Original Article

Identification of hub genes associated with bladder cancer using bioinformatic analyses

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Background: Bladder cancer (BLCA) is the ninth most common cancer worldwide, with high mortality and recurrence rates. Studies have increasingly reported that molecular diagnosis contributes to the early diagnosis and prognostic assessment of diseases. Thus, this study aims to find new biomarkers for the diagnosis and prognosis of BLCA.

Methods: The microarray datasets GSE147983 and The Cancer Genome Atlas (TCGA)-BLCA mRNA were obtained from the Gene Expression Omnibus (GEO) and TCGA. Differentially expressed genes (DEGs) were screened using the R "Limma" package. The "ClusterProfiler" package was used to conduct Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the DEGs. A DEG protein–protein interaction (PPI) network was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING) database and visualized using Cytoscape. The functional module was reanalyzed using Cytoscape's Molecular Complex Detection ("MCODE") plugin, and key genes related to BLCA were identified via the "cytoHubba" plugin. Gene Expression Profiling Interactive Analysis 2 (GEPIA2) and the Tumor Immune Estimation Resource (TIMER) were used to verify the correlation between hub gene expression and immunity. A survival analysis of hub genes was performed using the Kaplan–Meier Plotter online tool.

Results: A total of 355 DEGs were screened out, including 236 upregulated and 119 downregulated DEGs. Some of the GO terms and pathways, such as chromosome separation, cell cycle, and cell senescence, were found to be significantly enriched in the DEGs. The key genes were kinesin family member 11 (*KIF11*), DLG associated protein 5 (*DLGAP5*), non-SMC condensin I complex subunit G (*NCAPG*), cell division cycle 20 (*CDC20*), cyclin B2 (*CCNB2*), BUB1 mitotic checkpoint serine (*BUB1B*), TPX2 microtubule nucleation factor (*TPX2*), NUF2 component of NDC80 kinetochore complex (*NUF2*), kinesin family member 2C (*KIF2C*), and cyclin B1 (*CCNB1*). Nine of them were immune-related, including *KIF11*, *DLGAP5*, *NCAPG*, *CDC20*, *CCNB2*, *BUB1B*, *NUF2*, *KIF2C*, and *CCNB1*. Survival analysis showed that the overexpression of BUB1B, CCNB1, CDC20, and DLGAP5 significantly reduced overall survival (OS) in patients with BLCA.

Conclusions: This study provided a theoretical basis for elucidating the pathogenesis and evaluating the prognosis of BLCA by screening potential biomarkers of BLCA.

Keywords: Bladder cancer (BLCA); The Cancer Genome Atlas (TCGA); bioinformatics; immunological analysis

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Introduction

Bladder cancer (BLCA) is a major economic burden on society and the ninth most common cancer worldwide, with more than 500,000 new diagnoses and 200,000 deaths annually worldwide (1,2). The disease is more common in men, with a male-to-female ratio of 3:1, and disproportionately affects older adults, with a median age of 69 years for men and 71 years for women at diagnosis (3). Approximately 90–95% of BLCA cancers are urothelial cell carcinoma, while the remainder are non-urothelial tissue carcinoma (1,4). BLCA is associated with relapse and disease progression. The 5-year overall survival (OS) has been reported to be 90%, however, muscle invasive bladder cancer with high metastasis and a 5-year survival rate <50% (5). Unfortunately, there has been no significant progress in the treatment of BLCA in the last 30 years, and the disease is often diagnosed at an advanced stage (6). Thus, the diagnosis and treatment of BLCA requires improvement.

The combination of urine cytology and cystoscopy is the current gold standard for diagnosing BLCA (7). However, existing urine biomarkers are unreliable. Although cystoscopy is the most efficient and accurate diagnostic method, it is invasive and costly (8-10), and 5.5% of patients undergoing cystoscopy develop urinary tract infection (11). Therefore, there is an urgent need to discover new and reliable BLCA biomarkers.

The United States Food and Drug Administration (FDA) recently approved BTA stat (Polymedco), BTA TRAK (Polymedco), NMP22 enzyme-linked immunosorbent assay (Matritech), NMP22 BladderChek Test (Alere), uCyt (Scimedx), and UroVysion (Abbott Molecular) for use alongside cystoscopy for BLCA diagnosis and surveillance (12). However, the sensitivity and specificity of these reagents are lower than cystoscopy, and these biomarkers are typically used in combination with cystoscopy (11,13).

In this study, The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) databases were searched for potential BLCA diagnostic and therapeutic targets. Ten genes were revealed as potential BLCA biomarkers, including kinesin family member 11 (*KIF11*), DLG associated protein 5 (*DLGAP5*), non-SMC condensin I complex subunit G (*NCAPG*), cell division cycle 20 (*CDC20*), cyclin B2 (*CCNB2*), BUB1 mitotic checkpoint serine (*BUB1B*), TPX2 microtubule nucleation factor (*TPX2*), NUF2 component of NDC80 kinetochore complex (*NUF2*), kinesin family member 2C (*KIF2C*), and cyclin B1 (*CCNB1*). These markers were associated with the prognosis of patients with BLCA, and 9 were associated with immunity, including *KIF11*, *DLGAP5*, *NCAPG*, *CDC20*, *CCNB2*, *BUB1B*, *NUF2*, *KIF2C*, and *CCNB1*. Four of these genes (*BUB1B*, *CCNB1*, *CDC20*, and *DLGAP5*) were identified as prognostic predictors and novel therapeutic targets for patients with BLCA.

We present the following article in accordance with the STREGA reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1004/rc).

Methods

Microarray data

BLCA RNA sequence data and clinical information were downloaded from the GEO and TCGA databases. Hence, ethics committee approval or consent procedure was not required. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

The GSE147983 [GPL20301, Illumina HiSeq 4000 (Homo sapiens)] dataset containing 4 control and 4 BLCA tissue samples was obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The TCGA-BLCA mRNA dataset containing 19 normal and 414 tumor samples and related clinical data was downloaded from TCGA (https://portal.gdc.cancer.gov/).

Data processing

The online software GEO2R was used to analyze the differentially expressed genes (DEGs) in the GSE147983 dataset. The TCGA-BLCA mRNA dataset was processed using the "Limma" package in R version 4.0.4 (64 bit; The R Foundation for Statistical Computing, Vienna, Austria) (14). $P<0.05$ and $|Log FC| \geq 2$ were used as the cutoff criteria for BLCA-mRNA. Volcano maps were drawn using the "ggplot2" package in R, with $|log FC| \geq 1$ and $P<0.05$ as the screening criteria. The online tool Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to identify the overlapping DEGs in the 2 gene expression microarrays and determine upregulated and downregulated genes.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

To better understand the role of DEGs in biological

processes and signal transduction, the "clusterprofiler" R package was used to conduct GO and KEGG pathway analysis (15). P<0.05 was considered statistically significant.

Protein–protein interaction (PPI) network analysis

To further elucidate the molecular mechanisms of BLCA, the Search Tool for the Retrieval of Interacting Genes (STRING) online database (https://string-db.org/) was used to construct a DEG interaction network (16). The relationship between DEGs was then visualized using Cytoscape 3.7.2 software (17). The Cytoscape plugin app Molecular Complex Detection ("MCODE") was also used to reanalyze the clusters in the network according to the following parameters: degree cutoff =2, node score cutoff =0.2, k-core =2, max.depth =100. The top 2 modules were selected, and the top 10 hub genes were screened using the Cytoscape plugin "cytoHubba".

Gene expression analysis

The Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (http://gepia2.cancer-pku.cn/#index) (18) database was used to visualize hub gene expression in BLCA and paracarcinoma tissues. P<0.05 was considered statistically significant.

Genomic alteration of the 10 hub genes using the cBioPortal database

The cBioPortal database (https://www.cbioportal.org/) was used to study the genomic mutations of the 10 hub genes in BLCA. Genomic alteration types and alteration frequency in BLCA were analyzed (19,20). The genomic alterations of the 10 hub genes contained missense/splice/truncating mutations with unknown significance, deep deletion, and amplification.

Immune infiltration in BLCA with different somatic copy number alterations (SCNAs)

SCNA analysis of the 10 hub genes was conducted using the Tumor Immune Estimation Resource (TIMER; https://cistrome.shinyapps.io/timer/) (21). The "SCNA" module was used to compare the tumor infiltration levels among tumors with different SCNAs for the 10 hub genes. In TIMER, SCNAs are categorized into 5 groups by the Genomic Identification of Significant Targets in Cancer (GISTIC) version 2.0, including deep deletion [–2], arm-level deletion [–1], diploid/normal [0], arm-level gain [1], and high amplification [2]. Box plots were drawn to show the distributions of each immune subset at each copy number status in selected cancers. The infiltration level for each SCNA category was compared with the normal control using a two-sided Wilcoxon rank-sum test.

Survival analysis of the hub genes

A survival analysis of the hub genes was performed using the online Kaplan-Meier Plotter (http://kmplot.com/analysis/index.php?p=background) (22), which assesses the correlation between OS and different tumor genes.

Statistical analyses

The gene expression level and survival of BLCA were analyzed using GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA) and SPSS 22.0 (IBM Corp., Armonk, NY, USA). The data of the 2 groups were analyzed using a Student's *t*-test. The results are presented as mean ± standard deviation (SD). P<0.05 was considered a statistically significant difference.

Results

Identification of DEGs

Based on the inclusion criteria, 1,403 and 4,597 DEGs were extracted from the GSE147983 and TCGA-BLCA mRNA datasets using the R "Limma" package and visualized using volcano plots, respectively (*Figure 1A*,*1B*). Overlapping DEGs were identified via a Venn diagram. Compared with the normal bladder tissues, 355 common DEGs were discovered in the BLCA tissues, including 236 upregulated genes (P<0.05, log2FC ≥2) and 119 downregulated genes (P<0.05, log2FC ≤–2; *Figure 1C*).

GO and KEGG pathway analyses of DEGs

The R "ClusterProfiler" package was used to annotate and enrich the GO and KEGG pathways for the DEGs. The top 3 biological processes, cellular components, and molecule functions in the upregulated DEGs were muscle system process, regulation of muscle system process, and muscle contraction; myofibril, sarcomere, and contractile fiber; and heparin-binding, actin binding,

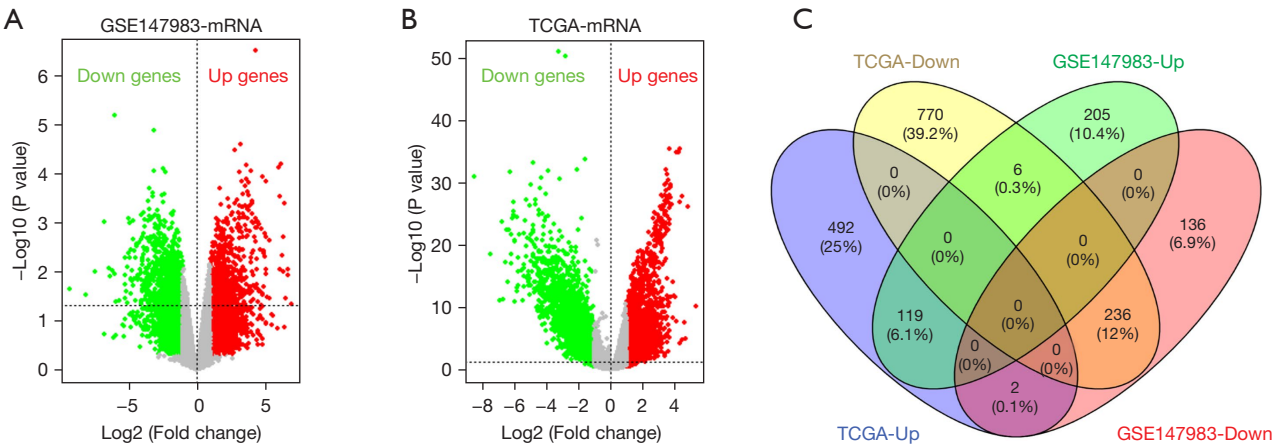

Figure 1 The identification of DEGs in the GSE147983 and TCGA-BLCA datasets. (A,B) Volcano plots of DEGs in BLCA based on the GSE147983 and TCGA-BLCA datasets; (C) overlapping genes between GSE147983 and TCGA-BLCA. DEGs, differentially expressed genes; TCGA, The Cancer Genome Atlas; BLCA, bladder cancer.

and glycosaminoglycan binding, respectively. The top 3 biological processes, cellular components, and molecule functions in the downregulated DEGs were enriched in chromosome segregation, nuclear division, and organelle fission; chromosome—centromeric region, chromosomal region, and kinetochore; and microtubule binding, tubulin binding, and microtubule motor activity, respectively (*Figure 2A*,*2B*). The top 3 KEGG pathways of the upregulated DEGs were hypertrophic cardiomyopathy, dilated cardiomyopathy, and vascular smooth muscle contraction, and the top 3 KEGG pathways of the downregulated DEGs were cell cycle, oocyte meiosis, and cellular senescence (*Figure 2C*,*2D*).

PPI network analysis and hub genes screening

The 355 overlapping DEGs were imported into the STRING database for PPI network analysis (*Figure 3*), and the hub genes inside the network were screened using Cytoscape 3.7.2. The top 10 genes with the highest degree of connectivity were regarded as the hub genes (*Figure 4A*). To further explore the associations within the PPI network, the top 2 modules inside the PPI network were extracted using the "MCODE" package in Cytoscape (*Figure 4B*,*4C*).

Expression levels of the 10 hub genes in BLCA

To assess the expression levels of the hub genes in BLCA tissues, GEPIA2 was used to determine their expressions. The expression of *BUB1B*, *CCNB1*, *CCNB2*, *CDC20*, *DLGAP5*, *KIF2C*, *KIF11*, *NCAPG*, *NUF2*, and *TPX2* was significantly increased in tumor tissues compared to that in the normal control, which was consistent with previous results (*Figure 5*).

Genetic alteration analysis of the 10 hub genes

Genomic mutations are closely related to tumorigenesis. Hence, the genomic mutations of the top 10 hub genes in BLCA were analyzed. The results showed that approximately 2.1%, 2.3%, 1.3%, 4%, 1%, 3%, 7%, 13%, 2.8%, and 2.4% of genetic alterations were presents in *KIF11*, *DLGAP5*, *NCAPG*, *CDC20*, *CCNB2*, *BUB1B*, *TPX2*, *NUF2*, *KIF2C*, and *CCNB1*, respectively, in BLCA, including missense/splice/truncating mutations/structural variants with unknown significance, amplification, and deep deletion (*Figure 6A*). Moreover, the genetic alteration type and frequency of the 10 hub genes showed significant differences in BLCA (*Figure 6B*), indicating that the genetic alterations of the 10 hub genes could play an important role in the tumorigenesis of BLCA.

The association of the SCNAs of the 10 hub genes with immune infiltration

The importance of immune surveillance in determining the prognosis of various types of cancers is widely accepted. To further explore the relationship between the genomic metrics of the 10 hub genes and the extent of immune infiltration in BLCA, the SCNAs of the 10 hub genes

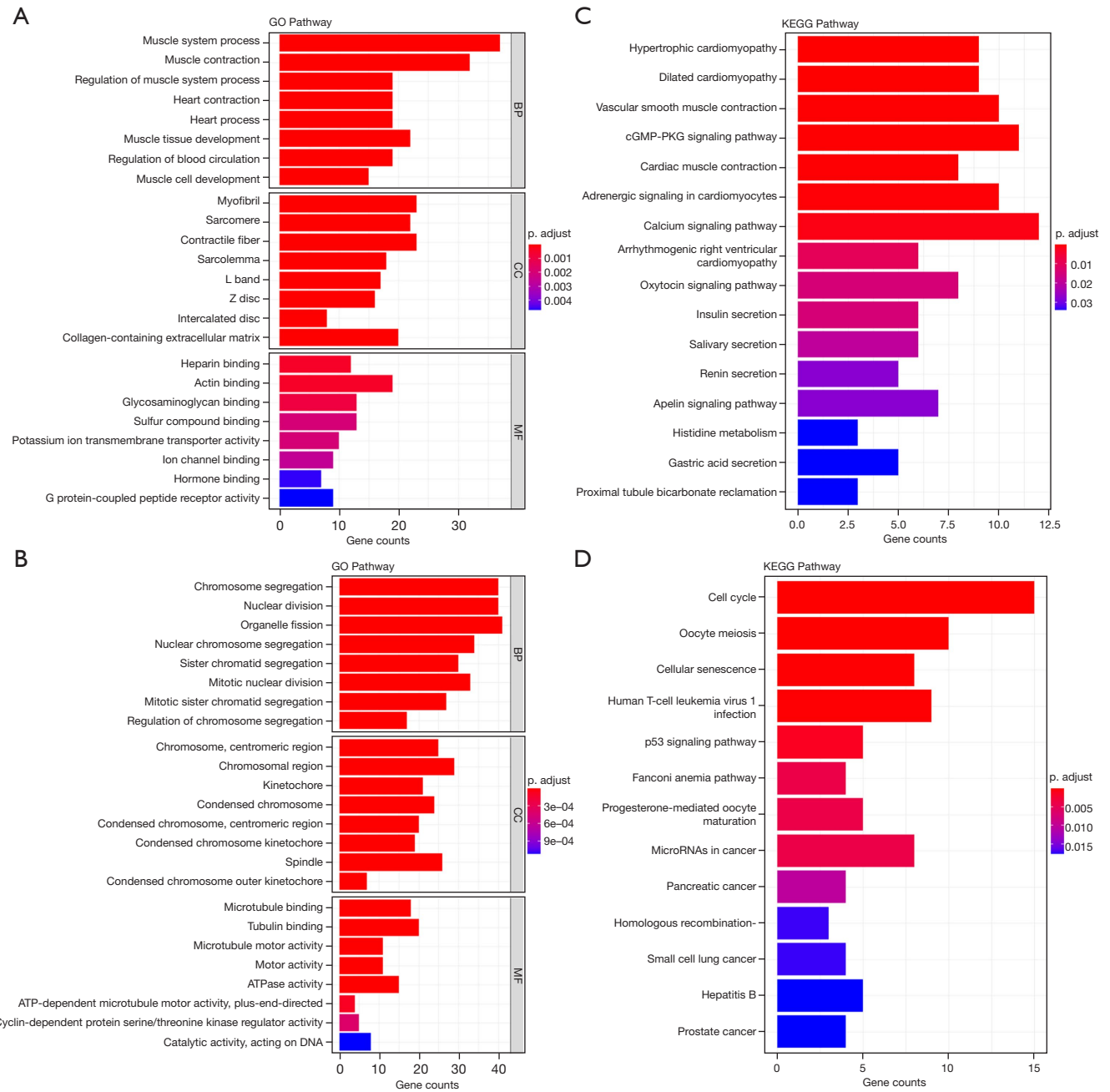

Figure 2 GO and KEGG pathway analysis. (A,B) The GO enriched pathways of the upregulated and downregulated DEGs; (C,D) the KEGG pathways of the upregulated and downregulated DEGs. P<0.05 was considered significantly enriched. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function; DEG, differentially expressed gene.

were defined using GISTIC 2.0 in TIMER. The results showed that immune cell enrichment was significantly different in BLCA with different gene SCNAs (*Figure* 7). Furthermore, BLCA with the SCNA of *BUB1B* showed decreased cytotoxic T cell ($CD8^+$ T), T helper cell ($CD4^+$ T), macrophage, neutrophil, and dendritic cell enrichment (*Figure* 7*A*); *CCNB1* decreased $CD8^+$ T cell and neutrophil cell enrichment (*Figure* 7*B*); *CCNB2* decreased dendritic

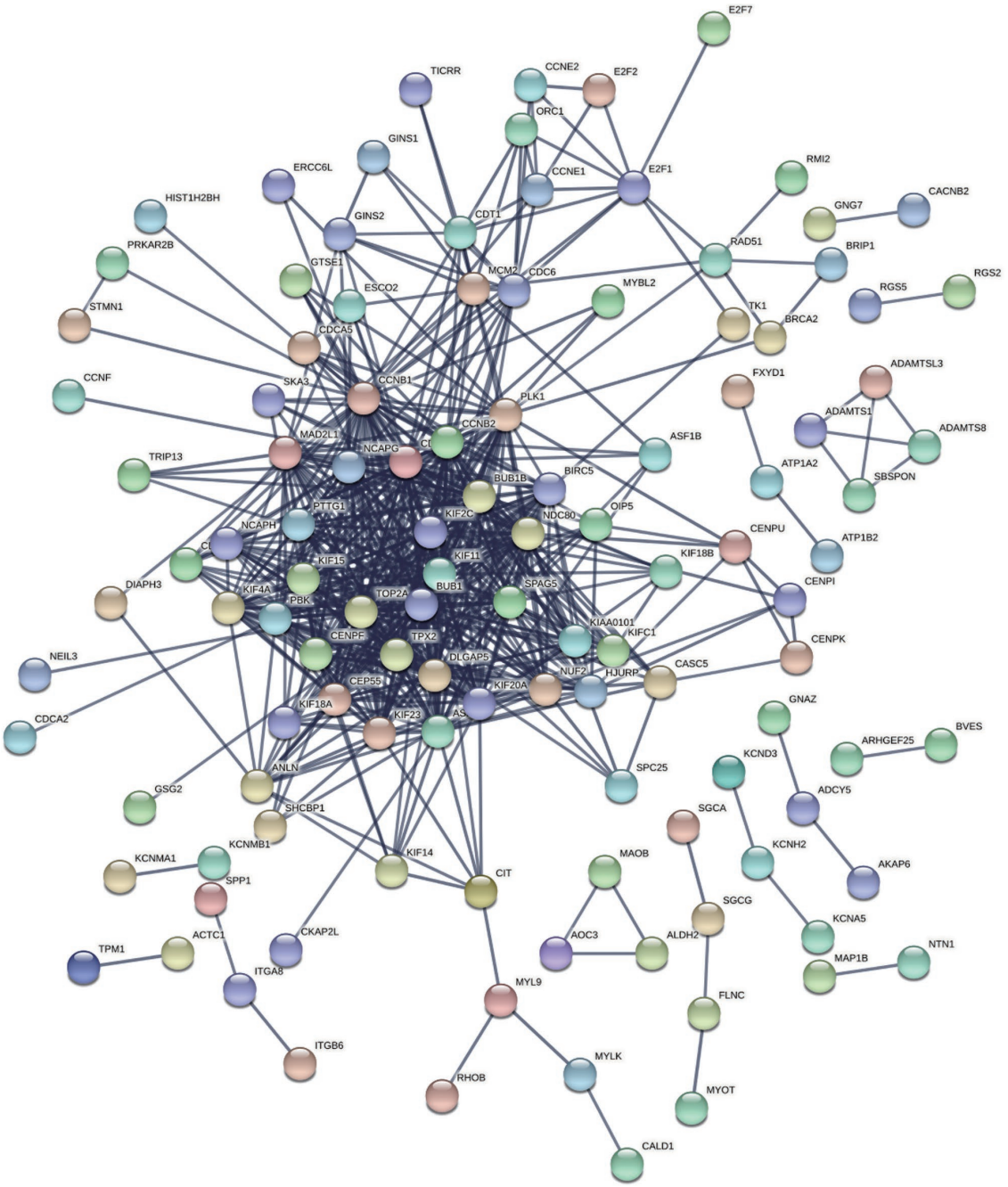

Figure 3 PPI networks of DEGs and the hub genes. PPI, protein–protein interaction; DEG, differentially expressed gene.

cell enrichment (*Figure 7C*); *CDC20* decreased CD8$^+$ T cell, CD4$^+$ T cell, and dendritic cell enrichment (*Figure 7D*); *DLGAP5* decreased B cell, CD8$^+$ T cell, CD4$^+$ T cell, neutrophil, and dendritic cell enrichment (*Figure 7E*); *KIF2C* decreased B cell, CD4$^+$ T cell, macrophage, and dendritic cell enrichment (*Figure 7F*); *KIF11* decreased enrichment in all 6 immune cell types (*Figure 7G*); *NCAPG* decreased B cell, CD4$^+$ T cell, and neutrophil cell enrichment (*Figure 7H*); and *NUF2* decreased CD4$^+$ T cell, neutrophil, and dendritic cell enrichment (*Figure 7I*). However, the immune cell enrichment showed no difference in BLCA with the SCNA of *TPX2* (*Figure 7J*). Therefore,

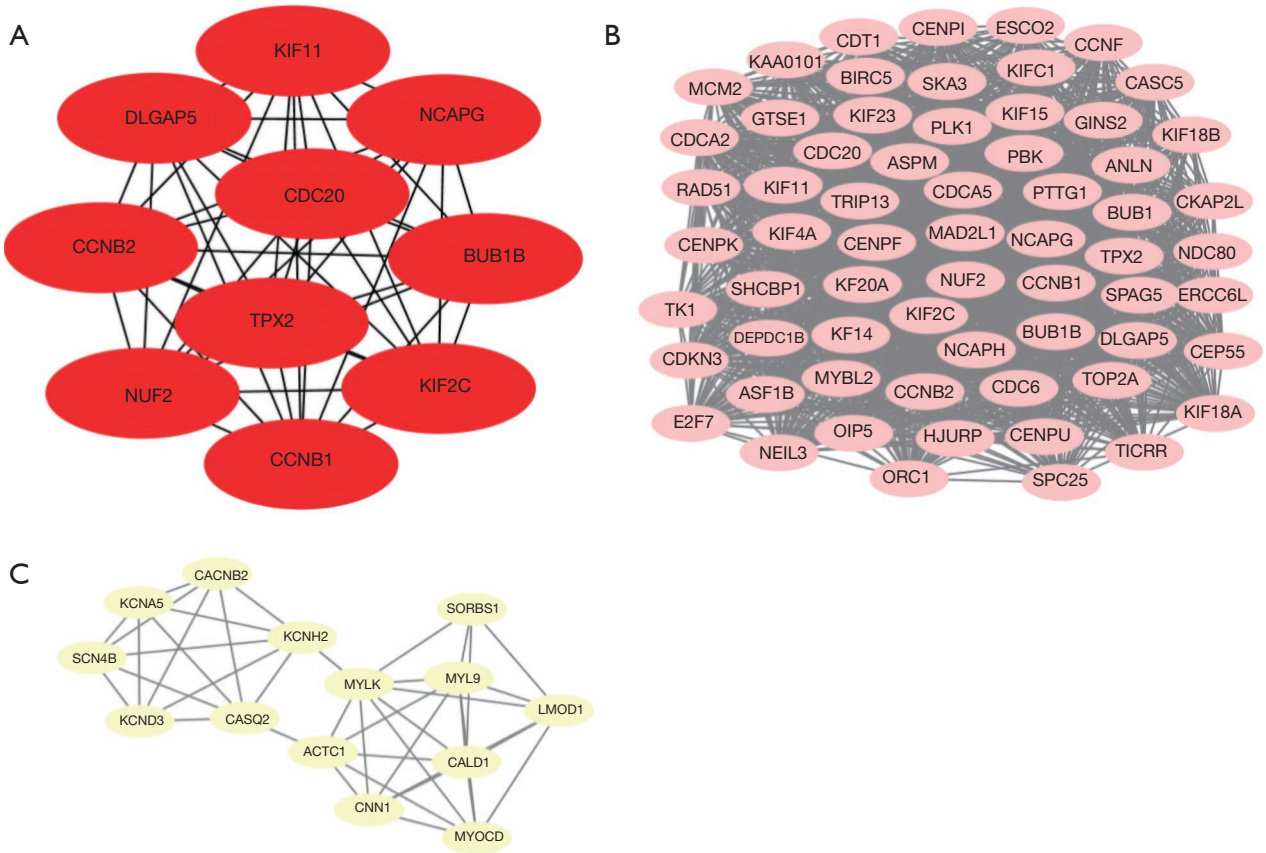

Figure 4 PPI network of DEGs. (A) PPI network of 10 hub genes identified using cytoHubba; (B,C) the top 2 modules inside the PPI network were identified using MCODE. PPI, protein–protein interaction; DEG, differentially expressed gene; MCODE, Molecular Complex Detection.

the genomic alterations of the 10 hub genes were strongly correlated with the extent of immune infiltration in BLCA.

Kaplan-Meier analysis of the hub genes

The clinical significance of the hub genes was analyzed using the Kaplan-Meier Plotter online software. The results showed that patients with higher *BUB1B*, and *CCNB1* expression levels had worse OS (P<0.05, *Figure 8A*,*8B*), while CCNB2 had better OS (P>0.05, *Figure 8C*). High *CDC20*, and *DLGAP5* expression levels had worse OS (P<0.05, *Figure 8D*,*8E*), whereas a significant change was not observed in the rest genes (*Figure 8F-8J*).

Discussion

BLCA is the ninth most common cancer worldwide. Its incidence varies by region, with more than 60% of cases occurring in less developed countries (23). Approximately 75% of patients are diagnosed with non-muscle-invasive BLCA, while the remainder have already progressed to the muscle-invasive stage at diagnosis (24). Although new diagnostic and treatment strategies have recently been developed, only a minimal improvement in the clinical efficacy of these strategies has been reported (25). Therefore, identifying new diagnostic markers, therapeutic targets, and treatment methods remains crucial to the diagnosis and treatment of BCLA.

This study used the GSE147983 and TCGA-BLCA mRNA datasets from the GEO and TCGA databases to conduct a comparative analysis of the DEGs in BLCA tissues and normal controls. *BUB1B*, *CCNB1*, *CCNB2*, *CDC20*, *DLGAP5*, *KIF2C*, *KIF11*, *NCAPG*, *NUF2*, and *TPX2* were found to be highly expressed in the BLCA tissues, suggesting their potential as diagnostic biomarkers for BLCA. Additionally, *BUB1B*, *CCNB1*, *CCNB2*, *CDC20*,

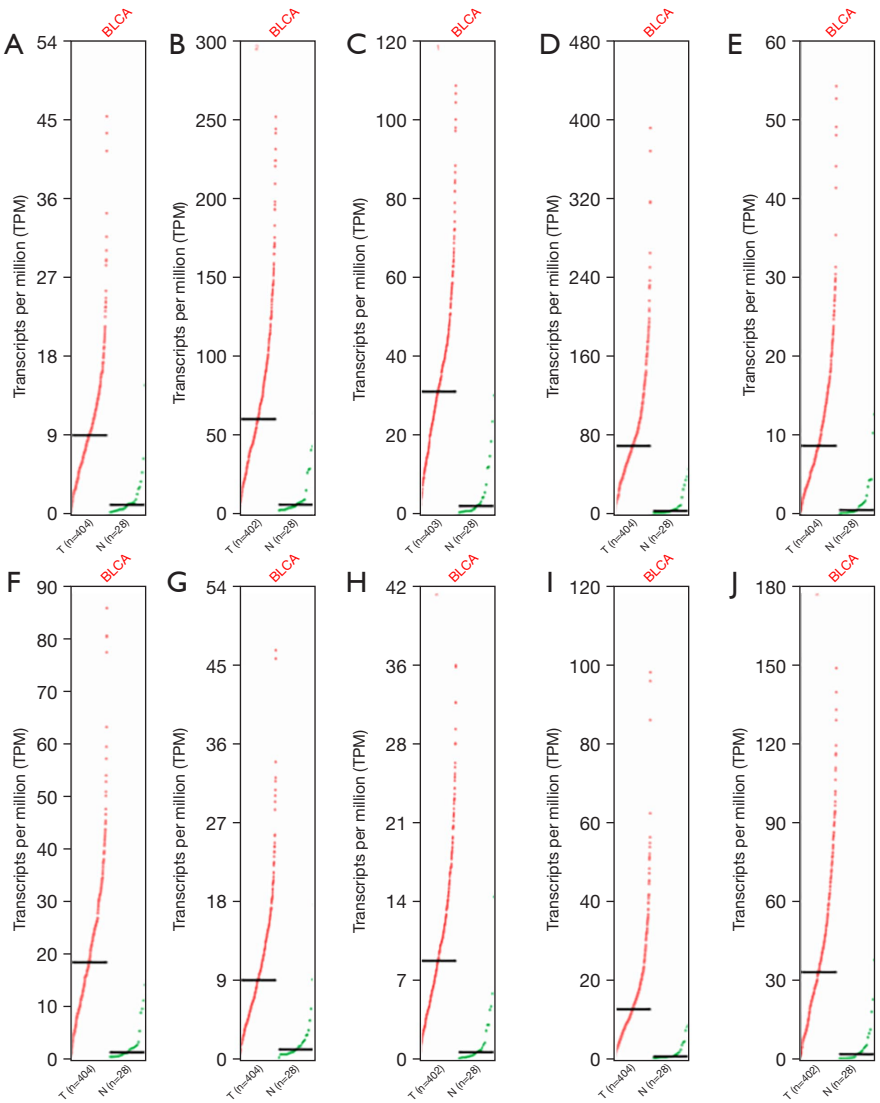

Figure 5 Expression levels of (A) *BUB1B*, (B) *CCNB1*, (C) *CCNB2*, (D) *CDC20*, (E) *DLGAP5*, (F) *KIF2C*, (G) *KIF11*, (H) *NCAPG*, (I) *NUF2*, and (J) *TPX2* in BLCA tissues *vs.* normal control. BLCA, bladder cancer; BUB1B, BUB1 mitotic checkpoint serine; CCNB1, cyclin B1; CCNB2, cyclin B2; CDC20, cell division cycle 20; DLGAP5, DLG associated protein 5; KIF2C, kinesin family member 2C; KIF11, kinesin family member 11; NCAPG, non-SMC condensin I complex subunit G; NUF2, NUF2 component of NDC80 kinetochore complex; TPX2, TPX2 microtubule nucleation factor.

DLGAP5, *KIF2C*, *KIF11*, *NCAPG*, and *NUF2* were found to be associated with immune cell infiltration. The high expression of *BUB1B*, *CCNB1*, *CDC20*, and *DLGAP5* was associated with worse OS in BLCA and could thus be considered an independent prognostic indicator for BLCA. These findings aid in identifying new diagnostic methods and treatment targets for BLCA, which could improve the prognosis of patients with BLCA.

BUB1B encodes a kinase involved in the spindle checkpoint function. Impaired spindle checkpoint function has been reported in many cancer types (26,27). Previous studies have reported that *BUB1B* is upregulated in BLCA (28,29), which is consistent with the results of this study. However, the immune role of *BUB1B* in BLCA remains undefined. Furthermore, *BUB1B* has been associated with immune infiltration in various tumors, such as prostate cancer, papillary renal cell carcinoma, and hepatocellular carcinoma (HCC) (30-32). In our immune infiltration

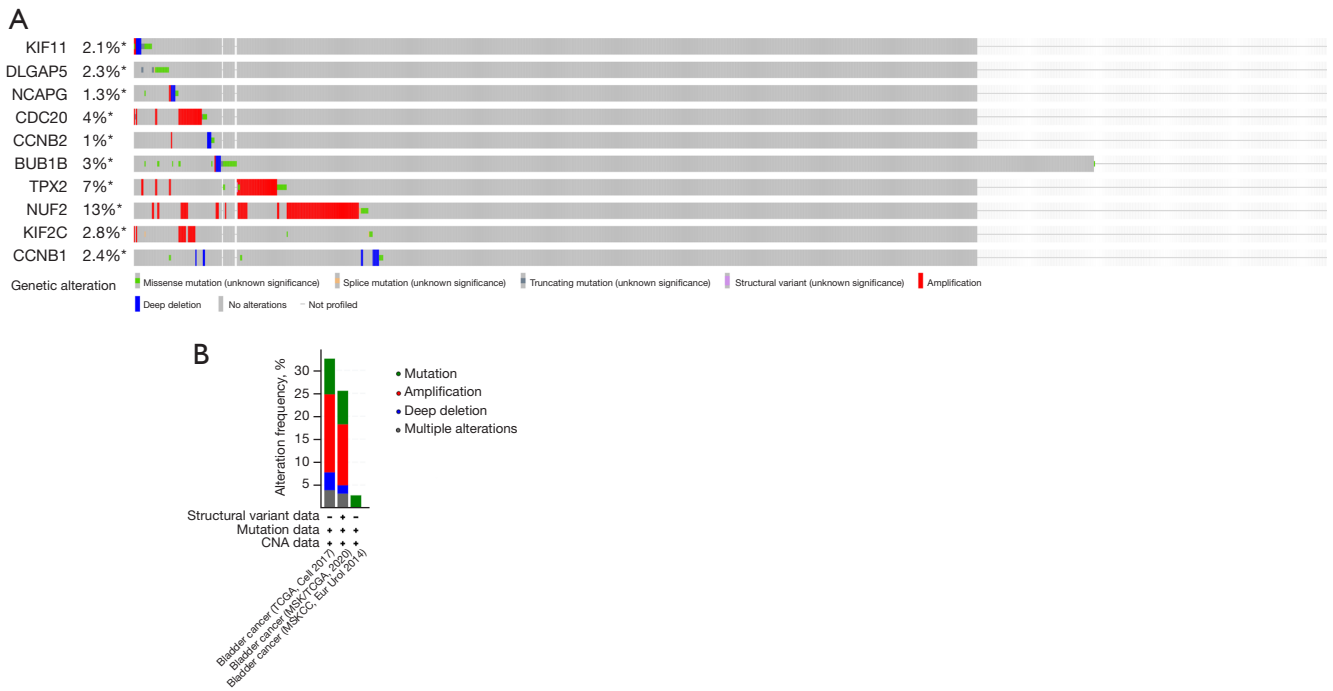

Figure 6 Genomic mutation of the 10 hub genes in BLCA using the cBioPortal database. (A) The genetic alterations of the 10 hub genes in BLCA, including missense/splice/truncating mutations/structural variants with unknown significance, amplification, and deep deletion, were analyzed. (B) The genetic alteration type and frequency of the 10 hub genes were studied in BLCA. Color images are available online. BLCA, bladder cancer; CNA, copy-number alterations.

analyses, *BUB1B* reduced $CD8^+$ T cell, $CD4^+$ T cell, macrophage, neutrophil, and dendritic cell enrichment, which validated the association of *BUB1B* with immune infiltration in BLCA.

CCNB1 and *CCNB2* are important components of the cyclin pathway and play a key role in the occurrence and development of cancer (33). *CCNB1* is involved in tumorigenesis and tumor development (34). Egloff *et al.* eluted *CCNB1*-derived peptides from major histocompatibility complex (MHC) class I molecules on tumor cells and revealed that this constitutively overexpressed protein was naturally processed into peptides that bind to MHC class I molecules and stimulate $CD8^+$ T cells (35). In our study, *CCNB1* decreased $CD8^+$ T cell and neutrophil cell enrichment, confirming the association of *CCNB1* with immune infiltration in BLCA. *CCNB2* overexpression has been reported to be related to poor prognosis in HCC (36) and to promote invasion and metastasis in BLCA (37). Ni *et al.* found that *CCNB2* could act as a biomarker and potential target for lung cancer treatment (38), while Xia *et al.* and Zou *et al.* reported the potential of *CCNB2* as a prognostic biomarker and its association with immune cell infiltration in HCC and breast cancer (39,40). In our study, *CCNB2* was highly expressed in BLCA and was associated with decreased dendritic cell enrichment, which suggested that *CCNB2* could affect the immune process of BLCA.

Various studies have reported the association of *CDC20* with the occurrence and development of different tumors, including BLCA (41). In most cancer types, *CDC20* expression is positively correlated with the infiltration of cancer-associated fibroblasts and myeloid-derived suppressor cells (41). Our analysis of the relationship between *CDC20* and tumor immunity revealed that *CDC20* decreased $CD8^+$ T cell, $CD4^+$ T cell, and dendritic cell enrichment in BLCA.

DLGAP5 protein, also known as hepatoma upregulated protein (HURP) or KIAA0008, was first identified as a cell cycle–regulated protein (42). Various studies have focused on the role of *DLGAP5* in the tumorigenesis of liver cancer (43), pancreatic cancer (44), lung cancer (45), and ovarian cancer (46). However, its role in BLCA remains unexplored. In this study, *DLGAP5* was highly expressed in BLCA, and the correlation between *DLGAP5* and B cell,

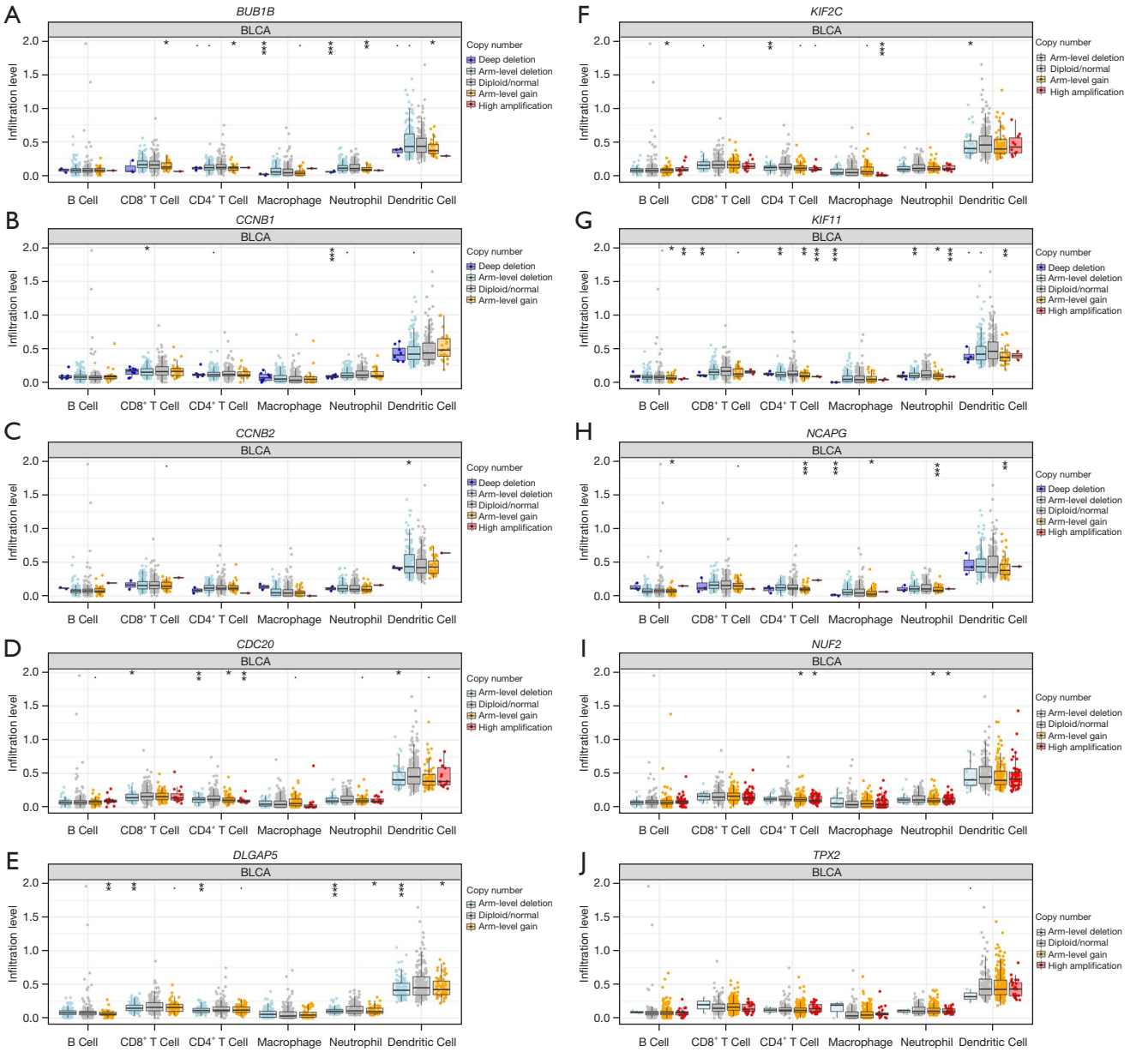

Figure 7 The association between the SCNAs of the 10 hub genes and immune cell infiltration in BLCA using GISTIC 2.0 in TIMER. (A) *BUB1B*, (B) *CCNB1*, (C) *CCNB2*, (D) *CDC20*, (E) *DLGAP5*, (F) *KIF2C*, (G) *KIF11*, (H) *NCAPG*, (I) *NUF2*, and (J) *TPX2*. In the genomic datasets, the SCNAs of the 10 hub genes were divided into 5 types: deep deletion, arm-level deletion, diploid/normal, arm-level gain, and high amplification. The infiltration of 6 immune cell types, including B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophage cells, neutrophil cells, and dendritic cells, were analyzed in BLCA. Color images are available online. *P<0.05, **P<0.01, ***P<0.001. SCNA, somatic copy number alteration; BLCA, bladder cancer; BUB1B, BUB1 mitotic checkpoint serine; CCNB1, cyclin B1; CCNB2, cyclin B2; CDC20, cell division cycle 20; DLGAP5, DLG associated protein 5; KIF2C, kinesin family member 2C; KIF11, kinesin family member 11; NCAPG, non-SMC condensin I complex subunit G; NUF2, NUF2 component of NDC80 kinetochore complex; TPX2, TPX2 microtubule nucleation factor.

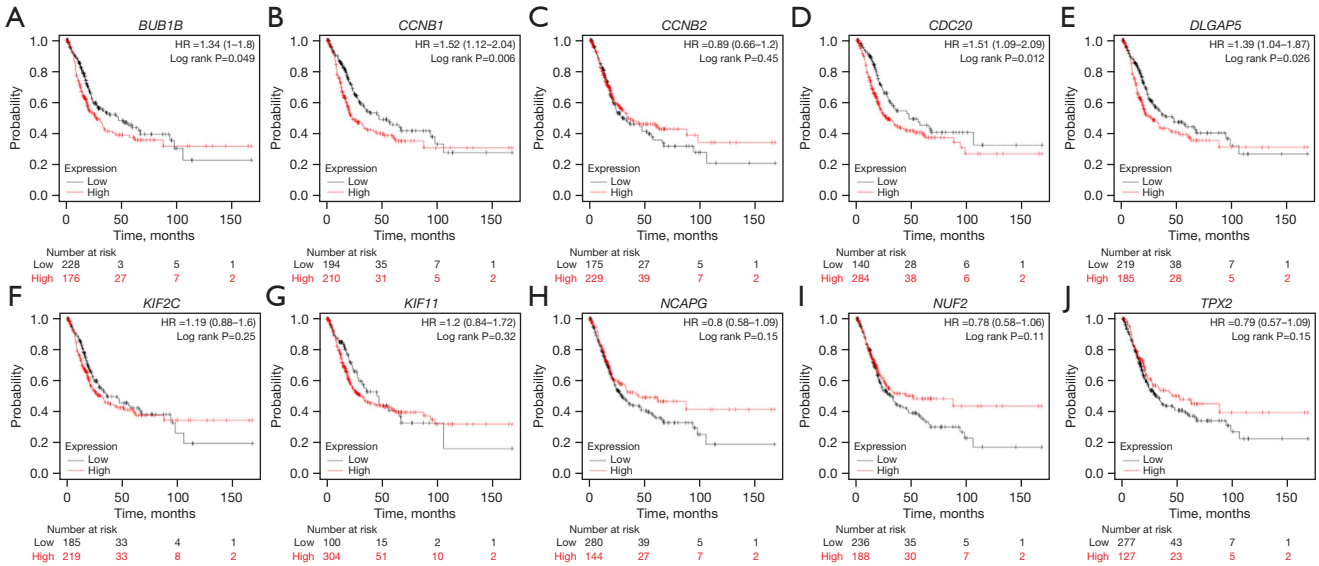

Figure 8 Kaplan-Meier analysis of the 10 hub genes: (A) *BUB1B*, (B) *CCNB1*, (C) *CCNB2*, (D) *CDC20*, (E) *DLGAP5*, (F) *KIF2C*, (G) *KIF11*, (H) *NCAPG*, (I) *NUF2*, and (J) *TPX2*. P<0.05 was considered statistically significant. BUB1B, BUB1 mitotic checkpoint serine; CCNB1, cyclin B1; CCNB2, cyclin B2; CDC20, cell division cycle 20; DLGAP5, DLG associated protein 5; KIF2C, kinesin family member 2C; KIF11, kinesin family member 11; NCAPG, non-SMC condensin I complex subunit G; NUF2, NUF2 component of NDC80 kinetochore complex; TPX2, TPX2 microtubule nucleation factor.

$CD8^+$ T cell, $CD4^+$ T cell, neutrophil, and dendritic cell enrichment was demonstrated.

KIF2C and *KIF11* belong to the kinesin family. The functions of *KIF2C* are related to the microtubule-dependent molecular motor and chromosome positioning processes, while the functions of *KIF11* are related to the centrosome separation and bipolar spindle establishment during cell mitosis processes (29). Their abnormal expression has been associated with the prognosis of BLCA (47,48). Additionally, *KIF2C* and *KIF11* have been reported to affect the immune microenvironment in some tumors (49,50), but their role in BLCA requires further elucidation. In the present study, *KIF2C* and *KIF11* were found to be associated with immune cell enrichment.

Reports on the role of *NUF2* and *NCAPG* in BLCA are scarce. This study revealed that *NUF2* and *NCAPG* play a critical role in BLCA diagnosis. Functional analysis showed that *NUF2* was associated with $CD4^+$ T cell, neutrophil, and dendritic cell enrichment in BLCA, while *NCAPG* was related to B cell, $CD4^+$ T cell, and neutrophil cell enrichment.

Previous trials have indicated that *TPX2* is associated with the metastasis and prognosis of BLCA (51,52). However, the present study observed that although *TPX2* was upregulated in BLCA tissues, higher expression levels were associated with better OS. Further validation of the expression status of *TPX2* revealed that *TPX2* expression was upregulated in BLCA. To date, only a few studies have reported the exact biological role of *TPX2* (51,52), and its potential mechanisms in the diagnosis, progression, and immunity of BLCA remain unclear.

Despite its advantages, this study has certain limitations, including the lack of *in vivo* and *in vitro* validations. Furthermore, although 10 hub genes were found to be upregulated in BLCA, the mechanism of upregulation was unclear. Therefore, further molecular studies are needed to determine the function of these central genes and their role in the progression of BLCA.

Conclusions

This study identified 10 potential biomarkers of BLCA, including *KIF11*, *DLGAP5*, *NCAPG*, *CDC20*, *CCNB2*, *BUB1B*, *TPX2*, *NUF2*, *KIF2C*, and *CCNB1*, which could be used as diagnostic indicators of BLCA. Nine of these genes were associated with immunity, including *KIF11*, *DLGAP5*, *NCAPG*, *CDC20*, *CCNB2*, *BUB1B*, *NUF2*, *KIF2C*, and *CCNB1*. Additionally, 4 of these genes (*BUB1B*,

CCNB1, *CDC20*, and *DLGAP5*) have the potential to be prognostic predictors and novel therapeutic targets for BLCA. However, further studies are required to validate these findings. Thus, this study provides a strong basis for the development of BLCA gene-targeted therapies.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1004/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1004/coif). XZ is from CheerLand Clinical Laboratory Co., Ltd. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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