Original Article

Identification of Potential Key Genes Linked to Gender Differences in Bladder Cancer Based on Gene Expression Omnibus (GEO) Database

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Abstract

Background: Growing evidence strongly indicates pivotal roles of gender differences in the occurrence and survival rate of patients with bladder cancer, with a higher incidence in males and poorer prognosis in females. Nevertheless, the molecular basis underlying gender-specific differences in bladder cancer remains unknown. The current study has tried to detect key genes contributing to gender differences in bladder cancer patients.

Materials and Methods: The gene expression profile of GSE13507 was firstly obtained from the Gene Expression Omnibus (GEO) database. Further, differentially expressed genes (DEGs) were screened between males and females using R software. Protein–protein interactive (PPI) network analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), and Kaplan–Meier survival analyses were also performed.

Results: We detected six hub genes contributing to gender differences in bladder cancer patients, containing IGF2, CCL5, ASPM, CDC20, BUB1B, and CCNB1. Our analyses demonstrated that CCNB1 and BUB1B were upregulated in tumor tissues of female subjects with bladder cancer. Other genes, such as IGF2 and CCL5, were associated with a poor outcome in male patients with bladder cancer. Additionally, three signaling pathways (focal adhesion, rheumatoid arthritis, and human T-cell leukemia virus infection) were identified to be differentially downregulated in bladder cancer versus normal samples in both genders.

Conclusion: Our findings suggested that gender differences may modulate the expression of key genes that contributed to bladder cancer occurrence and prognosis.

Keywords: Bioinformatics, bladder cancer, sex difference, survival analysis

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Introduction

Bladder cancer is ranked the tenth most frequent malignancy worldwide, with around 573,000 new cases and 213,000 deaths in 2020.[1] Several known modifiable risk factors such as tobacco smoking, dietary factors, environmental pollution, and occupational exposure to carcinogens, along with known non-modifiable risk factors such as race, age, gender, and genetic predisposition, are involved in the occurrence of

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bladder cancer.[2,3] According to the depth of tumor invasion, bladder cancer can pathologically be subdivided into two distinct categories, namely, non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC).[4] At present, NMIBC is the predominant category of bladder cancer, accounting for nearly 75% of new cases of this disease.[5] Cystoscopy and urine cytology are still the main tools to diagnose bladder cancer and postoperative follow-up. In 80% of diagnosed cases, NMIBC patients are conventionally treated by transurethral resection (TUR) of the bladder tumors. Although NMIBC generally has a favorable prognosis, a high recurrence rate after TUR is a significant challenge in managing bladder cancer patients. Approximately 25% of all newly diagnosed cases of NMIBC progress to invasive type or metastasis within five years after treatment.[6] Therefore, identifying diagnosis and treatment patterns in patients with bladder cancer is paramount to managing the disease.

An impressive character of bladder cancer is the effect of sexual disparity in disease occurrence and clinical outcomes. Indeed, the prevalence of bladder cancer in males is four times more than in female subjects.[7] However, women with bladder cancer present more aggressive disease and unfavorable clinical outcomes, including higher cancer recurrence rates and cancer-specific mortality.[8] It is suggested that differences in biological or social factors between genders might contribute to poor survival rates in females with bladder cancer; however, the molecular basis of sex-associated differences in bladder cancer needs more examination.[9,10]

Notably, multiple studies using bioinformatics analysis have screened several key genes and molecular mechanisms related to bladder cancer.[11,12] However, sex-biased genes and distinct pathways in bladder cancer remain obscure. Hence, the current study has tried to explore differences in the transcript levels of key genes and distinct pathways recognized among female and male subjects with bladder tumors using bioinformatic studies. High-throughput platforms and bioinformatic analyses are designed as a modern approach to investigate differentially expressed genes (DEGs) and distinct tumor-related pathways.[11,13] At present, there are few bioinformatic studies on all microarrays of cancerous and non-cancerous samples of bladder uploaded to tumor-related databases such as the Gene Expression Omnibus (GEO) database (available online: https://www.ncbi.nlm.nih.gov/geo). Therefore, in this survey, raw data for GSE13507 were extracted from the GEO database and analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, Gene Ontology (GO) analysis, protein–protein interaction (PPI) network, and Kaplan–Meier plotter survival analysis to recognize sex-affected genes and pathways related to tumorigenesis of bladder cancer.

Materials and Methods

Data source

We extracted an available gene expression profile (GSE13507) from the GEO database to recognize DEGs in bladder cancer. GSE13507 comprises 165 human bladder tumor tissue specimens (136 male and 29 female) and 51 normal bladder tissue samples (38 male and 13 female).

Data preprocessing and sex-associated DEGs screening

Initially, a raw dataset was preprocessed using the affy package in R. Then, a robust multi-array average (RMA) algorithm was employed for background adjustment, quantile normalization, and gene expression calculation.[14] DEGs between bladder tumor tissue and normal bladder tissue samples in females and males were calculated using the Linear Models for Microarray Data (LIMMA) package in R software. Genes with a $\log_2$ fold change (FC) value >1 and −1 and an adjusted P value < 0.05 were set as DEGs. For the observation of the screened DEGs, the Venn diagram and volcano plot were drawn using the Venn diagram and Ggplot2 packages of R, respectively.

Signaling pathway enrichment analysis

To analyze biological processes of DEGs, functional enrichment analyses of the DEGs were performed by KEGG and GO pathways using an online functional annotation tool through FunRich software (https://amp.pharm.mssm.edu/Enrichr).[15] The P value <0.05 was concerned as a cutoff criterion. The KEGG findings were graded according to Rich factor, the ratio of enriched DEG numbers to all gene numbers annotated in a certain pathway. Hence, the degree of pathway enrichment grows with the Rich factor increasing.

PPI network analysis

To evaluate the interaction information between the products of DEGs, the PPI network was generated using STRING online database (https://string-db.org/) with a combined score >0.4 and the topological analysis was conducted using Cytoscape tool (version 3.7.1).[16] Nodes and edges correspond to the proteins and interactions in the PPI network, respectively.

Validation of the hub genes

The UALCAN (http://ualca.n.path.uab.edu) database allows cancer researchers to provide available cancer transcriptomic data from The Cancer Genome Atlas (TCGA) consortium and MET500 transcriptome sequencing.[17] Relative expression of hub genes in the male and female tumors samples relative to normal samples was analyzed using the UALCAN database. The P value < 0.05 was chosen as a significant difference.

Survival analysis

Clinical outcome of each hub gene in bladder cancer patients was analyzed by the Kaplan–Meier plotter database (http://kmplot.com/analysis/). Sources for the survival information in this database are based on GEO, EGA, and TCGA databases. The hazard ratio (HR) was presented with 95% confidence intervals, and log rank P value was less than 0.05.

Results

Identification of DEGs

The gene expression profile of male and female subjects with bladder cancer and normal samples was obtained from

microarray analysis. As shown in Figure 1a, we recognized a total of 1004 DEGs in the tumor tissues versus normal tissues of female subjects, containing 240 upregulated and 763 downregulated genes. Additionally, we recognized a total of 284 DEGs in the cancerous tissues versus non-cancerous tissue samples of male subjects, including 22 upregulated and 262 downregulated genes [Figure 1b]. As depicted in Figure 1c, the Venn diagram showed 42 DEGs in male subjects, 761 DEGs in female subjects, and 242 DEGs in both male and female subjects.

PPI network construction

As demonstrated in Figure 2a, the PPI network in the males with bladder cancer revealed 225 nodes and 852 edges in the STRING database. The PPI network indicated 902 nodes and 6744 edges in women with bladder cancer [Figure 2b]. The genes with higher scores were considered as the hub genes. For the male and female subjects with bladder cancer, we represented the unique top ten hub genes for each female and male group, except two, ASPM and CDC20, indicated as shared hub genes.

Validation of the hub genes

The UALCAN database was utilized to confirm hub genes in bladder cancer, in which 19 normal and 408 bladder cancer specimens were evaluated. As can be seen in Figure 3a–f, ASPM, CDC20, BUB1B, and CCNB1 genes were upregulated, while CCL5 and IGF2 genes were downregulated in bladder tissues.

KEGG Pathways analysis

As revealed in Figure 4a, upregulated genes were enriched mainly in six pathways in females with bladder cancer, including cell cycle, cellular senescence, human T-cell leukemia virus 1 (HTLV-1) infection, progesterone-mediated oocyte maturation, DNA replication, and steroid biosynthesis. In contrast, downregulated genes were enriched in other signaling pathways in females with bladder cancer, including focal adhesion, PI3K-Akt signaling pathway, ECM–receptor interaction, vascular smooth muscle contraction, HTLV-1 infection, cGMP-PKG signaling pathway, protein digestion and absorption, MAPK signaling pathway, rheumatoid arthritis, and Apelin signaling pathway [Figure 4b]. In males, downregulated genes were involved in the Staphylococcus aureus infection, cell adhesion molecules, asthma, rheumatoid arthritis, focal adhesion, hematopoietic cell lineage, leishmaniasis, viral myocarditis, HTLV-1 infection, and intestinal immune network for IgA production pathways [Figure 4c]. Due to the low number of upregulated DEGs in male, no significant pathway was found in relation to these genes.

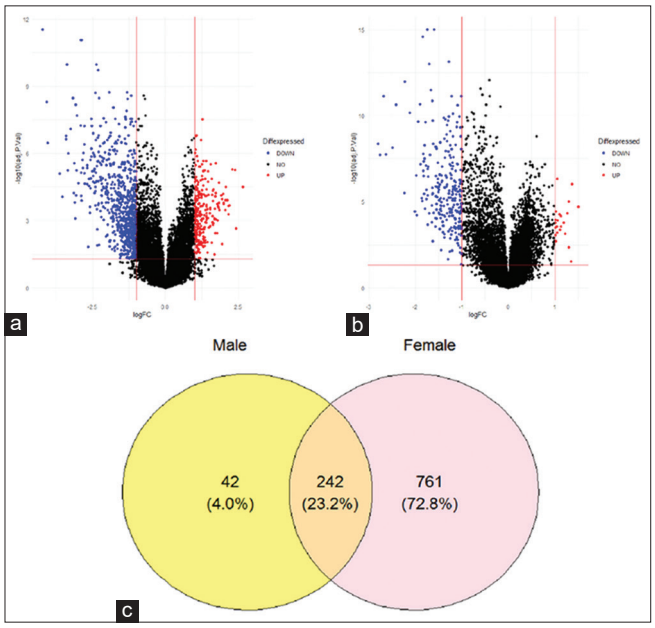

Figure 1: The volcano plots of DEGs for the GSE13507 dataset among cancerous and non-cancerous tissues of the bladder in (a) females and (b) males. The blue indicates downregulated genes, and the red indicates upregulated genes. (c) Venn diagram of the overlapping DEGs in the GSE13507 dataset between the male and female bladder cancer. DEGs: differentially expressed genes

Survival analysis of hub genes

We executed an overall survival analysis using the Kaplan–Meier plotter database to evaluate the prognostic value of hub genes screened in male and female bladder samples. Two specific hub male genes, IGF2 and CCL5, significantly represented male subjects with bladder cancer [Table 1, Figure 5a and b] in the PPI network. However, two hub genes, BUB1B and CCNB1, were revealed in female subjects with bladder cancer. We found two hub genes (ASPM and CDC20) in male and female subjects with bladder cancer [Table 1]. Furthermore, upregulation of IGF2 (HR = 1.34, P = 0.05), ASPM (HR = 1.37, P = 0.04), CDC20 (HR = 1.51, P = 0.012), BUB1B (HR = 1.34, P = 0.049), and CCNB1 (HR = 1.52, P = 0.006), as well as downregulation of CCL5 (HR = 0.71, P = 0.036), were associated with an unfavorable overall survival [Figure 5a–f].

HR represents the hazard ratio value, and also, the log-rank P column shows the significant values (log-rank $P \leq 0.05$).

Discussion

Bladder cancer is a heterogeneous urological malignant disease with gender-specific differences in incidence and outcomes. The incidence of bladder cancer in men is approximately four times higher than in women.[1] In contrast, it is well recognized that females with bladder cancer have a worse outcome than males.[8] At present, the information about gender differences-affected gene expression in bladder cancer

Table 1: Survival analysis of hub genes

Type	Name	HR	Log-rank P
Male	IGF2	1.34	0.05
	CCL5	0.71	0.036
Common	ASPM	1.37	0.04
	CDC20	1.51	0.012
Female	BUB1B	1.34	0.049
	CCNB1	1.52	0.006

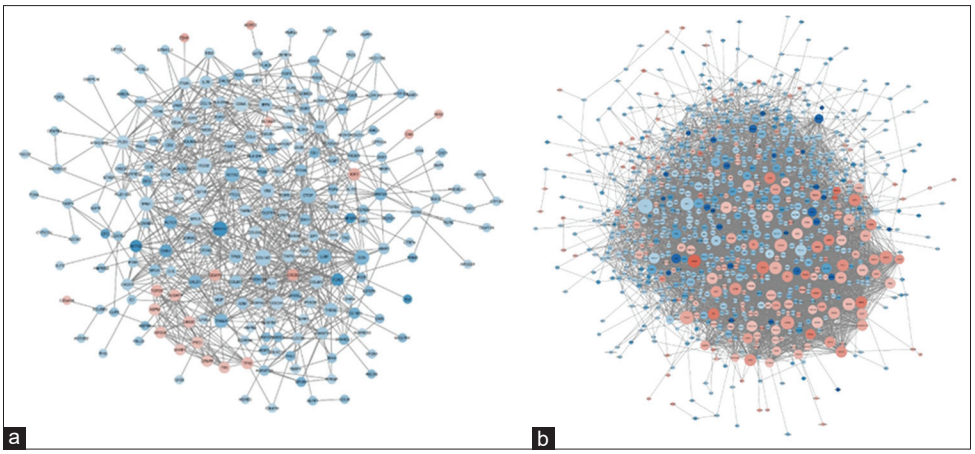

Figure 2: PPI network of DEGs in males (a) and females (b) with bladder cancer. Disconnected nodes are hidden in the network. The size of each node represents the degree of connectivity for identifying the key hub genes. The red nodes reveal upregulated genes, and the blue nodes indicate downregulated genes

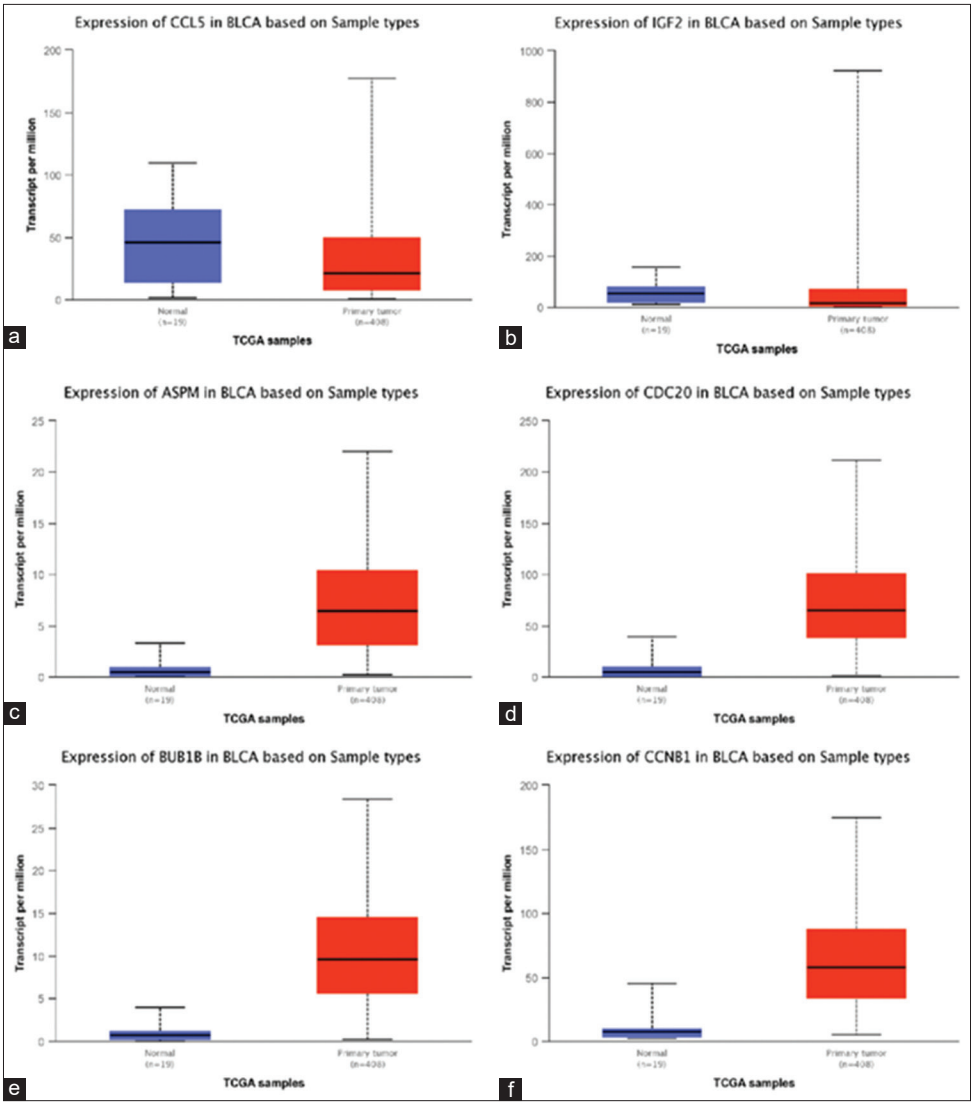

Figure 3: Relative expression of (a) CCL5, (b) IGF2, (c) ASPM, (d) CDC20, (e) BUBIB, (f) CCNB1 gene between normal and tumor tissue samples of the bladder. The $P < 0.005$ was set as a significant difference

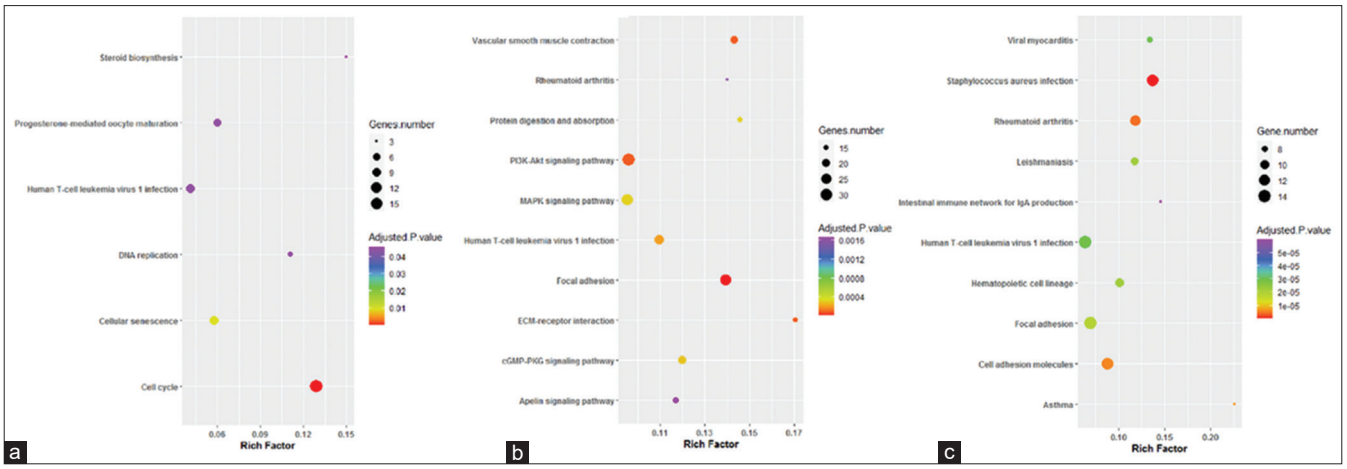

Figure 4: The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis for all DEGs of the female (a and b) and male (c) bladder cancer groups is represented by bubble plots, which represent the value of each signal activity by Rich factor score, as well as the number of genes consisted of by the size of each bubble. The adjusted *P* value, ≤ 0.05, was set as the cutoff. DEG: differentially expressed genes

is rare and might be considered in clinical significance. Therefore, the current bioinformatics study was designed to explore sex-associated key gene expression levels among bladder tumor tissues from male and female subjects. After data screening of GSE13507 from the GEO database, we investigated 29 females with bladder cancer and 13 healthy females and 136 males with bladder cancer, and 38 healthy males. A total of 240 upregulated and 763 downregulated DEGs were found in females, and 22 upregulated and 262 downregulated DEGs were found in males. In the PPI network analyses, IGF-2 and CCL-5 were found as hub genes in males, BUB1B and CCNB1 were found as hub genes in females, and ASPM and CDC20 were considered hub genes in both males and females.

CCNB1 is a critical cell cycle protein that regulates the transition from the G2 phase to mitosis. The overexpression of CCNB1 has been observed in various human malignancies, such as hepatocellular carcinoma and bladder cancer.[18,19] Additionally, it was also reported that suppressing the CCNB1 gene could inhibit cell growth.[19] In contrast, upregulation of CCNB1 is relevant to poor prognosis in various cancers, such as breast cancer.[19,20] Using the UALCAN database, we found that the CCNB1 level is overexpressed in bladder cancer tissues. We also showed that CCNB1 has differential expression in both genders. The high expression of CCNB1 was correlated with decreased survival in female patients. However, no considerable difference was found in the transcript levels of this gene in male subjects with bladder cancer, indicating that gender might influence the expression and activity of genes implicated in the cell cycle in bladder cancer.

BUB1B gene encodes a protein that is a key component of the mitotic spindle checkpoint complex for proper chromosome segregation. Extensive evidence has proven that BUB1B could contribute to tumor development. Overexpression of BUB1B is closely associated with tumor recurrence in patients with bladder cancer.[21,22] Our study found that BUB1B was upregulated in females and a higher level of BUB1B was strongly related to a worse prognosis.

IGF-2 is a polypeptide hormone similar to insulin abundantly produced by many tissues, particularly hepatocytes. Like insulin, IGF2 promotes cell proliferation and inhibits apoptosis.[23,24] Elevated IGF2 level is related to increased risk of developing different malignancies, including breast, ovarian, and prostate cancer.[25-27] The transcript level of IGF2 was previously elevated in bladder cancer patients.[28] According to the previous reports, in the current study, IGF2 expression was upregulated in male patients with bladder cancer, and a higher level of IGF2 was strongly related to a poorer prognosis.

CCL5 is a member of chemokine networks that promotes tumor progression by interacting with C-C chemokine receptor type 5 (CCR5).[29,30] Aldinucci *et al.*[30] reported the involvement of the CCL5/CCR5 axis in promoting tumor growth, extracellular matrix (ECM) remodeling, migration, and angiogenesis. In contrast, a previous study using bioinformatic analyses has shown that bladder cancer patients with a high expression level of CCL5 possessed an appreciably better prognosis.[31] In our study, CCL5 was notably downregulated in males with bladder cancer. We also revealed that lower expression of CCL5 was correlated with a worse survival rate in male patients, whereas no difference was found in the levels of this gene in female subjects with bladder cancer.

However, two overexpressed hub genes, ASPM and CDC20, were shared among both genders. Cell division cycle protein 20 (CDC20) gene encodes a protein that acts as a spindle assembly checkpoint protein during cell cycle progression.[32] The aberrant expression of CDC20 has been elucidated as a frequent event in various human malignancies and is coupled to chromosome aneuploidy, tumor occurrence, and poor prognosis.[32,33] Several studies have described that the CDC20 transcript level is exceedingly expressed in bladder cancer.[34,35] Using the UALCAN database in the current work, we found that the CDC20 level is overexpressed in bladder cancer

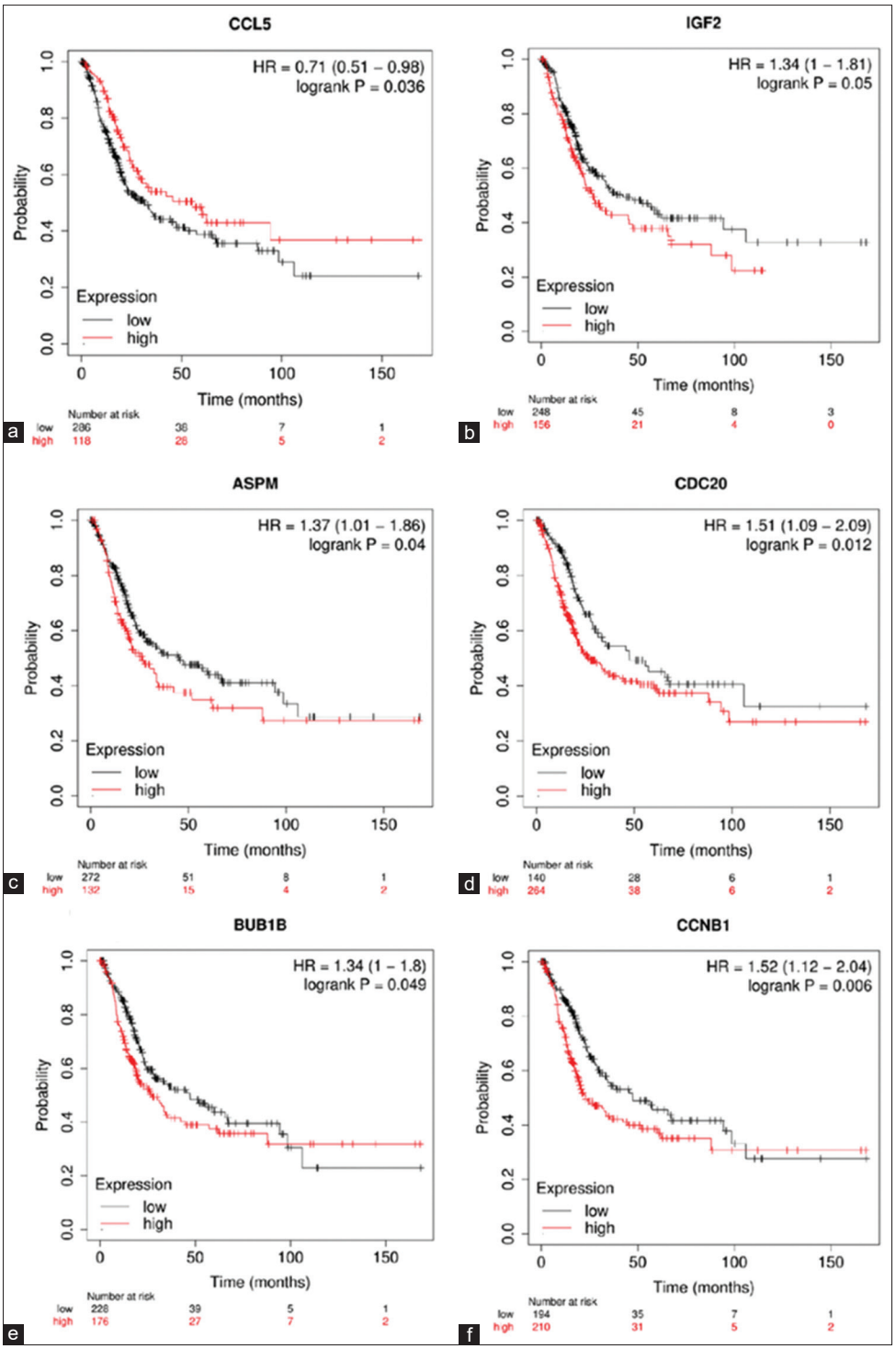

Figure 5: Prognostic values of (a) CCL5, (b) IGF2, (c) ASPM, (d) CDC20, (e) BUBIB, (f) CCNB1 gene in male and female subjects with bladder cancer using Kaplan–Meier plotter database

tissues. We also showed that CDC20 was overexpressed in both genders. The higher expression of CDC20 was related to worse survival in bladder cancer patients. In our study, ASPM was also recognized as another hub gene in the PPI network in both genders. ASPM, located on chromosome 1q31, encodes a protein involved in the mitotic spindle localization and function during cell replication. A gene expression analysis demonstrated that the ASPM transcript level is higher in embryonic tissues but is much lower in adult tissues. It has been pointed out that the ASPM gene is greatly upregulated in malignant tissues compared with normal tissues.[36-38] Gao *et al.*[39] highlighted that higher ASPM expression was associated with the poor survival rate in bladder cancer patients. Additionally, ASPM knockdown could markedly

suppress bladder cancer proliferation *in vitro* and in vivo. Following previous studies, our data showed that ASPM was a poor prognostic biomarker in subjects with bladder cancer.

Afterward, KEGG pathway analysis of DEGs indicated that focal adhesion, rheumatoid arthritis, and HTLV-I infection were enriched in female and male bladder cancers. KEGG analysis in male subjects revealed that DEGs were mainly enriched in the infection and inflammatory pathways. Chronic immune reactions and inflammatory responses widely thought to be led to carcinogenesis. Several studies have elucidated an association between bladder cancer occurrence and urinary tract bacterial infection. Microbial urine profiles of bladder cancer patients exhibited the existence of Staphylococcus aureus. This organism plays an important role in forming N-nitrosamines, which may lead to the initiation of tumorigenesis in bladder cancer patients.[40,41] DEGs in the females were enriched in the cellular pathways, including cell cycle and cell division. It has been investigated that the cell cycle plays a central role in regulating cell growth and that the impairment in the cell cycle regulation facilitates tumor growth and development.[42]

Taken together, the findings of the current study suggested that changes in the numerous hub genes linked to gender differences may play an important role in bladder cancer occurrence and prognosis. However, one of the limitations found in the present study was the lack of downregulation or upregulation mechanisms of hub genes in male and female patients with bladder cancer. Besides, the results from the bioinformatics lack corresponding experimental validation *in vitro* and *in vivo*.

Conclusion

Based on gender index, the current study revealed different gene expressions and pathways in bladder cancer patients. These hub genes were markedly correlated with the progression and prognosis of bladder cancer in both males and females. Further studies are necessary to corroborate the findings of the present study.

Ethics approval

This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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