Bioinformatics analysis of key biomarkers for bladder cancer

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Abstract. Bladder cancer (BC) is one of the most prevalent genitourinary cancers. Despite the growing research interest in BC, the molecular mechanisms underlying its carcinogenesis remain poorly understood. The microarray datasets GSE38264 and GSE61615 obtained from the Gene Expression Omnibus (GEO) database were analyzed and differentially expressed genes (DEGs) were identified, which were then verified using a dataset from The Cancer Genome Atlas (TCGA). By taking the intersection of the two microarray datasets, the common DEGs were identified and these were selected as candidate genes associated with BC. The DEGs were further subjected to Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis, and the protein-protein interaction network was constructed. Further module analysis was performed using STRING and Cytoscape. A total of 362 DEGs were identified, including 13 hub genes, and the GO analysis revealed that these genes were mainly enriched in extracellular matrix organization, positive regulation of cell proliferation, angiogenesis and peptidyl-tyrosine phosphorylation. The expression changes of PTPRC, PDGFRA, CASQ2, TGFBI, KLRD1 and MT1X in the different datasets indicated that these genes were involved in the development of BC. Next, the differential expression of these genes was verified in the TCGA dataset, and ultimately, these 13 genes were determined to be related to the occurrence and development of BC. Finally, the cancer tissues and adjacent tissues of patients with BC were collected and subjected to reverse transcription-quantitative PCR, the results of which were consistent with the bioinformatics prediction. The present findings provide several vital genes for the clinical diagnosis and treatment of BC.

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Introduction

Bladder cancer (BC) is the second most frequently occurring urinary system tumor and the mortality rate of BC is gradually increasing worldwide (1,2). The main risk factors for this cancer type include tobacco smoking and exposure to certain chemicals in the workplace and in the general environment (3-5). However, little is known about the molecular mechanisms underlying BC. Increasing evidence suggests that the occurrence and development of BC is related to gene mutations and abnormal gene expression. Studies have indicated that oxidative stress has an important role in BC (6-8). In the past decade, numerous BC biomarkers have been identified, including various tumor suppressor genes, oncogenes, growth factors, growth factor receptors, hormone receptors, proliferation and apoptosis markers, cell adhesion molecules, stromal factors and oncoproteins (9). Due to the lack of methods available for early diagnosis and the poor understanding of the molecular mechanisms of the occurrence and development of BC, patients are generally diagnosed when at advanced BC stages. Therefore, research on the molecular mechanisms of the occurrence and development of BC is particularly important to allow for early diagnosis and to provide early treatment interventions.

In recent years, microarray technology has been widely used in studies related to gene expression. Its application complements the methods of gene expression studies and strengthens research on disease susceptibility and disease pathology. After detecting the differences in gene expression, the next step is to find the biological functions of these differences and use bioinformatics analysis to screen for gene changes at the genomic level, so as to identify differentially expressed genes (DEG) and functional pathways involved in the occurrence and development of liver cancer. However, the analysis of a single microarray data set has limitations, and its results require to be further verified. Therefore, in the present study, following the method of Li *et al* (10) from 2017, gene chip datasets in the comprehensive gene expression omnibus (GEO) database were analyzed, common DEGs from the intersection of the two data sets were identified through a Venn diagram, and they were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The results provide a theoretical basis for further study of the molecular mechanisms of BC.

In the present study, the expression of BC-related genes and their impact on progression, malignancy and prognosis were examined. Through the analysis of two mRNA microarray data sets, a total of 362 DEGs, comprising 315 upregulated and 47 downregulated DEGs, were identified. Subsequently, 13 central genes were identified by using the Cancer Genome Atlas (TCGA) database and protein-protein interaction (PPI) network analysis. In conclusion, 362 DEGs and 13 hub genes were identified. Through various software analyses, it was indicated that these genes may be candidate biomarkers of BC. Among these hub genes, platelet-derived growth factor receptor α (PDGFRA) had the highest degree of connectivity.

Materials and methods

Screening of DEGs of BC in the GEO database. GEO (http://www.ncbi.nlm.nih.gov/geo) (11) is a global database of diseases, which contains a large amount of genomic data. The GSE38264 (12) and GSE61615 (13) gene chip data sets were downloaded from GEO (Affymetrix GPL570 platform; Affymetrix human genome U133 Plus 2.0 array). The gene chip was annotated using the DAVID website (http://david.ncifcrf.gov) (14). Overall, the GSE38264 dataset contains 28 BC tissue samples and 10 non-cancer samples, while the GSE6165 dataset contains two BC samples and two non-cancer samples.

Identification of DEGs. GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) was used to screen DEGs between BC and non-cancerous samples. GEO2R is an interactive network tool that allows users to compare two or more datasets in the GEO series (15). It may be used to analyze the online analysis tools of GSE38264 and GSE6165; |log fold change| >1.5 and P<0.001 were selected as cut-off criteria (16).

KEGG and GO enrichment analyses of DEGs. DAVID (http://david.ncifcrf.gov; version 6.8) (14) is an online bioinformatics database used to analyze gene function through GO and also allows for the identification of gene-related pathways using KEGG analysis (17). In order to further explore the biological processes and signaling pathways of these DEGs, functional analysis was performed (18). GO collects information about molecular function, biological process and cellular composition. KEGG pathway analysis is used to mine significant pathways related to DEGs and has prognostic significance. GO and KEGG are executed by the R package of clusterprofiler (19). A false discovery rate <0.05 was considered to indicate statistical significance.

Construction of the PPI network and analysis of modules. The STRING database, an online resource dedicated to organism-wide protein association networks (20), was used to construct the PPI network to provide an analysis of the functional interactions between proteins indicative of the underlying mechanisms of disease generation or development. The DEGs were analyzed using STRING by downloading data from the protein interaction network and the PPI of DEGs was constructed using Cytoscape (version 3.7.2), an open bioinformatics software platform used to construct a visualized protein interaction network (21). Cytoscape's plug-in molecular complex detection (Mcode) (version 2.0.0) was used to cluster a given network based on topology to find densely connected areas (22). Cytoscape was used to draw a PPI network and Mcode was used to identify the most important modules in the PPI network.

Table I. Primer sequences.

Gene/direction	Primer sequence (5'-3')
GAPDH	
Forward	TGCACCACCAACTGCTTAGC
Reverse	GGCATGGACTGTGGTCATGAG
KLRD1	
Forward	GTGAACAGAAAACTTGGAACGA
Reverse	ATAGATACTGGGAGAGTGCAGA
MT1X	
Forward	CCTGCAAGAAGAGCTGCTGC
Reverse	GCAGCTGCACTTGTCTGACG
PDGFRA	
Forward	GAAAATGAAAAGGTTGTGCAGC
Reverse	CTCTTCTTCAGACATGGGGTAC
PTPRC	
Forward	AAGTGCGGAAACAGAAGAGGTAGTG
Reverse	CAGGGTAGGTGCTGGCAATGAC
TGFBI	
Forward	ACTCAGCCAAGACACTATTTGA
Reverse	CTTGTATGGGCATCAATTGGAG

Retrieval of BC patient information from TCGA database. TCGA clinical data were downloaded from the Genomic Data Commons data portal (https://portal.gdc.cancer.gov/) (23). The clinical information of 412 patients with BC (anonymized) was downloaded from the TCGA database and the association between the hub gene and tumor stages was analyzed using R software for data exploration, statistical analysis and mapping (24).

Selection and analysis of hub genes. The plug-in biological network ontology tool (Bingo) (version 3.0.3) in Cytoscape was used to analyze the hub gene and visualize its biological processes (25). Using the National Center for Biotechnology Information (NCBI) genomics browser (https://www.ncbi.nlm.nih.gov/), a functional clustering of central genes was constructed (26). Kaplan-Meier plotter was used to analyze overall survival and disease-free survival associated with the expression of central genes. Using the Oncomine online database (http://www.oncomine.com) (27-29), the importance of key genes in other BC datasets was analyzed. The hierarchical clustering of central genes was performed using the University of California Santa Cruz Website (http://genome.ucsc.edu/).

Patients. Tumor and normal tissue samples were provided by three patients with squamous cell carcinoma of the bladder. In March 2022, three male patients aged 57, 54 and 59 years were hospitalized at the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China), all from Xinjiang, China. All 3 patients had painless and complete hematuria.

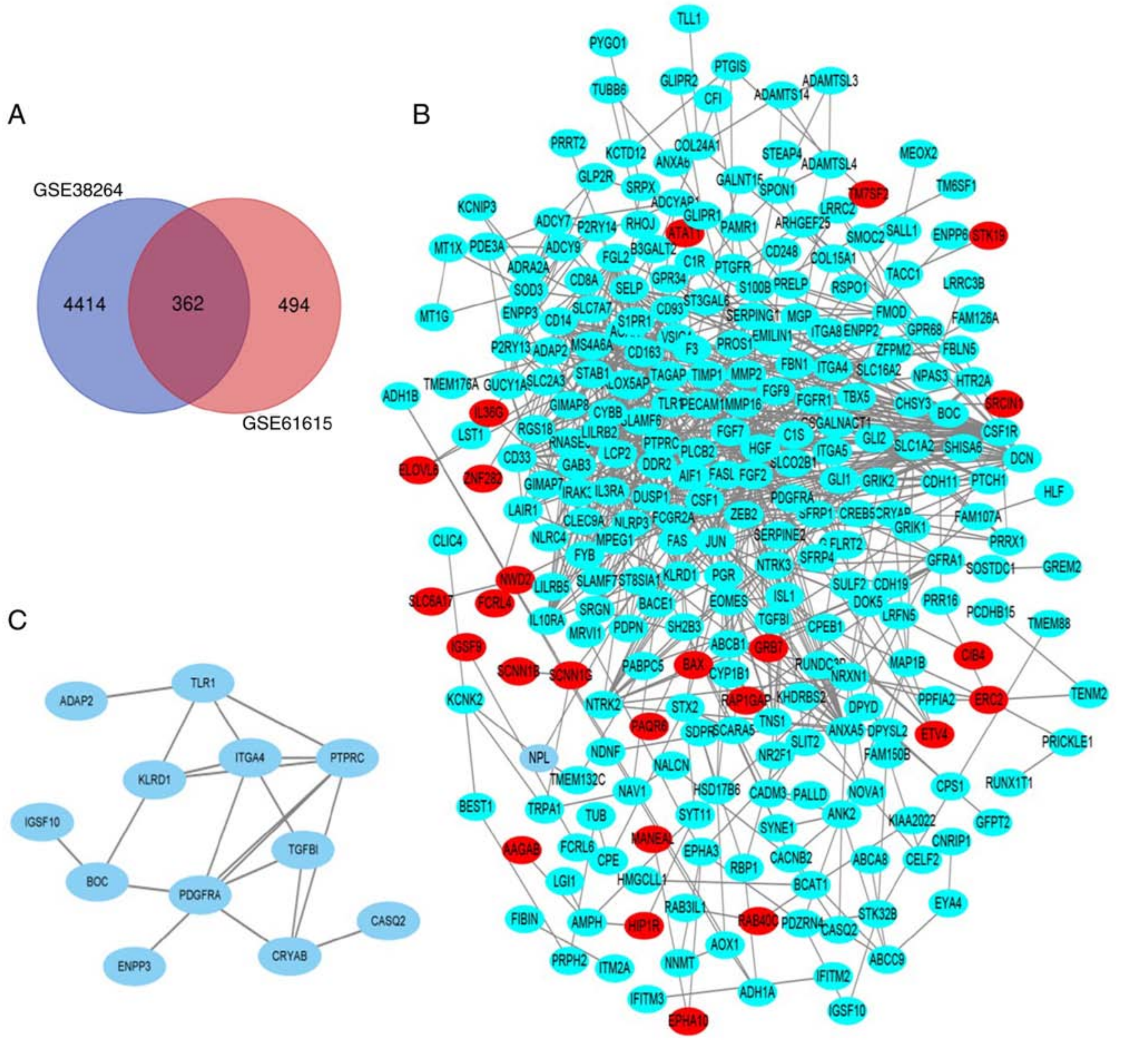

Figure 1. Venn diagram, PPI network and the most important modules of DEGs. (A) DEGs were selected from the mRNA expression datasets GSE38264 and GSE61615 using the selection criteria of fold change >2 and P-value <0.01. The two data sets had 362 overlapping DEGs. (B) The PPI network of DEGs was built using Cytoscape. The upregulated genes are marked in red and the downregulated genes in light blue. (C) The most important module was obtained from the PPI network with 12 nodes. PPI, protein-protein interaction; DEG, differentially expressed gene.

Differential expression of hub genes in patients by reverse transcription-quantitative (RT-q)PCR. The total RNA in the sample to be tested was extracted with TRIzol (Thermo Fisher Scientific, Inc.) and the purity and concentration of RNA were detected by a spectrophotometer. RNA was reverse transcribed into cDNA with an RT kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Real-time qPCR was performed with SYBR green real-time PCR reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions and the reaction time and temperature had been determined in a preliminary experiment. The PCR amplification conditions were as follows: Initial denaturation at 95˚C for 5 min, followed by 40 times cycles of 95˚C for 10 sec, 58˚C for 20 sec and 72˚C for 30 sec. GAPDH was used as the internal reference and the relative mRNA expression level of the gene to be tested was analyzed using the $2^{-\Delta\Delta Cq}$ method (30). Primers used for detection of gene expression are listed in Table I.

Immunohistochemical detection of transforming growth factor (TGF) β-induced (TGFBI) expression in BC. Paraffin-embedded tissues were sliced and dewaxed (10 min for xylene I/II; 5 min for 100% ethanol I/II; 10 sec for 95, 90, 85 and 75% ethanol. They were incubated with 3% H_2O_2 for 5-10 min at room temperature to eliminate the activity of endogenous peroxidase. Following rinsing with distilled water, they were soaked in PBS for 5 min, blocked with 5-10% normal goat serum (Shanghai Suolaibao Biological Co.) in PBS at room temperature for 10 min and the serum was drained off. The primary antibody to TGFBI (cat. no. PA5-82358; Thermo Fisher Scientific, Inc.) working solution (diluted with PBS at 1:200) was added drop-wise and incubated at 4˚C overnight. After washing with PBS,

Table II. GO and KEGG pathway enrichment analysis of DEGs in BC samples.

A, GO

Term	Description	Count in gene set	P-value
GO:0030198	Extracellular matrix organization	17	1.01×10^{-6}
GO:0008284	Positive regulation of cell proliferation	24	2.81×10^{-5}
GO:0001525	Angiogenesis	15	9.06×10^{-5}
GO:0018108	Peptidyl-tyrosine phosphorylation	12	1.58×10^{-4}
GO:0008201	Heparin binding	18	6.71×10^{-9}
GO:0005509	Calcium ion binding	36	1.50×10^{-7}
GO:0042803	Protein homodimerization activity	29	2.09×10^{-4}
GO:0005044	Scavenger receptor activity	6	1.84×10^{-3}
GO:0043565	Sequence-specific DNA binding	17	3.11×10^{-2}
GO:0005887	Integral component of plasma membrane	61	2.29×10^{-9}
GO:0005886	Plasma membrane	125	8.24×10^{-9}
GO:0005576	Extracellular region	61	2.42×10^{-7}
GO:0016021	Integral component of membrane	142	2.48×10^{-7}

B, KEGG

Term	Description	Count in gene set	P-value
Hsa04015	Rapl signaling pathway	14	3.24×10^{-4}
Hsa05200	Pathways in cancer	19	9.61×10^{-4}
Hsa04151	PI3K-Akt signaling pathway	15	1.02×10^{-2}
Hsa05205	Proteoglycans in cancer	10	2.05×10^{-2}
Hsa04010	MAPK signaling pathway	12	1.38×10^{-2}

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

an appropriate amount of biotin-labeled secondary antibody conjugated to HRP (cat. no. A-11001; Thermo Fisher Scientific, Inc.) working solution was added and samples were incubated at 37˚C for 30 min. Following washing with PBS for 5 min, an appropriate amount of horseradish enzyme (Shanghai Suolaibao Biological Co.) working solution was added with incubation at 37˚C for 10-30 min. Samples were washed with PBS for 5 min and the chromogenic agent diaminobenzidine was added for 3-15 min. Samples were fully rinsed with tap water, re-dyed with hematoxylin, dehydrated, cleared with xylene and sealed with neutral balsam. Slides were then observed under an inverted microscope (WMJ-9590; Nikon Corporation).

H&E staining of BC sections. Paraffin sections are dewaxed and rehydrated as follows: They dewaxed with xylene and rehydrated with an ethanol gradient and then distilled water. Hematoxylin was then used to stain the nuclei: The slices were stained with Harris hematoxylin for 3-8 min, washed with tap water, differentiated with 1% hydrochloric acid alcohol for several seconds, washed with tap water, turned back to blue with 0.6% ammonia and washed with running water. The sections were then stained with eosin for 1-3 min. Subsequently, the samples were dehydrated with an ethanol gradient, cleared with xylene. The slices were then slightly dried and sealed with neutral balsam, followed by observation under a microscope.

Statistical analysis. Statistical analysis was performed using R software (4.1.0) and GraphPad (version 8.0; GraphPad Software, Inc.). All data were expressed as the mean ± standard deviation and statistical analysis among different groups was performed by SPSS 24.0 software (IBM Corporation). Differences between groups were evaluated using one-way ANOVA with Tukey's post-hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of DEGs in BC. After standardizing gene expression values in the GeneChip datasets GSE38264 and GSE6165, 4,414 and 494 DEGs were screened, respectively. As indicated in the Venn diagram (Fig. 1A), the overlap between the two datasets contained 362 genes.

KEGG and GO enrichment analyses of DEGs. The DEGs were analyzed using functional analysis with the Web tool DAVID. GO analysis indicated that the changes in the category molecular function mainly included heparin binding, calcium ion binding, protein homodimerization activity, scavenger receptor activity and sequence-specific DNA binding (Table II). The enriched biological process terms of the DEGs were extracellular matrix organization, positive regulation of

Table III. GO pathway enrichment analysis of differentially expressed genes in the most significant module.

Pathway ID	Pathway description	Count in gene set	P-value
GO:1990405	Protein antigen binding	2	0.003254
GO:0005886	Plasma membrane	8	0.008012
GO:0005515	Protein binding	11	0.008428
GO:0005887	Integral component of plasma membrane	5	0.010799
GO:0046872	Metal ion binding	5	0.036499
GO:0007155	Cell adhesion	3	0.041053
GO:0009986	Cell surface	3	0.047841

GO, Gene Ontology.

cell proliferation, angiogenesis and peptidyl-tyrosine phosphorylation (Table II). In the category cellular component, the DEGs were mainly concentrated in the integral component of the plasma membrane, the plasma membrane, the extracellular region and the integral component of the membrane (Table II). KEGG pathway analysis suggested that the DEGs were mainly enriched in the Rap1 signaling pathway, pathways in cancer, the PI3K-Akt signaling pathway, proteoglycans in cancer and the MAPK signaling pathway.

Construction of the PPI network and analysis of the modules. Cytoscape was used to construct a PPI network of the different DEGs (Fig. 1B) and the most important module was obtained from the PPI network with 12 nodes (Fig. 1C). DAVID was used to analyze the most important module genes in Cellular Component and it was indicated that these genes were mainly plasma membrane and membrane components (Table III).

Selection and analysis of hub genes. Using Cytoscape Mcode, a total of 13 hub genes were selected. These 13 genes are listed in Table IV. The PPI network of the hub gene PDGFRA and its co-expressed genes was constructed using Cytoscape (Fig. 2A). The biological processes of the hub gene and its co-expressed genes are presented in Fig. 2B. Hierarchical cluster analysis revealed that the hub gene was able to distinguish liver cancer samples from noncancer samples (Fig. 2C). Kaplan-Meier curves were used to analyze the survival rate of the hub genes. It was indicated that patients with BC with higher expression of PDGFRA, Toll-like receptor (TLR)1, CASQ2, BOC, TGFBI, KLRD1, ADAP2, ITGA4, ENPP3, MT1X, IGSF10 and CRYAB had poor overall survival (Fig. 3). PTPRC, PDGFRA, CASQ2, TGFBI, KLRD1 and MT1X were significantly correlated with BC in different BC datasets (Fig. 4A-F). In the TCGA clinical database of patients with BC, PTPRC, PDGFRA, CASQ2, BOC, KLRD1, ADAP2, ITGA4, IGSF1 and CRYAB mRNA levels were associated with tumor grade (Fig. 5A-I).

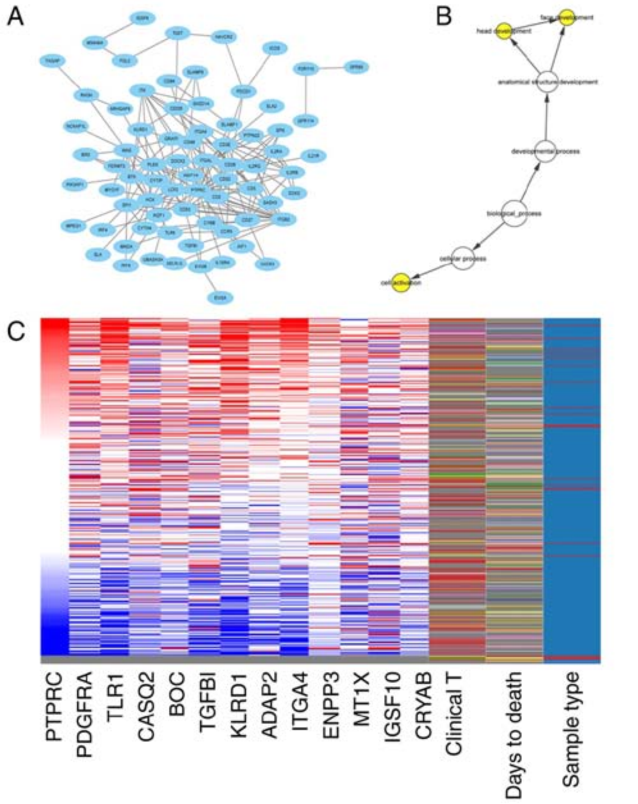

Figure 2. Interaction network and biological process analysis of hub genes. (A) The network of the hub gene and its co-expressed genes were analyzed using Cytoscape. (B) Biological process analysis for determining the central genes by using the plug-in biological network ontology tool (version 3.0.3) in Cytoscape. The color depth of the node refers to the corrected P-value of the body. The size of the nodes refers to the number of genes involved in the body. P<0.01 was considered to be statistically significant. (C) The hierarchical clustering of central genes was performed using the University of California Santa Cruz website. The upregulated genes are displayed in red and the downregulated ones in blue. In the category 'sample type', pink bars indicate non-cancerous samples and blue bars indicate breast cancer samples. In the category 'clinical T stage', light green to dark red represents clinical stage T_0-T_4. In 'days to death', light green to dark red represents the time of death from short to long.

RT-qPCR verifies hub genes. RT-qPCR was used to detect the expression of hub genes in cancerous and paracancerous tissues of patients with BC. The results indicated that the expression levels of the hub genes KLRD1, MT1X and PDGFRA in cancer tissues were significantly lower than those in adjacent tissues (Fig. 6A-C), while the expression levels of PTPRC and TGFBI were significantly higher than those in adjacent tissues (Fig. 6D and E).

Table IV. Functional roles of 13 hub genes with degree ≥10.

Gene symbol	Full name	Function
PTPRC	Protein tyrosine phosphatase receptor type C	Essential regulator of T- and B-cell antigen receptor signaling
PDGFRA	Platelet-derived growth factor receptor α	Mutations in this gene have been associated with idiopathic hypereosinophilic syndrome, somatic and familial gastrointestinal stromal tumors and a variety of other cancers
TLR1	Toll-like receptor 1	Associated with nasopharyngeal cancer
CASQ2	Calsequestrin 2	Mutations in this gene cause stress-induced polymorphic ventricular tachycardia
BOC	BOC cell adhesion-associated oncogene regulated	Component of a cell-surface receptor complex that mediates cell-cell interactions between muscle precursor cells, and promotes myogenic differentiation
TGFBI	Transforming growth factor β-induced	Mutations in this gene are associated with multiple types of corneal dystrophy
KLRD1	Killer cell lectin like receptor D1	Several transcript variants encoding different isoforms have been found for this gene
ADAP2	ArfGAP with dual PH domains	The gene is able to block the entry of certain RNA viruses
ITGA4	Integrin subunit α4	This gene is associated with gastrointestinal stromal tumors
ENPP3	Ectonucleotide pyrophosphatase/phosphodiesterase 3	Antibody drugs of ENPP3 may be used to treat advanced renal cell carcinoma
MT1X	Metallothionein 1X	High expression of this gene is related to the progression of hepatocellular carcinoma
IGSF10	Immunoglobulin superfamily member 10	High expression of this gene is related to the occurrence and development of breast cancer
CRYAB	Crystallin αB	CRYAB inhibits migration and invasion of bladder cancer cells through the PI3K/AKT and ERK pathways

All information is from the National Center for Biotechnology Information database.

Immunohistochemical detection of TGFBI and H&E staining. Immunohistochemical analysis of TGFBI protein indicated that the positive expression rate in tumor tissue was high (Fig. 6F). The H&E staining results of BC and normal tissues under the light microscope indicated that the tumor group exhibited irregular mitosis, while the nuclei of normal tissues were normal round, without any irregular mitosis (Fig. 6G).

Discussion

BC is one of the 10 most common tumor types. In recent years, mortalities from BC have increased (31,32). The main causes of BC include smoking, occupational exposure, diet, long-term use of certain drugs, infection and gene polymorphisms (33,34). However, the molecular mechanisms underlying BC have remained to be fully elucidated. The abnormal expression of the assembly factor for bundle microtubules, TEF transcription factor, PAR bZIP family member, chloride intracellular channel protein 1, zinc finger and the SCAN domain containing 16, c-myc or RAS, p53 or p21 genes, have been reported to be involved in BC (35-37). Furthermore, the loss of UTX, also known as lysine-specific demethylase 6A, and the activation of receptor tyrosine kinase fibroblast growth factor receptor 3, are reported to be BC-related (38). The treatment outcomes of patients with early undetected BC are poor and early effective diagnostic markers are urgently required. The application of multiple bioinformatics approaches contributes to the analysis of molecular changes in the development of BC and has also been used in the diagnosis of other diseases (39-42).

Through the analysis of two mRNA microarray datasets, a total of 362 DEGs, comprising 315 upregulated and 47 downregulated DEG, were identified in the present study. Enrichment analysis using GO and KEGG was performed to explore the interactions between DEGs. DEGs were mainly enriched in extracellular matrix organization, heparin binding and plasma membrane. In previous studies, the extracellular matrix has been found to have an important role in the occurrence and development of tumors, and may cause tumor invasion and migration (43-45). Furthermore, recent studies have indicated that heparin binding may significantly promote tumor growth (46,47). Furthermore, the results of the GO enrichment analysis suggested that at least 8 DEGs are involved in the

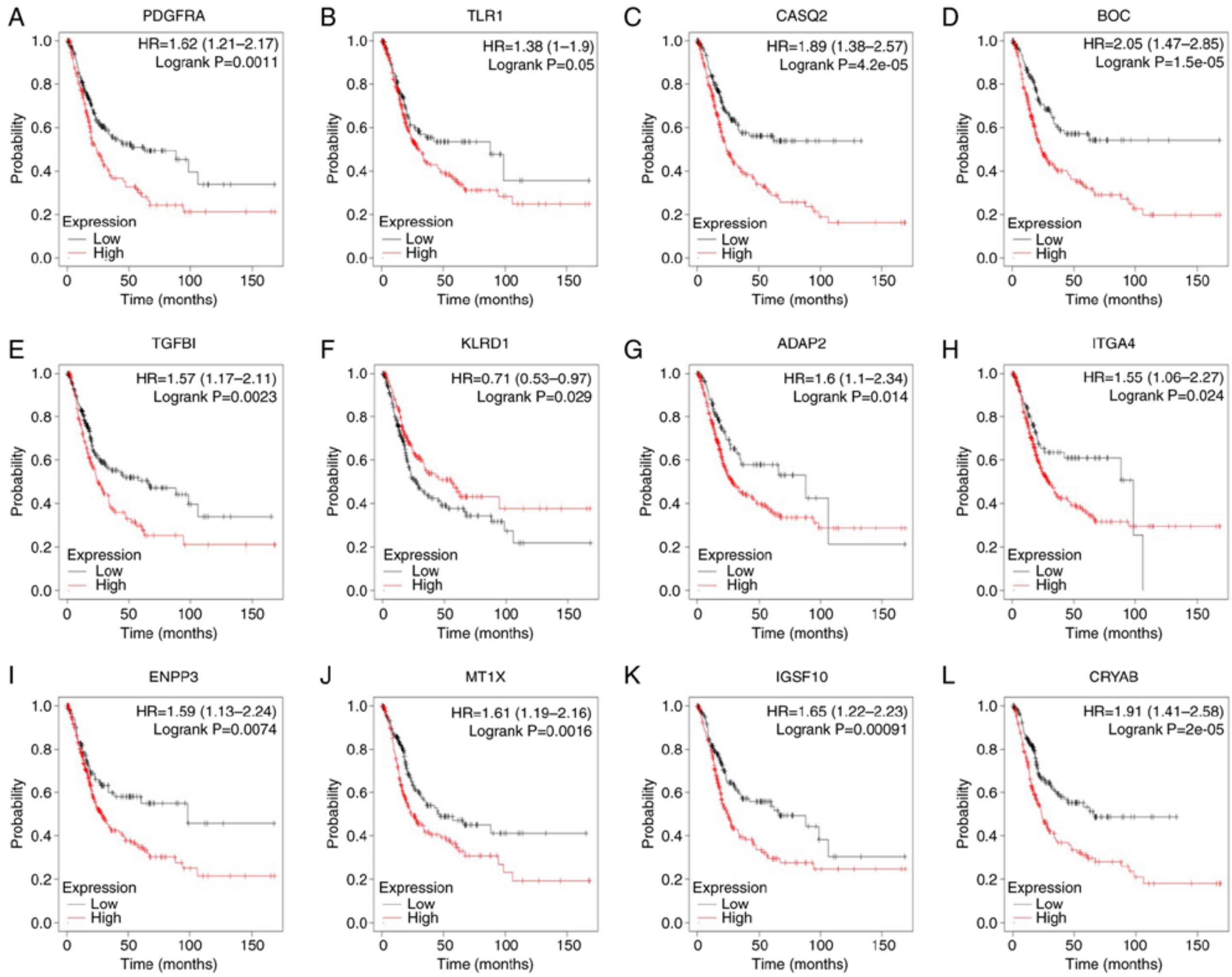

Figure 3. Kaplan-Meier plotter online platform was used to analyze overall survival associated with central genes. P<0.05 was considered statistically significant. Survival analysis for (A) PDGFRA, (B) TLR1, (C) CASQ2, (D) BOC, (E) TGFBI, (F) KLRD1, (G) ADAP2, (H) ITGA4, (I) ENPP3, (J) MT1X, (K) IGSF10 and (L) CRYAB in bladder cancer. HR, hazard ratio (presented with 95% CI).

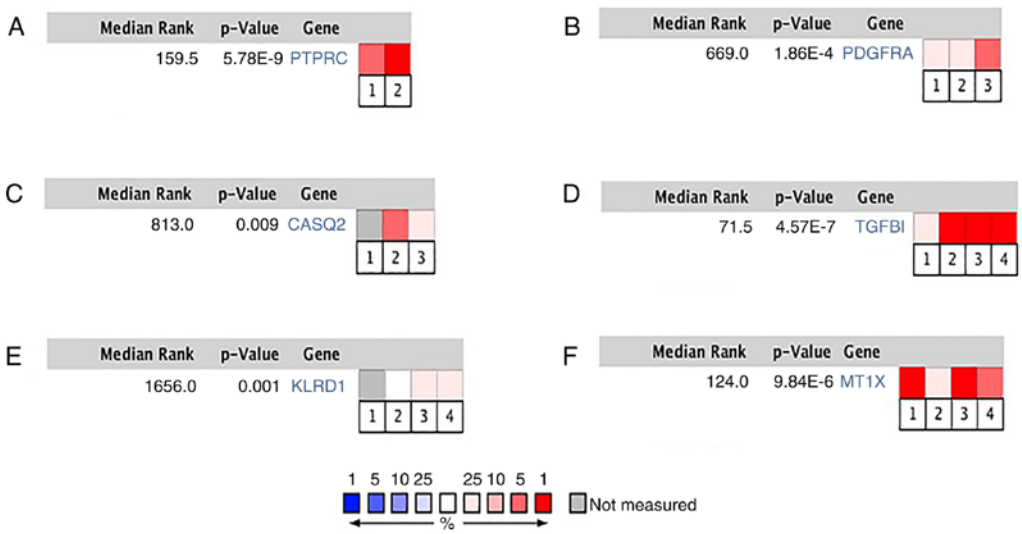

Figure 4. Hub gene expression in Blaveri Bladder, Dyrskjot Bladder, Sanchez-Carbayo Bladder and Stransky bladder datasets. (A) PTPRC in Blaveri Bladder and Sanchez-Carbayo Bladder datasets, (B) PDGFRA in Blaveri Bladder, Sanchez-Carbayo Bladder and Stransky bladder datasets, (C) CASQ2 in Blaveri Bladder, Sanchez-Carbayo Bladder and Stransky bladder datasets, (D) TGFBI in Blaveri Bladder, Dyrskjot Bladder, Sanchez-Carbayo Bladder and Stransky bladder datasets, (E) KLRD1 in Blaveri Bladder, Dyrskjot Bladder, Sanchez-Carbayo Bladder and Stransky bladder datasets, (F) MT1X in Blaveri Bladder, Dyrskjot Bladder, Sanchez-Carbayo Bladder and Stransky bladder datasets. Heat maps of PTPRC, PDGFRA, CASQ2, TGFBI, KLRD1 and MT1X gene expression in clinical bladder cancer samples vs. normal tissues. P<0.05 was considered statistically significant. 1-4 in the figure are respectively quoted from refs. 69-72. Data source cited in figure.

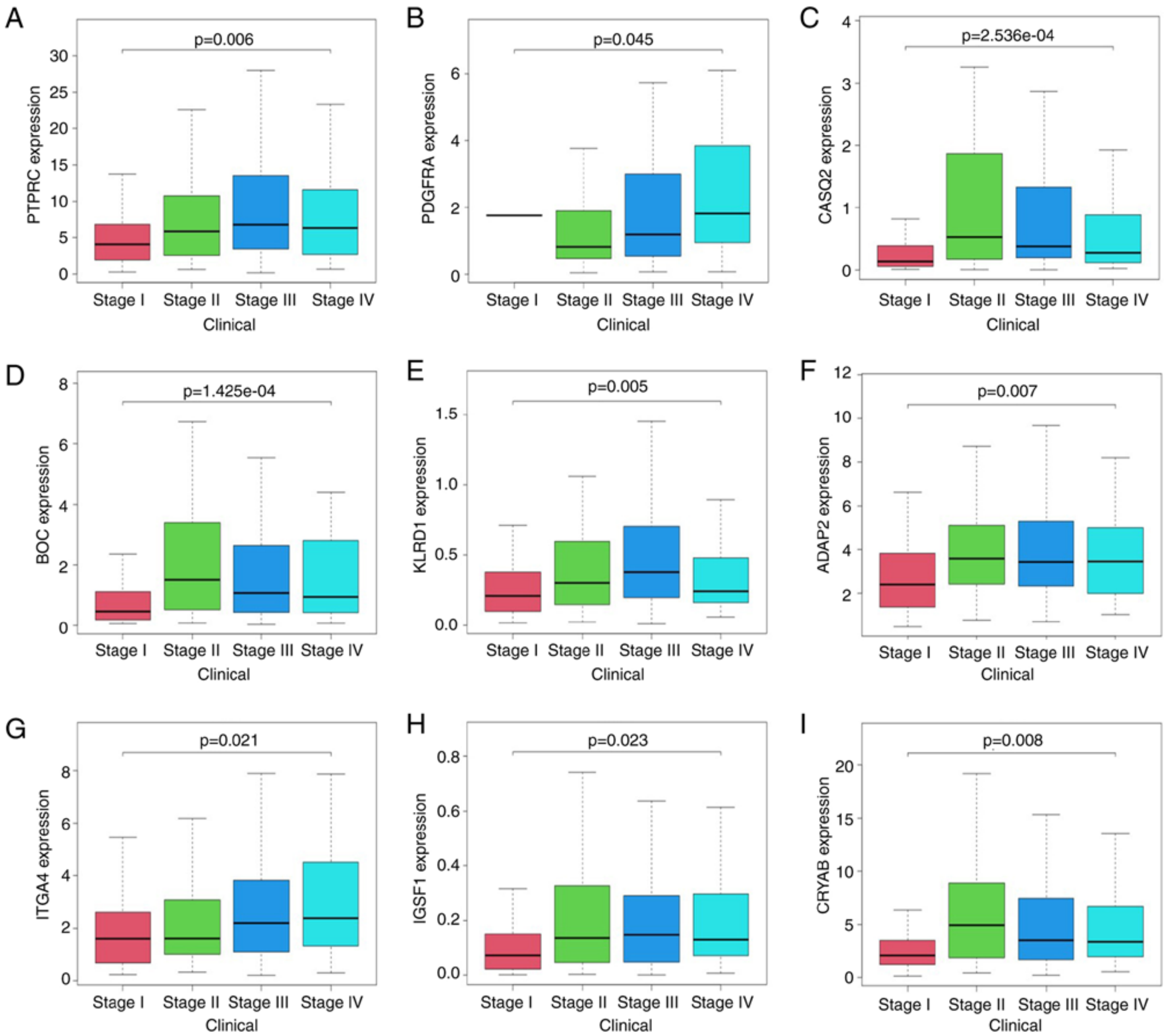

Figure 5. In The Cancer Genome Atlas dataset for clinical patients with bladder cancer, the expression of each gene was compared among different tumor stages. (A) PTPRC (P=0.006), (B) PDGFRA (P=0.045), (C) CASQ2, (D) BOC, (E) KLRD1 (P=0.005), (F) ADAP2 (P=0.007), (G) ITGA4 (P=0.021), (H) IGSF10 (P=0.023) and (I) CRYAB (P=0.008).

composition of the plasma membrane. The plasma membrane frequently has an important role in improving oxidative stress and particularly in tumors, the repair of the plasma membrane is dysfunctional (48-50). Consequently, the above evidence is consistent with the present results.

In the present study, 13 DEGs were selected as the central genes with a degree of connectivity of ≥10. Among these central genes, PDGFRA had the highest nodal degree (37). After extensive study of the literature, it was indicated that PDGFRA has an important role in wound healing and the occurrence and development of tumors. The gene mutation is obviously related to familial gastrointestinal stromal tumors and other cancers (51,52). Therefore, it may be considered a target for anticancer drugs, such as imatinib (53). In the present study, the PPI network analysis suggested that PDGFRA directly interacted with ENPP3, PTPRC, TGFBI, BOC and CRYAB, indicating the key role of PDGFRA in BC. This gene encodes a cell surface tyrosine kinase receptor for members of the PDGF family. These growth factors are mitogens for cells of mesenchymal origin. The identity of the growth factor bound to a receptor monomer determines whether the functional receptor is a homodimer or a heterodimer, composed of both PDGFRA and PDGFRB polypeptides. CRYAB is a ferroptosis-related gene and its high expression may lead to poor prognosis of gastric cancer and non-small cell lung cancer (54,55). It was reported that the mutation of PDGFRA is also related to gastric cancer (51). Therefore, it may be speculated that PDGFRA and CRYAB have a synergistic effect and high expression of PDGFRA and CRYAB may lead to poor prognosis of BC (51). Of note, these results are consistent with the present RT-qPCR results. Furthermore, in the present study, it was indicated that certain genes have a trend of gradual increase with the progression of the tumor stage, such as PTPRC, PDGFRA, KLRD1, ADAP2 and ITGA4.

ENPP3 is a molecular therapeutic target for renal cell carcinoma. It is expressed in renal tubules, activated basophils and mast cells. In cancer, ENPP3 is expressed in most

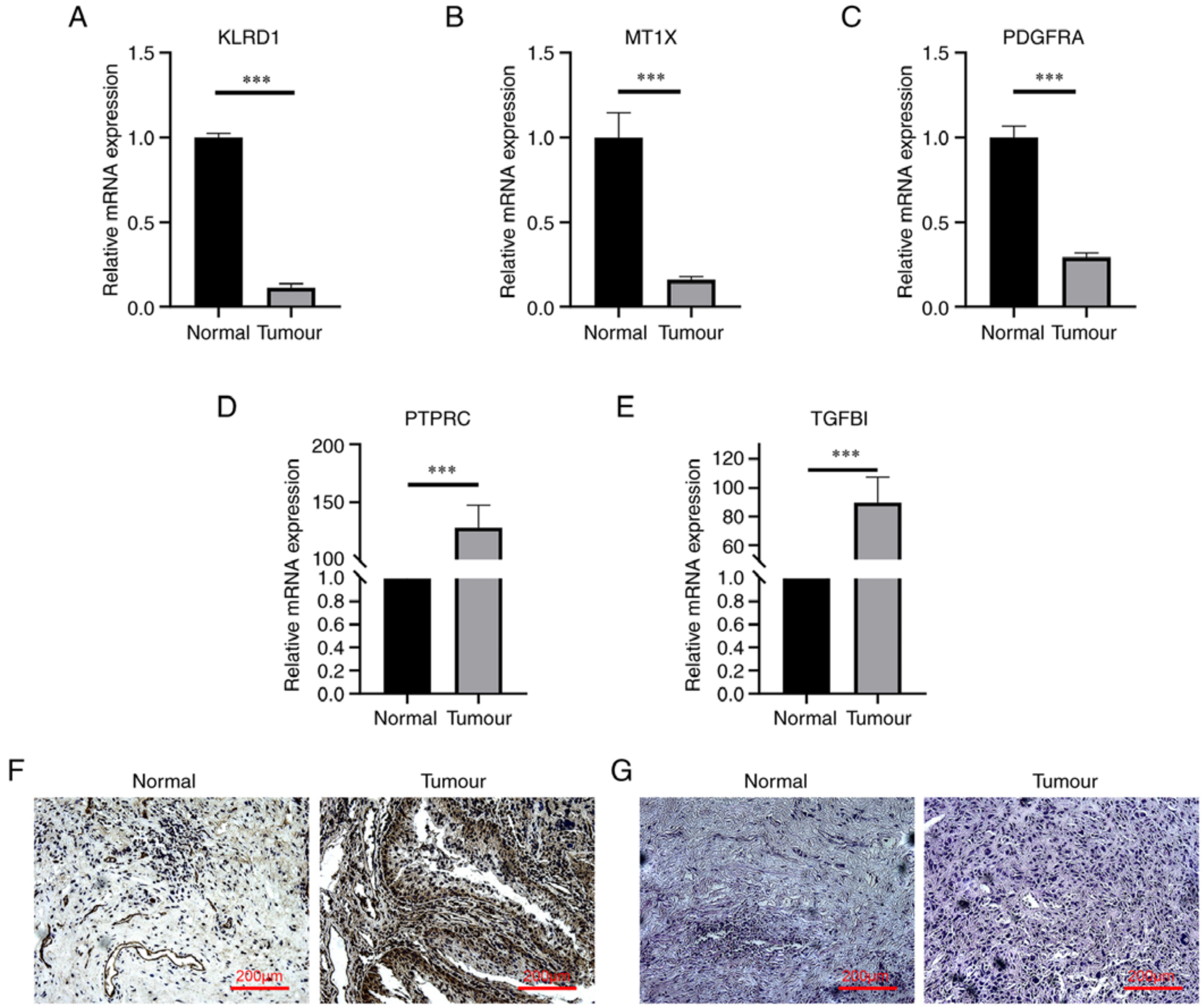

Figure 6. RT-qPCR was used to verify Hub genes. RT-qPCR was used to verify the expression of the differential genes (A) KLRD1, (B) MT1X, (C) PDGFRA, (D) PTPRC and (E) TGFBI in BC and normal tissues. (F) Immunohistochemistry was used to verify the expression of TGFBI in BC and normal tissues (scale bars, 200 μm). (G) H&E staining was used to compare the difference between BC and normal tissues (scale bars, 200 μm). ***P<0.001. BC, bladder cancer.

clear-cell histologies (94%), such as bladder tissue and kidney tissue. However, it still requires to be proven whether ENPP3 may be used as a molecular therapeutic target for BC (56-58). As expected, BOC has been reported in numerous tumor-related publications. BOC is highly expressed in early BC. It promotes a high level of DNA damage by increasing Sonic hedgehog signal transduction and ultimately affects the occurrence and development of BC (59). PRPC (CD45, leukocyte antigen) is a receptor-like protein tyrosine phosphatase expressed in all leukocytes. There are several glycoprotein isoforms, which are the result of the alternative splicing of exons 4, 5 and 6 (also known as A, B and C) of CD45 pre-mRNA, which has been reported to be associated with ovarian cancer (60). The TLR1 protein is a member of the TLR family. High expression of TLR1 has been found to have a significant correlation with the occurrence of gastric cancer (61). This gene can affect tumor promotion (such as pro-inflammatory, angiogenesis, and anti-apoptosis) or anti-tumor immunity (62). A recent study of CASQ2 found that CASQ2 is a conventional marker of leiomyosarcoma (63). TGFBI may be induced by human adenocarcinoma cells and secreted TGF-β (64). Previous reports have revealed that TGFBI acts as a tumor suppressor gene in various tumor types, including lung and breast cancer (65-67). The expression of MT1X changes in oral cancer and may predict cancer metastasis and the treatment effect in patients (68). The genes identified in the present study provide predictive markers for clinicians to diagnose BC in the future and provide directions for experimental research. These diagnostic markers still require to be experimentally verified.

In conclusion, the present study set out to identify DEGs that may be associated with BC. A total of 13 hub genes were identified and through various bioinformatics analyses, these genes were determined to serve as potential diagnostic markers of BC; however, the biological function of these genes in BC still requires further investigation.

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Availability of data and materials

TCGA mRNA expression and clinical data were downloaded from the TCGA public database (https://portal.gdc.cancer.gov/) and the GEO mRNA expression and clinical data were downloaded from the GEO public database (https://www.ncbi.nlm.nih.gov/geo/).

Authors' contributions

LW and XY completed the experiments. BS and LL were involved in the study conception and design. BS and LW performed the bioinformatics analysis. LL and XY wrote and edited the manuscript. BS and LL checked and confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Written informed consent was provided by the three patients who donated their BC tissues. Ethical review was performed and the protocol was approved by the ethics committee of Xinjiang Medical University (Urumqi, China; review no. XJYKDXR20220106001).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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