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Original Article

Identification of hub genes and pathways in bladder cancer using bioinformatics analysis

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Abstract: Bladder cancer (BC) is the most common malignant tumor of urinary tract system. The aim of this study was to investigate the genetic signatures of bladder cancer (BC) and identify its potential molecular mechanisms. The gene expression profiles of GSE3167 (50 samples, including 41BC and 9 non-cancerous urothelial cells) was downloaded from the GEO database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) were performed to identify enriched pathways, and a protein-protein interaction (PPI) network was used to identify hub genes and for module analysis. Moreover, we conducted expression and survival analyses to screen and validate hub genes. In total, 1528 DEGs were identified in bladder cancer (BC), including 1212 up-regulated genes and 316 down-regulated genes. Up-regulated differentially expressed genes (DEGs) were significantly enriched in negative regulation of macromolecule metabolic process, macromolecule catabolic process, proteolysis and regulation of cell death, while the down-regulated differentially expressed genes (DEGs) were mainly involved in cell surface receptor linked signal transduction, ion transport, cell-cell signaling and defense response. The top 10 hub genes with the highest degrees were selected from the PPI network. These genes included HSP90AA1, MYH11, MYL9, CNN1, ACTC1, RAN, ENO1, HNRNPC, ACTG2 and YWHAZ. From sub-networks, we found these genes were involved in the proteasome, pathways in cancer and cell cycle. Hence, the identified DEGs and hub genes may be beneficial to elucidate the mechanisms underlying BC.

Keywords: Bladder cancer, microarray analysis, differentially expressed genes, protein-protein interaction network

Introduction

Bladder cancer (BC) is one of the most frequently occurring urogenital malignancy of urinary tract system, with 79,030 new cases and an estimated 16,870 death in the United States in 2017 [1]. Like other cancers, BC is considered as a heterogeneous disease in which gene mutations [2], cellular context [3, 4], and gender [5, 6] lead to tumor initiation, progression, and metastasis. Although a number of cancer-associated genes and cellular pathways have been proven to participate in the occurrence and development of BC [7, 8], a lack of knowledge regarding the accuracy of early diagnosis, therapeutic and progression for BC limits the ability to treat advanced disease. Therefore, to investigate the molecular mechanisms, including the proliferation and apoptosis of BC is crucial for the diagnostic and treatment strategies.

The high-throughput platforms such as gene microarray technology are widely applied in medical oncology and assessing tumor development [9-11]. At present, numerous gene expression profiling studies have been performed using microarray technology to select differentially expressed genes (DEGs) in BC samples [12-14]. As we known, comparative analysis of the DEGs in independent research appears to a relatively limited degree of overlap, and no reliable biomarker profile for cancerous samples. Now, microarray technology combining bioinformatics methods have been using to identify differentially expressed genes (DEGs) in BC and non-cancerous urothelial cells [15].

In current study, the original data (GSE3167) was downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) to screen differentially expressed genes (DEGs). Then, the hub genes, modules key pathways and survival analysis, were identified using comprehensive bioinformatics methods. In this study, we aimed to identify the candidate genes and associated pathways of BC since it may be helpful to explore the potential candidate bio-

markers for diagnosis, prognosis, and treatment.

Materials and methods

Data source

The GSE3167 gene expression profiles were downloaded from the GEO database (GEO, http://www.ncbi.nlm.nih.gov/geo/). This data set was based on the on the GPL96 platform (HG-U133) Affymetrix Human Genome U133A Array and were deposited by Professor Dyrskjøt L *et al* [16]. We selected 50 samples, including 41 BC and 9 non-cancerous urothelial cells samples from the GSE3167 dataset.

Identification of DEGs

GEO2R is an interactive online tool, which allows for comparing two or more groups of samples in order to identify genes that are differentially expressed across experimental conditions [17]. We used a classical *t test* to identify DEGs with cut-off criteria of $P < 0.05$ and $|logFC| \geq 0.5$ were considered to be statistically significant.

Functional enrichment analysis of DEGs

Gene Ontology (GO) analysis has become a common useful approach for biological functional studies of high-throughput genome or transcriptome data [18, 19]. To describe gene biological functional studies, GO commonly provides three categories, including biological process (BP), cellular component (CC) and molecular function (MF) categories [20]. KEGG (http://www.genome.jp/) is an usual bioinformatics database resource for the systematic analysis of gene functions, which contains information on gene networks in various organisms [21]. In the present study, GO analysis and KEGG pathway enrichment analyses were available in the Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/.) [22]. $P < 0.05$ was considered to indicate a statistically significant difference.

Protein-protein interaction (PPI) network and modules analysis

Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/) is an online tool to assess protein-protein interaction (PPI) information, including direct (physical) and indirect (functional) associations [23]. In order to evaluate the interactive relationships among DEGs, STRING was using to evaluate the PPI information, and combined score > 0.4 were selected as significant. Then, the PPI networks were visualized using the Cytoscape software [24]. The plug-in Molecular Complex Detection (MCODE) was used to screen the significant modules with established scores of > 3 and nodes of > 4 in Cytoscape. Then, pathway enrichment analysis was performed for DEGs in the modules. $P < 0.05$ was considered to have significant differences.

Expression and survival analyses of hub genes

To screen the hub genes, CytoHubba plugin was utilized to explore PPI network hub genes in Cytoscape software [24]. The prognostic significance of the identified hub genes was analyzed using GEPIA. Gene Expression Profiling Interactive Analysis (GEPIA) is an online tool to analyze the RNA sequencing expression and survival analyses from The Cancer Genome Atlas (TCGA) and the Genotype Tissue Expression (GTEx) databases [25]. To evaluate the overall survival (OS) rate of patients, the method of Kaplan-Meier was performed in GEPIA. $P < 0.05$ was considered to have significant differences.

Results

Identification of DEGs

A total of 1528 DEGs were selected from GSE3167 data sets by using GEO2R analysis, including 1212 up-regulated genes and 316 down-regulated genes (**Figure 1**). With criteria of the false discovery ratio < 0.05 and $|\log_2 FC| \geq 0.5$.

GO terms analysis of DEGs

We uploaded all DEGs to the online software DAVID, and mapped the up-regulated and down-regulated genes by using GO terms and the KEGG pathways. The top five significant GO terms of the BP, CC and MF categories enriched by the up-regulated and down-regulated DEGs were identified (**Table 1**). GO analysis results demonstrated that the up-regulated DEGs were mainly involved in biological processes (BP), including negative regulation of macromolecule

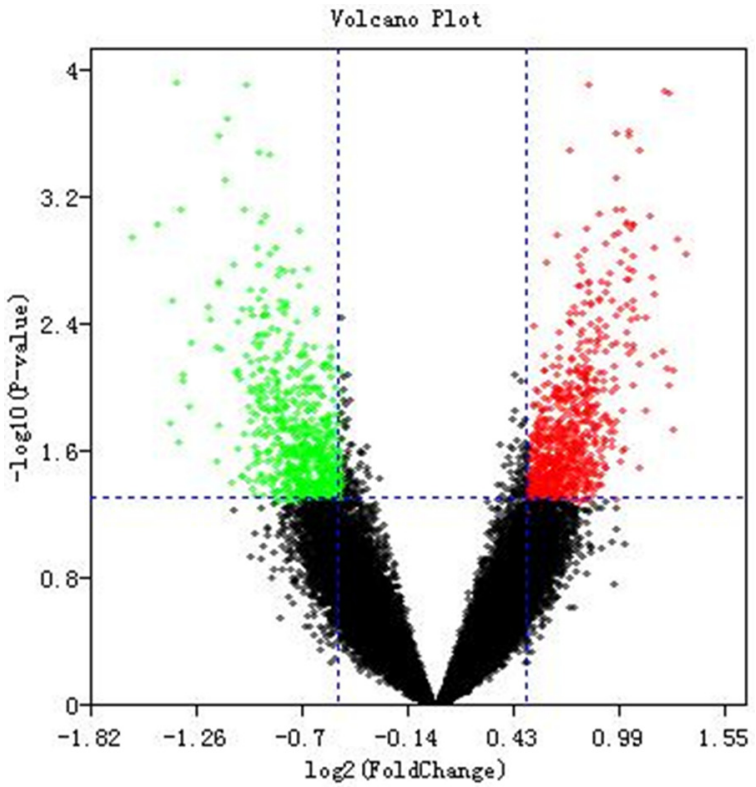

Figure 1. DEG analysis of the GSE3167 data set. DEGs were identified using GEO2R analysis. Green indicates down-regulated genes, red indicates up-regulated genes and black indicates genes with unchanged expression. DEG, differentially expressed gene; FC, fold-change; Down, down-regulated; Not, no change; Up, up-regulated.

metabolic process, macromolecule catabolic process, proteolysis, and regulation of cell death (**Table 1**); whereas the down-regulated DEGs were mainly associated with cell surface receptor linked signal transduction, ion transport, cell-cell signaling, and defense response (**Table 1**). For GO cell component (CC), the up-regulated DEGs were mainly involved in membrane-enclosed lumen, non-membrane-bounded organelle, intracellular non-membrane-bounded organelle, organelle lumen, and ribonucleotide binding; and the down-regulated DEGs were enriched in plasma membrane, plasma membrane part, extracellular region, and intrinsic to plasma membrane (**Table 1**). Additionally, molecular function (MF) analysis also showed that the up-regulated DEGs were significantly involved in nucleotide binding, purine nucleotide binding, purine ribonucleotide binding, and ribonucleotide binding; and the down-regulated DEGs were enriched in plasma membrane, plasma membrane part, extracellular region, and intrinsic to plasma membrane (**Table 1**).

GO analysis of up-regulated and down-regulated genes of the GSE3167 data set

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

KEGG pathway analysis of DEGs

GO terms of the BP, CC and MF categories enriched by the up-regulated and down-regulated DEGs were identified (**Table 1**). The top five significant KEGG pathway enrichment are shown in **Table 2**, which showed that the up-regulated genes were significantly enriched in Huntington's disease, Pathways in cancer, Spliceosome, Alzheimer's disease and Parkinson's disease, while the down-regulated genes were mainly enriched in Vascular smooth muscle contraction, Calcium signaling pathway, Drug metabolism, Dilated cardiomyopathy, and Retinol metabolism.

PPI network and module analyses

The String with combined scores > 0.4 was selected for constructing PPI networks. Then, the entire PPI networks were identified by MCODE, the top three modules were chosen (**Figures 2**-**4**). Moreover, KEGG analysis revealed that the genes were mainly enriched in 'proteasome', 'pathways in cancer' and 'cell cycle' (**Tables 3**-**5**). The top 10 genes in the MCC method were chosen by CytoHubba plugin and sequentially ordered as follows: HSP90AA1, MYH11, MYL9, CNN1, ACTC1, RAN, ENO1, HNRNPC, ACTG2, YWHAZ (**Figure 5**).

Expression level and survival analysis of hub genes in patients with BC

To validate the hub genes identified, the KM-plot was perform the association analysis of mRNA expression and OS rate in patients with BC. As presented in **Table 6** and **Figure 5**, the five up-regulated hub genes showed no significant differences compare to those in the

Table 1. Gene ontology analysis of differentially expressed genes associated with bladder cancer

Expression	Category	Term	Count	%	*P* Value
Up-regulated	GOTERM_BP_FAT	GO:0010605~negative regulation of macromolecule metabolic process	120	11.152416	1.99E-18
	GOTERM_BP_FAT	GO:0009057~macromolecule catabolic process	117	10.873606	6.63E-15
	GOTERM_BP_FAT	GO:0006508~proteolysis	112	10.408922	1.13E-05
	GOTERM_BP_FAT	GO:0010941~regulation of cell death	111	10.315985	1.99E-11
	GOTERM_BP_FAT	GO:0043067~regulation of programmed cell death	111	10.315985	1.60E-11
	GOTERM_CC_FAT	GO:0031974~membrane-enclosed lumen	254	23.605948	1.71E-32
	GOTERM_CC_FAT	GO:0043228~non-membrane-bounded organelle	252	23.420074	3.05E-11
	GOTERM_CC_FAT	GO:0043232~intracellular non-membrane-bounded organelle	252	23.420074	3.05E-11
	GOTERM_CC_FAT	GO:0043233~organelle lumen	247	22.95539	7.18E-31
	GOTERM_CC_FAT	GO:0070013~intracellular organelle lumen	245	22.769517	1.33E-31
	GOTERM_MF_FAT	GO:0000166~nucleotide binding	235	21.840149	3.60E-13
	GOTERM_MF_FAT	GO:0017076~purine nucleotide binding	177	16.449814	6.45E-06
	GOTERM_MF_FAT	GO:0032555~purine ribonucleotide binding	175	16.263941	1.01E-06
	GOTERM_MF_FAT	GO:0032553~ribonucleotide binding	175	16.263941	1.01E-06
	GOTERM_MF_FAT	GO:0001883~purine nucleoside binding	151	14.033457	1.25E-05
Down-regulated	GOTERM_BP_FAT	GO:0007166~cell surface receptor linked signal transduction	47	17.216117	0.0080642
	GOTERM_BP_FAT	GO:0006811~ion transport	27	9.8901099	9.14E-04
	GOTERM_BP_FAT	GO:0007267~cell-cell signaling	24	8.7912088	3.40E-04
	GOTERM_BP_FAT	GO:0006952~defense response	23	8.4249084	0.001132
	GOTERM_BP_FAT	GO:0006812~cation transport	22	8.0586081	6.90E-04
	GOTERM_CC_FAT	GO:0005886~plasma membrane	87	31.868132	0.0012027
	GOTERM_CC_FAT	GO:0044459~plasma membrane part	69	25.274725	3.19E-07
	GOTERM_CC_FAT	GO:0005576~extracellular region	48	17.582418	0.0143998
	GOTERM_CC_FAT	GO:0031226~intrinsic to plasma membrane	46	16.849817	4.90E-07
	GOTERM_CC_FAT	GO:0005887~integral to plasma membrane	44	16.117216	1.72E-06
	GOTERM_MF_FAT	GO:0008092~cytoskeletal protein binding	24	8.7912088	2.36E-05
	GOTERM_MF_FAT	GO:0003779~actin binding	19	6.959707	1.60E-05
	GOTERM_MF_FAT	GO:0046873~metal ion transmembrane transporter activity	15	5.4945055	0.0018227
	GOTERM_MF_FAT	GO:0005506~iron ion binding	14	5.1282051	0.0028998
	GOTERM_MF_FAT	GO:0009055~electron carrier activity	12	4.3956044	0.0017116

Table 2. KEGG pathway analysis of DEGs associated with bladder cancer

Pathway ID	Term	%	*P*-Value
Up-regulated DEGs			
hsa05016	Huntington's disease	3.9962825	2.09E-09
hsa05200	Pathways in cancer	3.9962825	0.0081602
hsa03040	Spliceosome	3.0669145	2.20E-08
hsa05010	Alzheimer's disease	3.0669145	1.05E-05
hsa05012	Parkinson's disease	2.9739777	1.20E-07
Down-regulated DEGs			
hsa04270	Vascular smooth muscle contraction	3.6630037	0.0013481
hsa04020	Calcium signaling pathway	3.6630037	0.0247659
hsa00982	Drug metabolism	2.5641026	0.00346
hsa05414	Dilated cardiomyopathy	2.5641026	0.022477
hsa00830	Retinol metabolism	2.1978022	0.0090598

healthy controls (P > 0.05). The low expression of MYH11, CNN1, ACTC1 and ACTG2 were significantly associated with a good prognosis in normal patients compared to BC patients (P < 0.05) (**Figure 5**).

Discussion

Bladder cancer (BC) is one of the most frequently occurring urogenital malignancy of urinary tract system, with 79,030 cases and

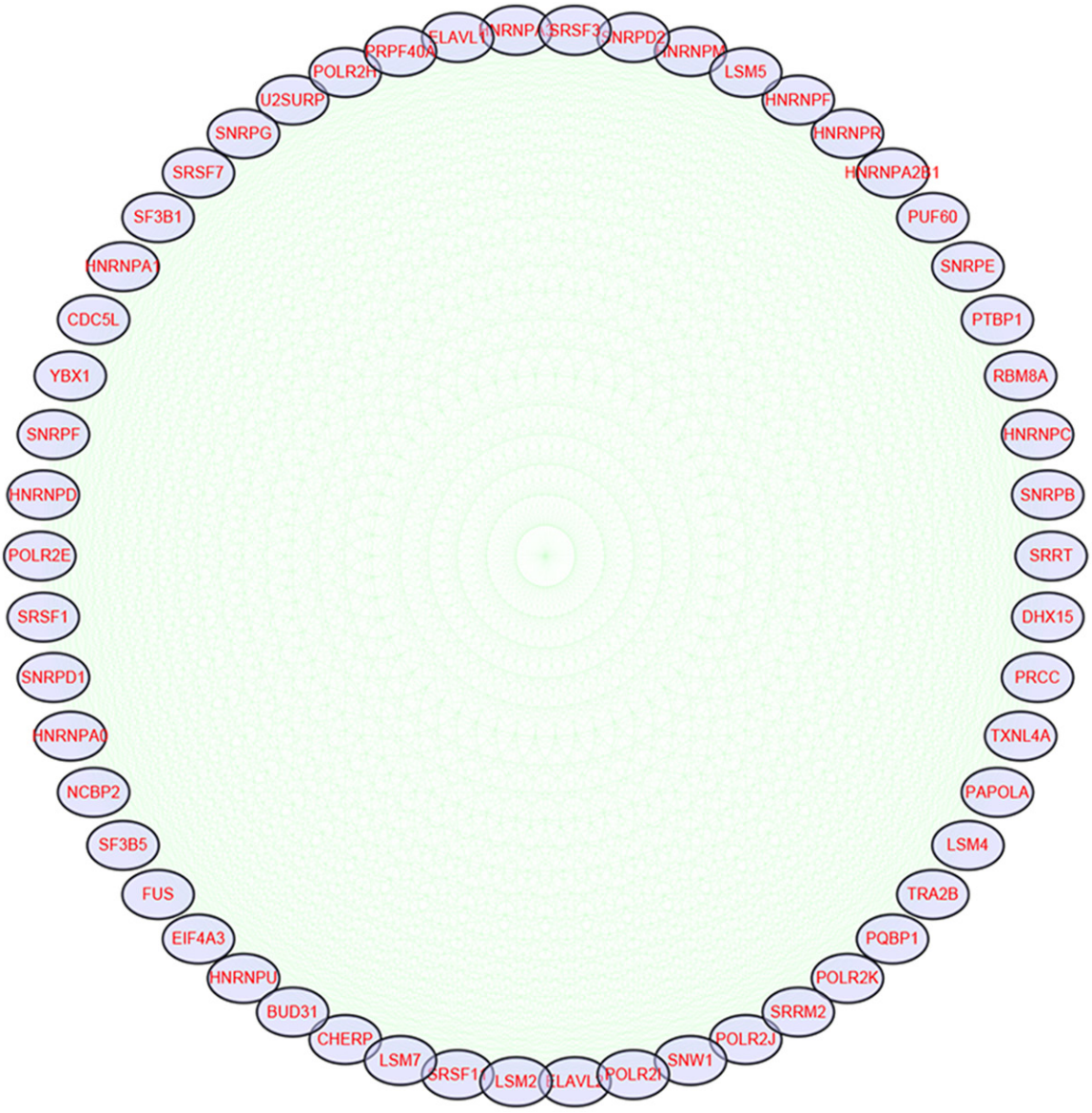

Figure 2. Top 1 module from the Protein-protein interaction network.

16,870 deaths are expected making it the most common type of cancer in the in the United States in 2017 [1]. Understanding the molecular mechanism of BC is of great importance for diagnosis and treatment. With the development of microarray technology and high-throughput sequencing technology, it is now easier to discovery the genetic alterations in the progression of diseases, and has been widely adopted to predict potential diagnosis and therapeutic targets for Bladder cancer [26, 27].

In the present study, DEGs between Bladder cancer (BC) and healthy samples were selected, and a series of bioinformatics analytical methods applied to determine the hub genes and pathways associated with BC. In our study, a total of 1528 DEGs were extracted from GSE3167 data sets, including 1212 up-regulated genes and 316 down-regulated genes. In order to understand the interactions of DEGs, we further performed bioinformatics analysis, including GO enrichment, KEGG pathway, PPI network and survival analyses, revealed that BC-associated genes and pathways may serve an important role in the progression of bladder cancer.

The GO term analysis showed that up-regulated genes were enriched in the negative regulation

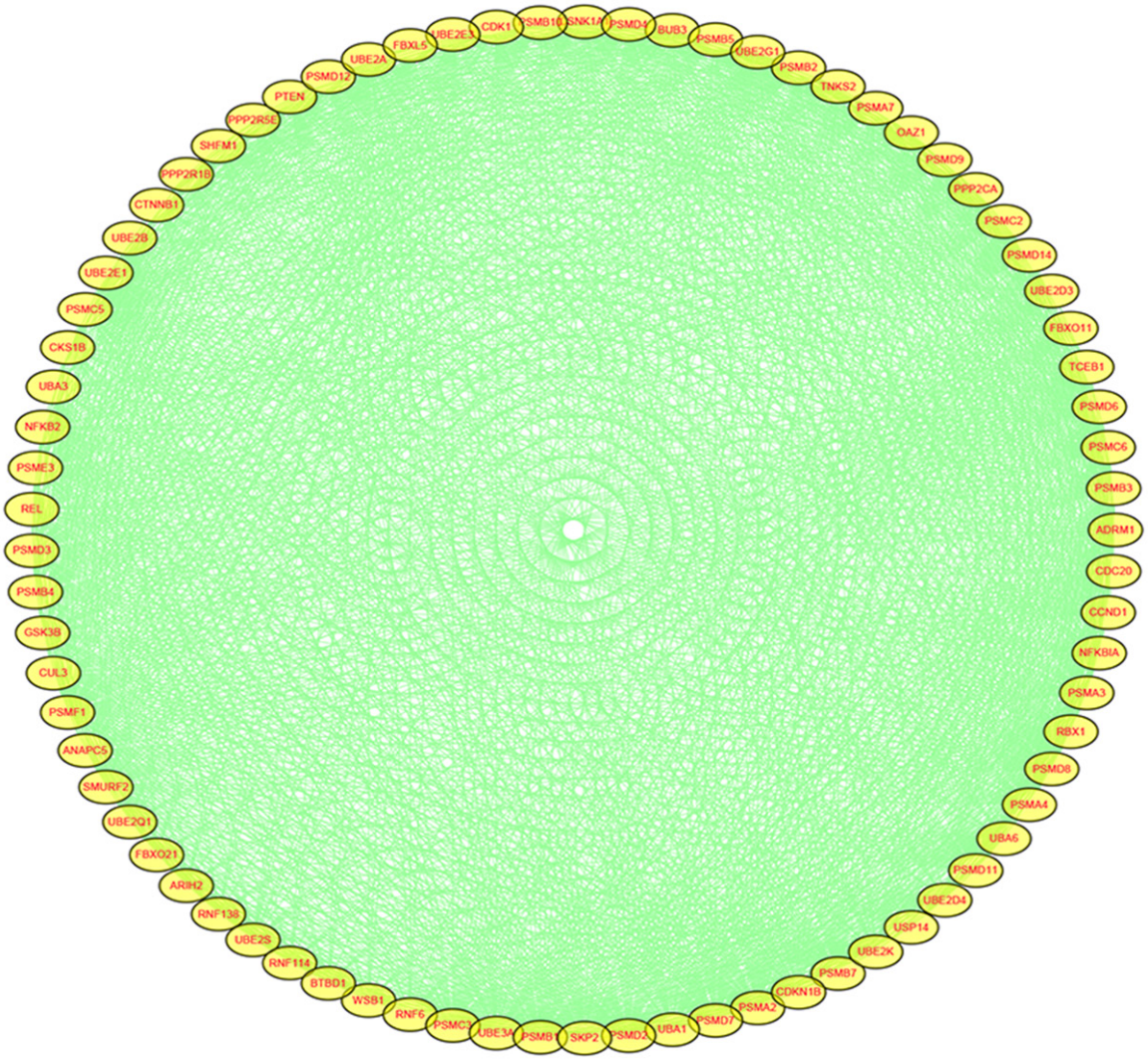

Figure 3. Top 2 module from the Protein-protein interaction network.

of macromolecule metabolic process and regulation of cell death, while the down-regulated genes were mainly involved in cell surface receptor linked signal transduction, cell-cell signaling and defense response, which may be involved in the occurrence of cancer. KEGG pathway analysis indicated that the functions of the up-regulated genes were significantly enriched in Huntington's disease, Pathways in cancer, Spliceosome, Alzheimer's disease and Parkinson's disease, while the down-regulated genes were mainly enriched in Vascular smooth muscle contraction, Calcium signaling pathway, Drug metabolism, Dilated cardiomyopathy and Retinol metabolism. Several studies showed that the calcium signaling pathway may play an important role in the occurrence and development of urinary bladder cancer [28, 29].

Furthermore, we also constructed the PPI network with DEGs, then selected significant modules and the top degree hub genes. Analysis of the top three modules from the PPI network indicated that the proteasome, cell cycle and pathways in cancer may associate with bladder cancer. The significant key genes in the PPI networks, including MYH11, ACTG2, ACTC1 and CNN1 are may all potential diagnostic indicators for bladder cancer.

The MYH11 (myosin heavy chain 11) gene product is a subunit of a hexameric protein that consists of two heavy chain subunits and two pairs of non-identical light chain subunits. A previous study demonstrated MYH11 mutations appear to contribute to human intestinal cancer [30]. And Li et al showed that the MYH11 may be a

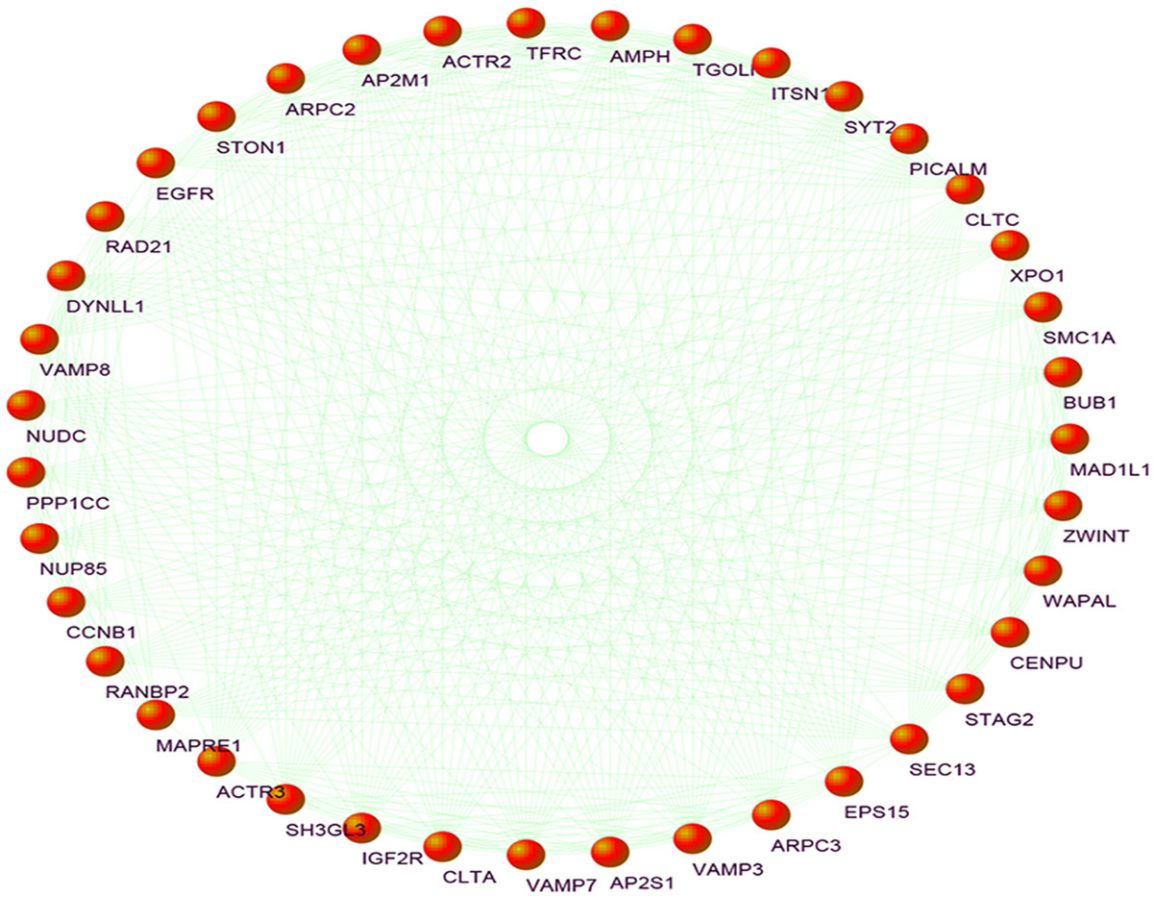

Figure 4. Top 3 module from the Protein-protein interaction network.

biomarker for bladder cancer [31]. In addition, Li M et al revealed that when the MYH11 was disrupted, which may lead to the bladder developing lesions in mouse [32]. In our study, we discovered that the elevated MYH11 expression is related to better OS in normal patients. Hence, we speculated that MYH11 may participate in the occurrence and development of bladder cancer.

ACTG2 (Actin, Gamma 2, Smooth Muscle, Enteric) is a Protein Coding gene [33, 34]. Diseases associated with ACTG2 include Visceral Myopathy and Chronic Intestinal Pseudoobstruction [35]. Previous studies have demonstrated that ACTG2-related disorders are a subset of visceral myopathy with variable involvement of the bladder and intestine [36-38]. Additionally, Thorson W et al revealed that ACTG2 transcripts were primarily found in murine urinary bladder and intestinal tissues, and ACTG2 mutations may lead to congenital distended bladder [36]. Moreover, our results additionally demonstrated that a high ACTG2 expression was associated with a better OS rate. Therefore, this gene may be an essential marker for the diagnosis and prognosis of BC, and more investigation needs to be confirmed.

ACTC1 was originally characterized by Kramer PL et al [39] and is known to encodes cardiac muscle alpha actin [40]. Previous studies have demonstrated that ACTC1 may be associated with Dilated Cardiomyopathy [41], Hypertrophic Cardiomyopathy [42, 43], Left Ventricular Noncompaction [44] and atrial septal defects [45]. In addition, Matsson H, et al [45] revealed that ACTC1 knockdown and a reduction in the atrial septa, which suggested that the ACTC1 gene has a role during development. Furthermore, Zaravinos A et al [46] revealed that ACTC1 may modulate the invasive abilities of BC cells, and future investigation needs to confirm the implication of these genes in urinary

Table 3. Top 1 module from the Protein-protein interaction network

Term	*P* Value	Genes
Spliceosome	1.27106E-42	NCBP2, CHERP, TRA2B, LSM7, SNRPD1, SNRPD2, SF3B5, BUD31, HNRNPA3, HNRNPM, SF3B1, RBM8A, DHX15, LSM5, PQBP1, LSM4, LSM2, HNRNPC, PRPF40A, SNW1, CDC5L, HNRNPA1, HNRNPU, EIF4A3, SNRPB, SNRPF, SNRPE, PUF60, TXNL4A, SNRPG
Huntington's disease	0.033797	POLR2H, POLR2E, POLR2K, POLR2J, POLR2I
Purine metabolism	0.019984	POLR2H, POLR2E, POLR2K, POLR2J, POLR2I
Pyrimidine metabolism	0.003842	POLR2H, POLR2E, POLR2K, POLR2J, POLR2I
RNA degradation	5.74E-04	PAPOLA, LSM7, LSM5, LSM4, LSM2
RNA polymerase	3.43E-05	POLR2H, POLR2E, POLR2K, POLR2J, POLR2I

Table 4. Top 2 module from the Protein-protein interaction network

Term	*P* Value	Genes
Proteasome	2.57E-43	PSMB10, SHFM1, PSMA7, PSMB5, PSMF1, PSMB4, PSMB7, PSMB1, PSMB3, PSMB2, PSMD2, PSMD3, PSMD4, PSMD6, PSMD7, PSMD8, PSMA2, PSMD14, PSMC6, PSMC5, PSMD12, PSMD11, PSMC3, PSMA4, PSMC2, PSMA3, PSME3
Pathways in cancer	0.001097	CKS1B, CCND1, CDKN1B, GSK3B, SKP2, NFKBIA, NFKB2, TCEB1, PTEN, RBX1, CTNNB1
Cell cycle	2.41E-05	CDK1, CCND1, CDKN1B, ANAPC5, GSK3B, SKP2, CDC20, BUB3, RBX1
Ubiquitin mediated proteolysis	3.35E-04	CUL3, ANAPC5, SKP2, CDC20, SMURF2, TCEB1, RBX1, UBE2E1
Wnt signaling pathway	6.06E-04	CSNK1A1, PPP2R1B, CCND1, GSK3B, PPP2CA, PPP2R5E, RBX1, CTNNB1
Oocyte meiosis	6.55E-04	PPP2R1B, CDK1, ANAPC5, PPP2CA, CDC20, PPP2R5E, RBX1
Small cell lung cancer	0.00129	CKS1B, CCND1, CDKN1B, SKP2, NFKBIA, PTEN
Prostate cancer	0.001672	CCND1, CDKN1B, GSK3B, NFKBIA, PTEN, CTNNB1
Endometrial cancer	0.0141	CCND1, GSK3B, PTEN, CTNNB1

Table 5. Top 3 module from the Protein-protein interaction network

Term	*P* Value	Genes
Huntington's disease	5.65E-28	UQCRC2, NDUFB4, ATP5E, CLTA, UQCRC1, NDUFB7, AP2S1, COX7B, NDUFAB1, COX5A, CLTC, ATP5G3, UQCR10, COX6B1, ATP5O, NDUFS2, AP2M1, ATP5J, NDUFA2, NDUFA3, COX7A2, ATP5F1, SDHB, NDUFV1, SDHC, NDUFV2, SDHD, ATP5C1, COX6A1
Oxidative phosphorylation	3.78E-27	UQCRC2, NDUFB4, ATP5E, UQCRC1, NDUFB7, COX7B, NDUFAB1, COX5A, ATP5G3, UQCR10, COX6B1, ATP5O, ATP5I, NDUFS2, ATP5J, NDUFA2, COX7A2, NDUFA3, ATP5F1, SDHB, NDUFV1, SDHC, SDHD, NDUFV2, ATP5C1, COX6A1
Parkinson's disease	1.05E-25	UQCRC2, ATP5E, NDUFB4, NDUFA2, NDUFA3, UQCRC1, COX7A2, NDUFB7, COX7B, NDUFAB1, ATP5F1, COX5A, ATP5G3, SDHB, UQCR10, SDHC, NDUFV1, SDHD, NDUFV2, COX6B1, ATP5C1, COX6A1, ATP5O, NDUFS2, ATP5J
Alzheimer's disease	4.81E-23	UQCRC2, ATP5E, NDUFB4, NDUFA2, NDUFA3, UQCRC1, COX7A2, NDUFB7, COX7B, NDUFAB1, ATP5F1, COX5A, ATP5G3, SDHB, UQCR10, SDHC, NDUFV1, SDHD, NDUFV2, COX6B1, ATP5C1, COX6A1, ATP5O, NDUFS2, ATP5J
Cardiac muscle contraction	1.17E-05	UQCRC2, UQCR10, COX7A2, UQCRC1, COX7B, COX6B1, COX6A1, COX5A
Endocytosis	0.010559	EPS15, SH3GL3, CLTA, TFRC, AP2S1, CLTC, AP2M1
Cell cycle	0.03804	MAD1L1, RAD21, BUB1, SMC1A, STAG2
Citrate cycle (TCA cycle)	0.039489	SDHB, SDHC, SDHD

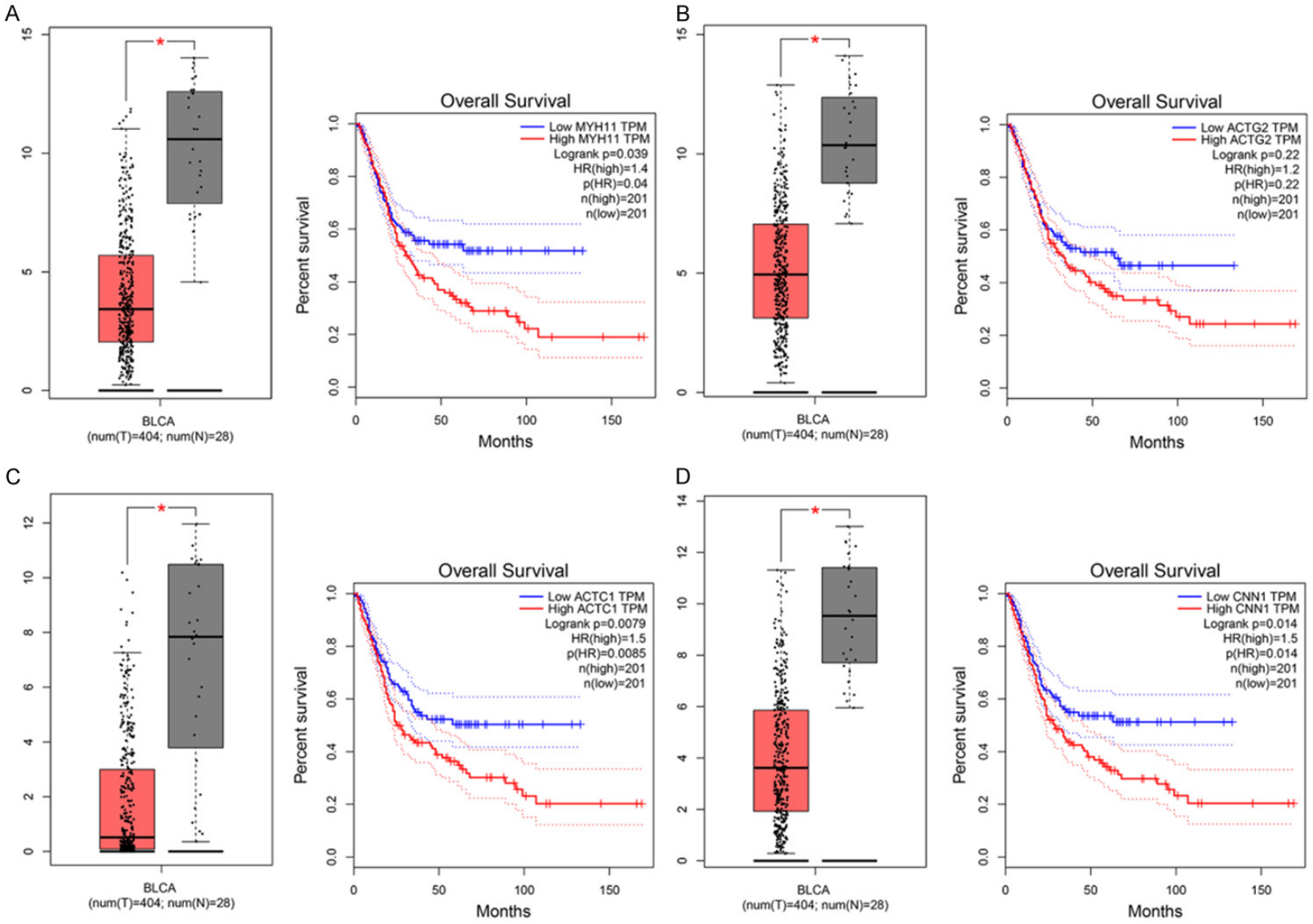

Figure 5. Expression and survival analysis of MYH11, CNN1, ACTC1 and ACTG2.

Table 6. Association between mRNA expression of hub genes and overall survival in patients with bladder cancer

Gene name	End point	*P*-value	Hazard ratio
HSP90AA1	Overall survival rate	0.78	1.0
RAN	Overall survival rate	0.06	1.3
ENO1	Overall survival rate	0.02*	1.4
HNRNPC	Overall survival rate	0.93	1.0
YWHAZ	Overall survival rate	0.07	1.3
MYH11	Overall survival rate	0.04*	1.4
MYL9	Overall survival rate	0.06	1.3
CNN1	Overall survival rate	0.01*	1.5
ACTC1	Overall survival rate	0.01*	1.5
ACTG2	Overall survival rate	0.22*	1.2

Association between mRNA expression and overall survival rate in patients with bladder cancer (using the KMplot database). P < 0.05 was used as the threshold. *considered to have significant differences. HR: hazard ratio.

bladder cancer. In the present study, ACTC1 acts as a tumor-suppressor in BC, which may be a useful marker of BC.

CNN1 plays a tumor-suppressive role in ovarian cancer [47] and it is a structural molecular signature of cancer initiation and progression [48]. CNN1 functions as a tumor suppressor gene and it is an indicator of cell migration in primary cultured invasive hepatocellular carcinoma cells [49]. Furthermore, Liu Y et al [50] also revealed that CNN1 may plays a tumor-suppressive role in bladder cancer, and therefore is a potential candidate biomarker and therapeutic target for invasive BC. Our KM analysis results also revealed that high CNN1 expression was significantly associated with a better OS rate. Due to these findings, the expression level of CNN1 may be a useful prognostic marker of BC.

In conclusion, bioinformatics analysis identified hub genes and pathways that may have important roles in the occurrence, development and prognosis of BC. The key nodes identified in the PPI network constructed with these DEGs and genes involved in the significant module, including MYH11, CNN1, ACTC1 and ACTG2, may be important in the development of BC, and may play a tumor-suppressive role in the pathogenesis of BC. Moreover, further biological experimental evidence is required in order to confirm the function of the identified gene in BC.

Disclosure of conflict of interest

None.

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