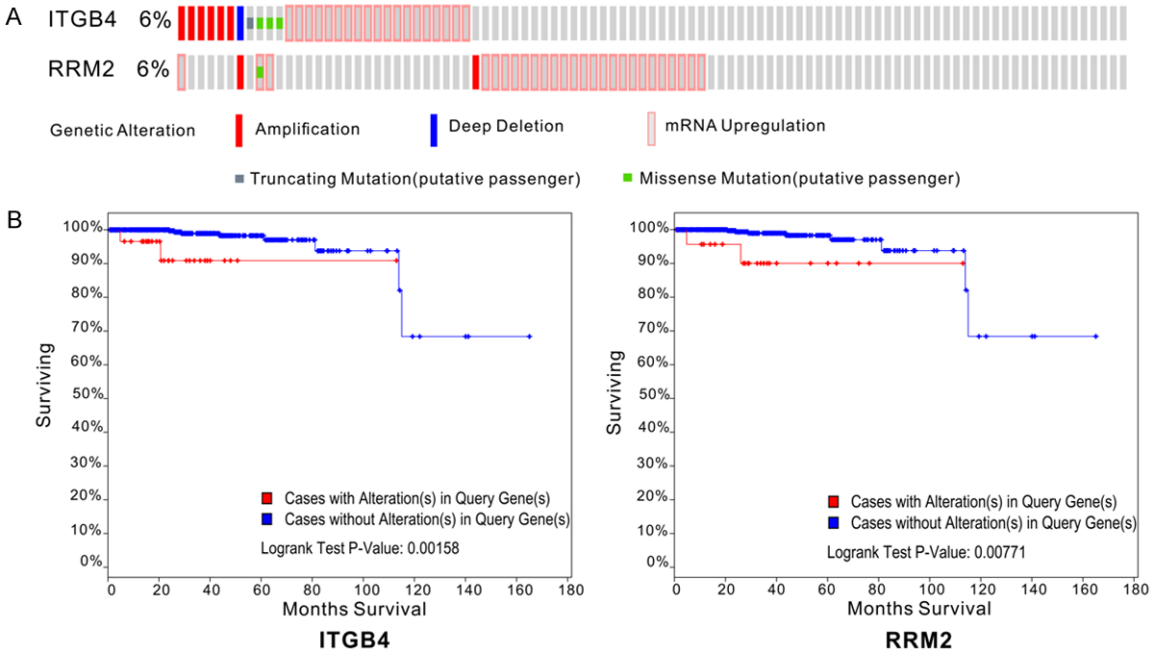

Figure 5. Genetic alterations and the prognostic value of differentially expressed genes in prostate cancer. A. Genetic alterations: Red represents amplification, blue represents deep deletion, pink represents mRNA upregulation, gray represents truncating mutation (putative passenger) and green represents missense mutation (putative passenger). B. Kaplan-Meier curves of two hub genes between group with alterations and group without alterations. The Kaplan-Meier survival curves showed the significant prognostic value of ITGB4 and RRM2 alteration regarding survival. Red line represents cases with alterations in query genes. Blue line represents cases without alterations in query genes. The x-axis indicates overall survival time (months) and the y-axis represents the survival rate. These curves were downloaded from cBioPortal.

types, including gastric, lung, colon and breast cancer [19]. BIRC5 levels are correlated with Ras signaling signature expression, which is upregulated in PC [20]. Danilewicz *et al.* had confirmed that the immunoexpression of survivin is augmented in PC compared with

benign prostatic hyperplasia, and positively correlated with parameters of tumor aggressiveness [21]. These results suggest that BIRC5 may be used as a therapeutic target in PC treatment. TOP2A encodes topoisomerase IIα, which is a ribozyme that controls DNA topology and cell cycle progression. This enzyme is a marker of cell proliferation in normal and tumor tissue [22]. Previous studies have reported that increased expression of TOP2A is linked to shortened survival in breast, ovary, brain, skin and small cell lung cancers [23-27]. Sullivan *et al.* suggested that increased TOP2A is a strong predictor of advancing stages and tumor grade in PC [28]. De Resende *et al*. suggested that TOP2A protein expression levels may act as a prognostic index for patients with PC [22]. Annexin A1 (ANXA1) is a Ca^{2+}-binding protein in the invasive stages of PC. Bizzarro *et al*. indicated that ANXA1 may be a pivotal mediator of hypoxia-related metastasis-associated processes in PC [29]. D'Acunto *et al*. reported that the expression of ANXA1 is a contributing factor to the promotion of apoptosis in PC [30]. ANXA1 may increase the accuracy of prognostication as a biomarker of PC following radical prostatectomy [31]. Therefore, ANXA1 is important in PC and may be used as a prognostic indicator. Lysophosphatidic acid (LPA) is a growth factor in many cells, including prostate and ovarian cancer-derived cell lines [32]. In both *in vivo* and *in vitro* systems, LPA has been demonstrated to be involved in multiple aspects of cancer progression, including cell proliferation, growth, survival, migration, invasion and progression of angiogenesis [33-35]. LPA and LPA receptor 1 (LPAR1), mediated by activation of nuclear factor-κB, promotes proliferation, survival and migration of PC cells [36, 37]. HärmäV *et al*. suggested that LPAR1 and Gα (12/13) signaling regulates cellular motility and invasion with epithelial maturation in PC [38]. These results suggest that LPAR1 has a potential therapeutic benefit in PC. Integrin (ITGB)8 is one of the members of the integrin family. ITGB8 has been shown to be upregulated in some cancers, including head and neck cancer, hepatocellular carcinoma, ovarian cancers and melanoma cell lines, in addition to primary non-small lung cancer samples and brain metastases from several epithelial cancers [39-41]. Furthermore, a six-gene expression signature biomarker, which includes ITGB8, may predict the occurrence of lung metastasis from breast cancer [42]. Rutkowski *et al*. reports that the overexpression of EPH Receptor (Eph) B4 leads to aggressive phenotypes in PC cells. The study additionally revealed that EphB4 regulates ITGB8 expression [43]. However, the role of ITGB8 in the motility of PC cells remains to be fully elucidated.

ITGB4 is also a member of the integrin family. Aberrant expression of integrin subunits has been implicated in the malignant phenotype of a variety of cancers [44]. Brendle *et al*. suggests that ITGB4 may influence tumor aggressiveness and survival, and it may have prognostic value in breast cancer [45]. In pancreatic ductal adenocarcinoma, ITGB4 overexpression promotes cell scattering and motility, downregulates E-cadherin and upregulates vimentin expression. Masugi *et al*. revealed that IGTB4 has a potential role in the regulation of cancer invasion and epithelial-mesenchymal transition [46]. Kettunen *et al*. demonstrated that the ITGB4 is upregulated in malignant pleural mesothelioma (MM) suggesting that the ITGB4 has link with the development of MM [47]. ITGB4 is important in PC migration and expansion of prostate tumor progenitors [48]. Kawakami *et al.* reports that ITGB and vinculin may be useful markers for the progression of PC associated with taxane resistance, and this result may provide a basis for the diagnosis of PC [49]. In the present study, it was demonstrated that the PC patients with ITGB4 alterations exhibited a poorer survival rate compared with those without the genetic alterations. This result suggests that the mutation in ITGB4 reduces the survival rate of patients with PC. Ribonucleotide reductase is required for DNA synthesis and repair [50], and is responsible for the *de novo* conversion of the ribonucleoside diphosphates to deoxyribonucleoside diphosphates. The ribonucleotide reductase M2 subunit (RRM2) determines malignant cellular behavior in a range of human cancers [51], such as colorectal cancer, oral squamous cell carcinoma, nasopharyngeal carcinoma, hepatocellular carcinoma, adrenocortical cancer, pancreatic adenocarcinoma and breast cancer. In the present study, the PC patients with an RRM2 alteration had a lower survival rate compared with patients without alteration. Notably, Huang *et al*. dem-

onstrated that RRM2 is important in the proliferation and invasion of PC, which suggests that RRM2 may act as a novel biomarker for assessment of patients with low-risk PC [52]. The results of the present study are consistent with the results of previous studies. RRM2 may have an important role in the progression of PC, however this requires further study, in order to verify the specific molecular marker role of RRM2 in the diagnosis of patients with low-risk PC.

The module analysis result of the PPI network demonstrated that the development of PC was associated with neuroactive ligand-receptor interaction, the p53 signaling pathway and the glutathione metabolism pathway. The neuroactive ligand-receptor interaction signaling pathway is a collection of receptors and ligands on the plasma membrane that are associated with intracellular and extracellular signaling pathways [53]. Fang *et al*. and Liu *et al*. used bioinformatics to demonstrate that the neuroactive ligand-receptor interaction signaling pathway is associated with progression of bladder cancer and renal cell carcinoma [54, 55]. Myers *et al*. revealed that five pathways were enriched in prostate tumors in members of the African-American population, including the neuroactive ligand-receptor interaction signaling pathway, via protein analysis [56]. In accordance with the results of previous studies, the present study demonstrated that neuroactive ligand-receptor interactions are involved in the progression of PC, however the specific molecular mechanisms in PC require further investigation. Furthermore, previous studies have demonstrated that glutathione metabolism is associated with PC. Glutathione peroxidase 1 polymorphism is involved in prostate carcinogenesis [57]. Glutathione exhibits an important role in survival mechanisms of PC cells [58] and this pathway is linked to the antineoplastic function and recrudescence in PC [59, 60]. The p53 gene is the most common mutant gene in human tumors, and the primary function of the p53 protein is to prevent the cells into the DNA synthesis period and make it stagnation in the G1 phase to repair damaged DNA [61, 62]. The mutation rate of the p53 gene in patients with primary PC is 10-20%, whereas the rate in the progression of PC is 42% and is closely related to malignant features such as bone metastasis and androgen dependence [63, 64]. Kluth *et al*. analyzed tissue microarrays including 11,152 prostate cancer samples using immunohistochemistry and fluorescence in situ hybridization, and demonstrated that p53 may be a useful clinical molecular feature of PC [65]. These results indicate that p53 has an important role in the progression and prognosis of PC. *Therefore,* monitoring and *blocking of* neuroactive ligand-receptor interaction, p53 signaling pathway, and glutathione metabolism pathway *are* promising therapeutic strategies for future investigation and treatment of PC patients.

Conclusion

In conclusion, the present study identified various DEGs by using comprehensive bioinformatics analysis. Furthermore, various hub genes and pathways involved in the progression of prostate cancer, which may be predictors or therapeutic targets for PC were identified. However, lack of experimental verification is a limitation of the present study. Further experimental research is necessary to fully elucidate the mechanisms underlying PC tumorigenesis.

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Disclosure of conflict of interest

None.

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