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Original Article

Prognostic Significance and Diagnostic Potential of High E2F3 mRNA Expression in Bladder Urothelial Carcinoma

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Abstract:

Background: Bladder urothelial carcinoma (BLCA) is a lethal malignancy with poor prognosis and low survival rates, and it is difficult to diagnose early. Transcription factor E2F3 plays an important role in tumor growth. This study explored the prognostic value of E2F3 in BLCA.

***Methods*:** We analyzed the E2F3 mRNA expression using the data from TCGA-BLCA cohort. Areceiver operating characteristic(ROC) curve was performed to assessed the diagnostic value of E2F3 mRNA expression in BLCA. Chi-square test was used to analyzed the correlation between clinicalcharacteristics and E2F3 mRNA expression. Kaplan-Meier analysis and Cox analysis were performed to determine the prognostic value of E2F3 mRNA expression in BLCA. Gene set enrichment analysis(GSEA) was conducted using TCGA database.

***Results*:** Our results revealed that E2F3 mRNA expression was higher in BLCA tissues than in normal tissues. E2F3 presented a moderate diagnostic value in BLCA. High E2F3 mRNA expression was correlated with T classification, Distant metastasis, Pathological grade of BLCA. High E2F3 mRNA expression was associated with poor overall survival. Furthermore, Multivariate analysis revealed that E2F3 was an independent risk factor for BLCA patients. PI3k akt pathway, Notch3 pathway, cancer pathway, β- catenin pathway, bladder cancer and β- catenin transactivating complex were differentially enriched in the phenotype that positively correlated with E2F3.

***Conclusion*:** In conclusion, our results suggest that E2F3 was a promising diagnostic and prognostic biomarker for BLCA.

Keywords: E2F3; TCGA; bioinformatics; diagnosis; prognosis; Bladder urothelial carcinoma (BLCA)

Introduction

Bladder urothelial carcinoma (BLCA) is one of the leading causes of cancer-related deaths worldwide [1]. Despite the improvements in therapeutic strategies, BLCA, especially advanced BLCA, still exhibits a high mortality rate [2, 3]. The main cause of the poor prognosis is tumor metastasis, in which angiogenesis plays a key role [4,5]. Therefore, searching for new sensitive biomarkers are important for early diagnosis of BLCA.

E2F transcription factor 3 (E2F3) encodes a member of a small family of transcription factors that function through binding of dimerization partner (DP) interaction partner proteins[6,7]. The encoded protein recognizes a specific sequence motif in DNA and interacts directly with the retinoblastoma protein (pRB) to regulate the expression of genes involved in the cell

cycle[8,9]. Altered copy number and activity of this gene have been observed in a number of human cancers. Feng et reported that E2F3 could promote cancer growth and was overexpressed through copy number variation in human melanoma[10]. Pei et al concluded that the E2F3/miR-125a/DKK3 regulatory axis promoted the development and progression of gastric cancer[11,12]. However, the functional role and prognostic significance of E2F3 in BLCA are still not fully understood.

In this study, we compared the E2F3 mRNA expression between BLCA tissues and normal tissues. We evaluated the correlations between clinical characteristics and E2F3 mRNA expression of BLCA patients. We investigated the biological pathways that related to E2F3 using GSEA analysis. Our results suggest that E2F3 was a promising diagnostic and prognostic biomarker for BLCA.

Materials and Methods

Clinical Caracteristics in The Cancer Genome Atlas

A dataset of 407 samples (375 BLCA and 32 normal adjacent gastric tissue samples) containing RNA sequencing and clinical data was obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov) [13].

GSEA

In this study, we analyzed the correlations between E2F3 mRNA expression and all genes by R (v.3.6.1), then performed GSEA analysis using the cluster Profiler packagein R. ES>1,P<0.05,and FDR <0.25 were considered tobe statistically significant.

Statistical Analysis

Receiver operating characteristic (ROC) curves were used to qualify the discrimination of the signature by evaluating the area under the curve (AUC). The R package “pROC” was applied to plot the ROC curve [14]. Kaplan-Meier analysis was performed to investigate the predictive value of the signature using the R package “survival” [15].

Results

High E2F3 expression in BLCA Patients

We analyzed the transcription levels of E2F3 based on TCGA database. We found that E2F3 mRNA expression was significantly higher in BLCA tissues than that in normal tissues(P=4.78e-05,P=3.81e-06,respectively) (Figure 1).

Furthermore, different E2F3 mRNA expression were observed in groups based on T classification, distant metastasis and Pathological Grade. The E2F3 mRNA expression of patients with T3/T4 classification were higher than that of patients with T1/T2 classification (P=4.253e-05). Patients with a positive distant metastasis had higher E2F3 mRNA expression than patients with a negative status(P=3.646e-04). High pathological grade groups(I/II) had higher E2F3 mRNA expression than low pathological groups(III/IV,)(P=1.24e-07)(Figure 2).

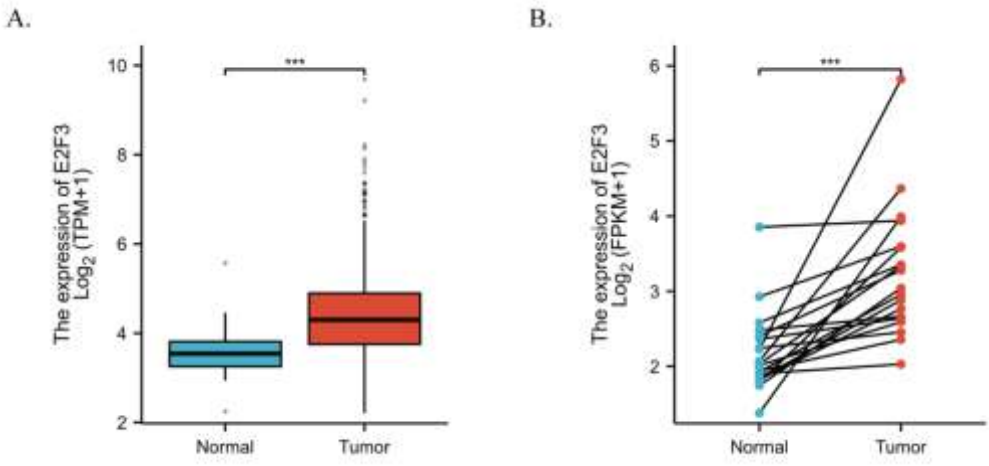

Figure 1 E2F3 mRNA expression in BLCA patients. (A)E2F3 mRNA expression in BLCA tissues and normal issues(P=4.78e-05) (B)E2F3 mRNA expression in BLCA tissues and adjacent tissues (P=3.81e-06).

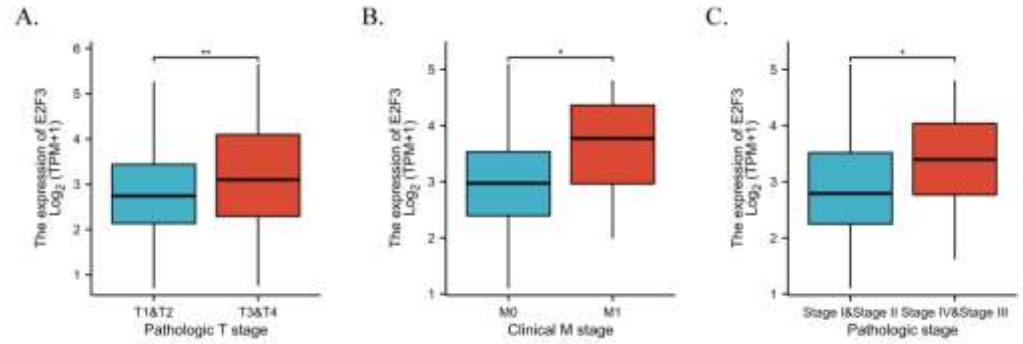

Figure 2 Association with E2F3 mRNA expression and clinicopathologic characteristics. (A) T classification (P=4.253e-05), (B) Distant metastasis (P=3.646e-04), (C) Pathological grade (P=1.24e-07).

Diagnostic Value of E2F3 mRNA Expression in BLCA

To determine the diagnostic value of E2F3, we plotted ROC curves in 375 cases of BLCA with matched normal adjacent tissue. The results showed that the Area Under the Curve (AUC) of E2F3 was 0.832. The diagnostic capability of E2F3 was also analyzed in different stages. The results showed that the AUC values of E2F3 in stages I, II, III, and IV were 0.675, 0.773, 0.776, and 0.918, respectively(Figure 3).

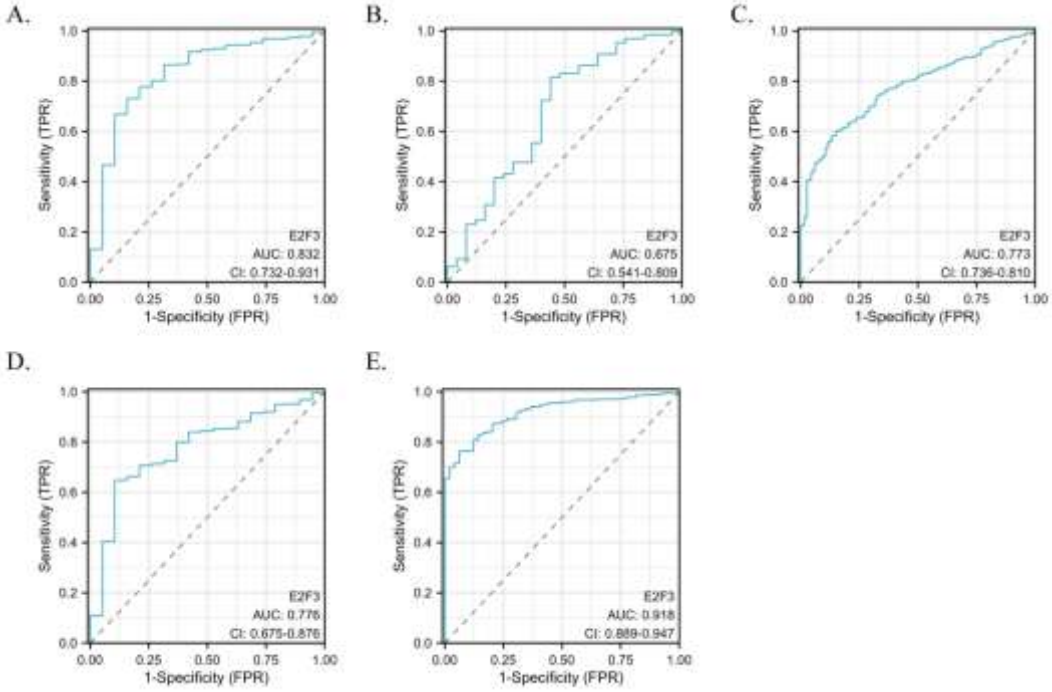

Figure 3 ROC curve of E2F3 mRNA expression in BLCA cohort. (A) ROC curve of E2F3 mRNA expression in normal and tumor, (B-E) Subgroup analysis for stage I, II, III and IV , respectively.

Correlation between E2F3 and Clinical Characteristics of BLCA Patients

Clinical and gene expression data of 375 primary tumors and 32 normal samples were download from TCGA database, including patients'age, gender, grade, overallsurvival, stage, distant metastasis, survival status, T classification and lymph nodes. To further understand how E2F3 participate in the tumorigenesis of BLCA, we analyzed the association between E2F3 and BLCA clinical features. We found that the E2F3 expression level is closely associated with the T classification ($p < 0.05$). However, no significant association was identified between E2F3 and patients' other clinical features(Table 1). These

data further highlight the importance of E2F3 in BLCA tumorigenesis.

Table 1 Correlation between E2F3 and clinicopathologic factors in BLCA

Characteristic	levels	Low expression of E2F3	High expression of E2F3	p
n		187	188	
Gender, n (%)	Female	75 (20%)	59 (15.7%)	0.098
	Male	112 (29.9%)	129 (34.4%)	
Age, n (%)	<=65	75 (20.2%)	89 (24%)	0.222
	>65	109 (29.4%)	98 (26.4%)	
Pathologic stage, n (%)	Stage I	27 (7.7%)	26 (7.4%)	0.671
	Stage II	52 (14.8%)	59 (16.8%)	
	Stage III	80 (22.7%)	70 (19.9%)	
	Stage IV	17 (4.8%)	21 (6%)	
T stage, n (%)	T1	14 (3.8%)	5 (1.4%)	0.029
	T2	32 (8.7%)	48 (13.1%)	
	T3	82 (22.3%)	86 (23.4%)	
	T4	56 (15.3%)	44 (12%)	
N stage, n (%)	N0	60 (16.8%)	51 (14.3%)	0.288
	N1	42 (11.8%)	55 (15.4%)	
	N2	42 (11.8%)	33 (9.2%)	
	N3	35 (9.8%)	39 (10.9%)	
M stage, n (%)	M0	167 (47%)	163 (45.9%)	0.230
	M1	9 (2.5%)	16 (4.5%)	
Histologic grade, n (%)	G1	4 (1.1%)	6 (1.6%)	0.123
	G2	78 (21.3%)	59 (16.1%)	
	G3	102 (27.9%)	117 (32%)	
Age, median (IQR)		68 (59, 73.25)	67 (57.5, 73)	0.357

A High E2F3 Level Correlates with a Poor BLCA Prognosis

We evaluated the overall survival (OS) of the 375 BLCA patients. Univariate analysis showed that T stage, N stage, M stage, E2F3 level, age and TNM stage were associated with poor prognosis (p<0.05). Meanwhile, the results of the multivariate survival analysis showed that E2F3 expression and age were independent prognostic factors in BLCA patients (Table 2, p<0.05). To further evaluate the prognostic value of E2F3 in BLCA patients, we plotted the patient survival curve based on E2F3 expression levels. The OS of patients with high E2F3 expression was significantly shorter than those with low E2F3 expression (p=0.001, Figure 4A). A subgroup survival analysis was performed to evaluate whether E2F3 expression affects other prognostic factors. The results showed that elevated E2F3 was significantly associated with OS of clinical stage I/II, clinical stage III/IV, M0, M, T1/T2(Figure 4B-F).

Table 2 Correlations between overall survival and mRNA expression of E2F3 analyzed by univariate and multivariate Cox regression

Characteristics	Total(N)	Univariate analysis			Multivariate analysis	
		Hazard ratio (95% CI)	P value		Hazard ratio (95% CI)	P value
T stage	362					
T1	18	Reference				
T2	78	6.725 (0.913-49.524)	0.061		3.499 (0.439-27.898)	0.237
T3	167	9.548 (1.326-68.748)	0.025		4.976 (0.565-43.798)	0.148
T4	99	9.634 (1.323-70.151)	0.025		4.823 (0.534-43.588)	0.161

N stage	352					
N0	107	Reference				
N1	97	1.629 (1.001-2.649)	0.049		1.196 (0.595-2.405)	0.616
N2	74	1.655 (0.979-2.797)	0.060		1.445 (0.617-3.384)	0.397
N3	74	2.709 (1.669-4.396)	<0.001		1.895 (0.802-4.475)	0.145
M stage	352					
M0	327	Reference				
M1	25	2.254 (1.295-3.924)	0.004		1.058 (0.437-2.563)	0.900
Pathologic stage	347					
Stage I	50	Reference				
Stage II	110	1.551 (0.782-3.078)	0.209		1.132 (0.397-3.226)	0.817
Stage III	149	2.381 (1.256-4.515)	0.008		1.088 (0.274-4.313)	0.905
Stage IV	38	3.991 (1.944-8.192)	<0.001		2.359 (0.592-9.389)	0.223
Histologic grade	361					
G1	10	Reference				
G2	134	1.648 (0.400-6.787)	0.489			
G3	217	2.174 (0.535-8.832)	0.278			
E2F3	370					
Low	182	Reference				
High	188	1.608 (1.151-2.247)	0.005		1.730 (1.186-2.524)	0.004
Gender	370					
Female	133	Reference				
Male	237	1.267 (0.891-1.804)	0.188			
Age	367					
<=65	163	Reference				
>65	204	1.620 (1.154-2.276)	0.005		1.826 (1.261-2.645)	0.001

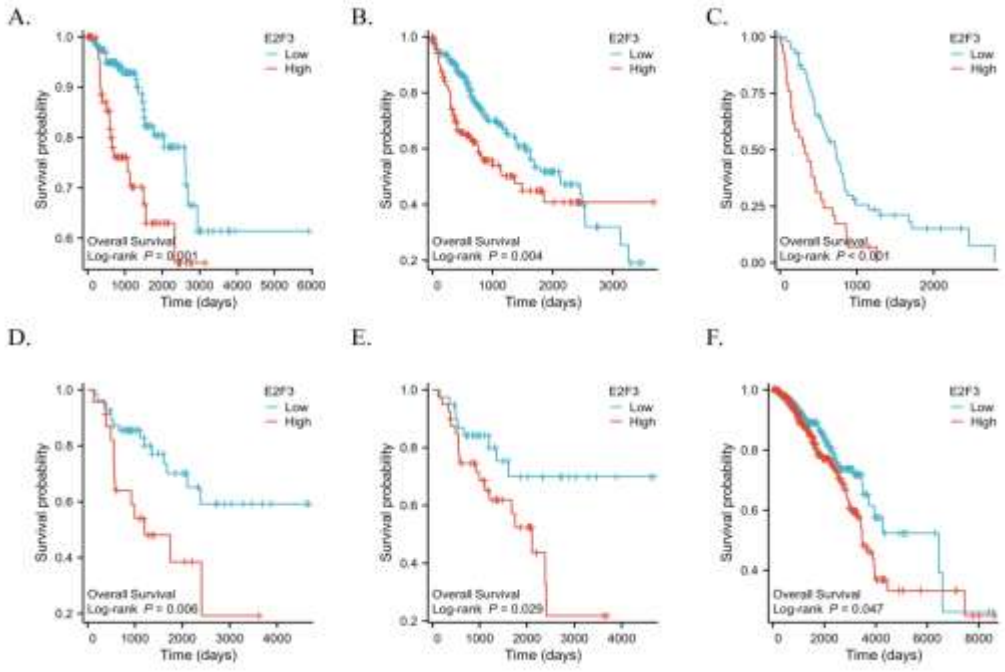

Figure 4: Results of Kaplan-Meier survival analysis in BLCA patients. (A) Kaplan-Meier curves of overall survival rate of BLCA in all cases based on E2F3 expression levels. (B, C,D,E, F) Subgroup

analysis of the overall survival in BLCA cases of (B) clinical stage I/II, (C) clinical stage III/IV, (D) M0, (E) M, (F) T1/T2 based on E2F3 expression levels.

GSEA Identifies a E2F3-Related Signaling Patbway

GSEA was performed to identify the signaling pathwaysthat activated in BLCA. The results showed that PI3k akt pathway, Notch3 pathway, cancer pathway, β- catenin pathway, bladder cancer and β- catenin transactivating complex were differentially enriched in the positively(Figure 5).

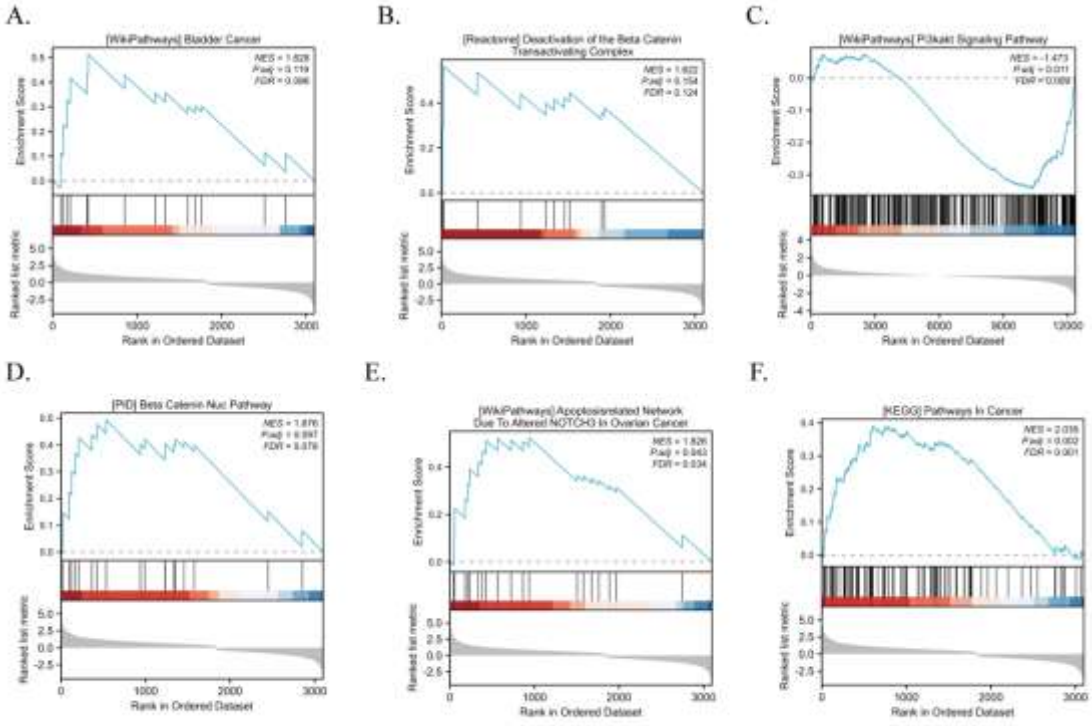

Figure 5 Enrichment plots by GSEA. (A) bladder cancer, (B) β- catenin transactivating complex, (C) PI3k akt pathway, (D) β- catenin pathway, (E) Notch3 pathway, (F) cancer pathway.

Discussion

The promotive role of E2F3 in human cancer has been well documented in a variety of cancers, such as prostate cancer [16], breast cancer [17], and BLCA [18]. In addition, elevated E2F3 has been observed in multiple malignancies, including lymphoma [19] and BLCA [18]. However, how it is associated with BLCA and the underlying mechanism have not been elucidated. Accumulating evidence has revealed the postive regulatory effect of AKT signaling on E2F3 in tumor progression [20,21]. Therefore, targeting E2F3 production is an important anticancer strategy.

In this study, our results confirmed that E2F3 mRNA expression was higher in BLCA tissues than in normal tissues. High E2F3 mRNA expression was associated with T classification. Moreover, high E2F3 mRNA expression correlated with poor overall survival. In addition, E2F3 was an independent prognostic factor of BLCA.

Moreover, our univariate and multivariate COX regression analysis revealed, for the first time, that the expression of E2F3 is an independent predictor of BLCA prognosis. This is consistent with previous studies that have shown that E2F3 have also been associated with poor prognosis in colorectal cancer, hepatocellular carcinoma, and esophageal squamous cell carcinoma by promoting tumor growth and metastasis [22-25]. Our results also showed that high expression of E2F3 was associated with low OS, suggesting that E2F3 is a molecular biomarker for outcome prediction in BLCA.

High E2F3 mRNA expression was significantly

correlated with poor prognosis in different tumors[26,28]. E2F3 was overexpressed in gastric cancers[29,30]. E2F3 deletions suppresses the invasion and migration of ESFT and hepatocellular carcinoma (HCC) cells[31,32]. Consistently, we found that E2F3 mRNA expression was significantly associated with poor overall survival, we further investigated the relation between clinical characteristics and E2F3 mRNA expression in BLCA patients, we found that T classification, Distant metastasis, Pathological grade were highly correlated with E2F3 mRNA expression. The potential mechanism may involve in PI3k akt pathway, Notch3 pathway, cancer pathway, β- catenin pathway, bladder cancer and β- catenin transactivating complex.

Our group and others have shown a role for E2Fs in tumorigenesis and tumor maintenance[33]. For example, we showed that E2F3 is overexpressed in breast cancer, and maintains higher rates of centrosome amplification and dysregulated mitosis, which promotes aneuploidy and chromosome instability to initiate and sustain tumors[34-36]. Recent studies have shown a novel role for E2Fs in tumor progression, including epithelial-to-mesenchymal transition (EMT) signaling, invasion, and metastasis[37,38]. Ultimately, EMT results in the acquisition of mesenchymal features that allow cells to invade and migrate through the ECM[39-41]. EMT is a process that occurs naturally in embryological development but is observed in pathological states such as cancer and fibrosis. Although EMT signaling has been thoroughly studied, several knowledge gaps remain, such as identifying key EMT drivers in cancer and developing strategies to target these to stop the metastatic process. E2F3 plays an important role in tumor metastasis.

Overall, our study verified the value of E2F3 mRNA expression in diagnosis and prognosis of BLCA patients. Nevertheless, this study also has shortcomings. First, the cohort for bioinformatic analysis was small, so a larger sample size is needed to verify our results. Second, we need to collect complete follow-up data to confirm the impact of E2F3 on the survival and prognosis of BLCA. Another disadvantage is that the current research mainly focuses on the correlation between E2F3 and AKT, but the specific tumor suppression mechanism is still elusive. Therefore, we need to study the mechanism of E2F3 in promoting tumor progression and BLCA metastasis in the future.

Conclusions

Our results confirmed that E2F3 mRNA expression was upregulated in BLCA tissues. Moreover, higher E2F3 mRNA expression was correlated with poor overall survival

of BLCA patients. In addition, E2F3 mRNA expression was an independent prognostic factor of BLCA, our data also show that E2F3 has the potential to diagnose and predict the treatment outcome of BLCA and may become a new biomarker of BLCA.

Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to limitations of ethical approval involving the patient data and anonymity but are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

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