

A Prognostic Biomarker for Bladder Cancer Correlated with Immune Infiltration Is PAEP

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Abstract

Background: A major cause of cancer death worldwide is bladder cancer, which is the most common malignant tumor of the urinary tract. PAEP is a member of the kernel lipocalin superfamily whose members share relatively low sequence similarity but have highly conserved exon/intron structure and three-dimensional protein folding. Most lipocalins are clustered on the long arm of chromosome 9. The purpose of this study was to clarify the correlation between PAEP expression level and bladder cancer. **Methods:** In the TCGA database, we obtained clinical and RNA sequencing data of 431 BLCA patients, including 412 BLCA tissues and 19 normal bladder tissues in the study. Analyses of bioinformatics were conducted in this study to determine the role of PAEP in bladder cancer. A quantitative real-time PCR method was used to quantitate the gene expression profile. Additionally, the effect of PAEP on tumor immune infiltration and prognosis was analyzed. **Results:** PAEP was a poor prognostic biomarker of bladder cancer because it was significantly upregulated. bladder cancer patients with higher PAEP expression had poor outcomes. An AUC of 0.780 was calculated from the area under the ROC curve. PAEP was associated with T stage, pathologic stage, Histologic grade and Subtype of bladder cancer patients, and served as an independent predictor of overall survival in bladder cancer patients. Functional enrichment analysis revealed PAEP was obviously enriched in pathways connected with carcinogenesis and immunosuppression. The expression of PAEP was significantly associated with tumor immune cells and immune checkpoints according to ssGSEA and Spearman correlation analysis. **Conclusions:** In this study, we screened and detected a mRNA, PAEP is a prognostic and immune-related biomarker in BLCA, which may contribute to the early diagnosis and treatment of BLCA.

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Keywords

PAEP, Bladder Cancer, Immune Cell Infiltration, Immune Checkpoint, Prognosis

1. Introduction

Bladder cancer is the 10th most common cancer in the world, with about 573,000 new cases and 213,000 deaths [1]. Smoking and specific occupational exposure are the most definite risk factors [2]. Bladder cancer has a high incidence and high mortality in urinary system tumors [3] [4] [5]. Cystoscopy is still the gold standard technique for the preliminary diagnosis of bladder cancer [6]. Traditional invasive examination cannot be used as a routine physical examination item in clinical practice because of its invasiveness, pain experience and high examination cost. Most BLCA patients are already in the advanced stage of the disease, or patients are resistant to chemotherapy [7] [8]. Eventually, distant metastasis occurred [9] [10]. The prognosis and response to chemotherapy and checkpoint inhibitors in patients with bladder cancer (BC) vary greatly, and the treatment that benefits the patient the most should be selected [11] [12]. Immunotherapy and molecular-targeted therapy have been proven to be beneficial for patients with bladder cancer in recent years [13] [14] [15]. Therefore, in order to improve the early diagnosis rate of BLCA patients, the effectiveness of immunotherapy and prolong survival, it is particularly important to identify useful biomarkers.

PAEP is a member of the kernel lipocalin superfamily whose members share relatively low sequence similarity but have highly conserved exon/intron structure and three-dimensional protein folding. Most lipocalins are clustered on the long arm of chromosome 9 [16]. The glycoprotein encoded by was previously known as pregnancy-associated endometrial α -2-globulin, placental protein 14, but has been officially named as progesterone-associated endometrial protein [17] [18] [19]. Previous studies have shown that PAEP is highly expressed in the inner layer of the endometrium, which is induced by progesterone and can be used as an immunosuppressive protein [20]. Studies have found that PAEP is highly expressed in many tumors: The high expression of PAEP in breast cancer plays a role in inhibiting tumors. PAEP is a marker of good prognosis in breast cancer [21] [22] [23]. PAEP is highly expressed in lung cancer. Knockdown of PAEP leads to significant up-regulation of immune system regulatory factors (such as PDL1, CXCL5, CXCL16, MICA/B and CD83) and proliferation stimulators EDN1 and HBEGF, and reduced migration of tumor cells cultured in vitro; PAEP is a serum and tissue biomarker for metastatic and advanced non-small cell lung cancer [24] [25]. PAEP inhibits the migration and invasion of HEC1-B cultured in vitro and is an independent biomarker for poor prognosis of endometrial cancer [26] [27]. Detection of

PAEP by plasma proteomics to determine the occurrence of ovarian cancer can be used as a high-precision candidate biomarker, and it is also a biomarker for predicting the poor prognosis of patients with advanced ovarian cancer [28] [29]. PAEP is highly expressed in colon cancer. Down-regulation of PAEP expression can reduce the proliferation and migration ability of colon cancer cell lines DLD1, SW480 and obtain oxaliplatin tolerance [30]. PAEP is an oncogenic gene of melanoma, which is associated with tumor microenvironment and tumor mutation load, and affects the progression and invasive phenotype of melanoma [31] [32] [33].

PAEP is associated with clinical outcomes in patients with bladder cancer [34]. However, there are few studies on the role of PAEP in BLCA immune infiltration. The purpose of this study is to determine whether PAEP is related to the prognosis and immune infiltration of BLCA, and to provide an important molecular basis for early non-invasive diagnosis and immunotherapy of BLCA.

2. Methods

2.1. Bladder Cancer Data Collection

In TCGA (<https://portal.gdc.cancer.gov/>) [35], we obtained clinical and RNA sequencing data of 431 BLCA patients, including 412 BLCA tissues and 19 normal bladder tissues in the study, as well as clinical data such as age, gender, T stage, N stage, M stage, pathological stage, tumor grade, and lymphatic vascular invasion.

2.2. Analysis of DEmRNA

DEmRNAs with the absolute value of log2 fold change ($|\log FC|$) > 1.5 and $P_{adj} < 0.05$ were obtained using the “Limma” package [36], and mRNA co-expressed with target genes were obtained by R language [37]. We used the “ggplot2” package [38] to visualize the volcano map of mRNA and the heat map of the target gene and its co-expressed mRNA.

2.3. Survival Analysis

The “Survival” package was used to analyze DEmRNA related to the prognosis of BLCA patients [39]. BLCA patients were divided into high-expression groups and low-expression groups according to the median of DEmRNA expression.

2.4. Identification of the Target Gene Associated with Immunity

Immune-related genes were obtained using the ImmPort (<https://www.immport.org/shared/home>) database. Then, Venn overlap analysis was used to map the interaction between prognosis-related DEmRNA and immune-related genes [40]. Finally, PAEP was determined as the target gene. Immune-related genes are shown in Supplementary 2.

2.5. Expression Profile Analysis of PAEP

UCSC XENA database was used to analyze PAEP expression (<https://xenabrowser.net/datapages/>), “ggplot2” package was used to visualize PAEP expression from TCGA, and “pROC” package was used for ROC analysis [41].

2.6. Functional Enrichment Analysis

In order to study the function of PAEP, gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis and gene set enrichment analysis (GSEA) were performed [42]. Automated processing of GO and KEGG entries using the “cluster Profiler” package [43]. BLCA patients were divided into low expression group and high expression group by PAEP expression median. Then, GSEA _ 4.2.3 software was used for analysis.

2.7. Immune Correlation Analysis of PAEP

The ssGSEA algorithm [44] was used to evaluate 24 immune cells in BLCA tissue samples. Spearman analysis was used to evaluate PAEP and immune cells and immune checkpoint-programmed cell death protein 1 (PD-1) -PDCD1, cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death ligand 1 (PD-L1), lemur tyrosine kinase 3 (LMTK3), lymphocyte activation gene 3 (LAG3) -CD223, hepatitis A virus-cell receptor 2 (HAVCR2) -CD366, T cell immune receptor with Ig and ITIM domains (TIGIT), indoleamine 2. The correlation of 3-dioxygenase 1 (IDO1) expression. We use the “ggplot2” package and the “pheatmap” package [45] to visualize the correlation. We used the Wilcoxon rank sum test to evaluate the enrichment of immune infiltrating cells in BLCA patients with high PAEP expression and low PAEP expression.

2.8. Statistical Analysis

In SPSS23.0 software, the expression of PAEP in normal bladder tissue and BLCA tissue was detected by the Wilcoxon rank-sum test and Wilcoxon signed-rank test. Chi-square test was used to analyze the correlation between PAEP expression and clinicopathological parameters. The effects of PAEP expression and clinicopathological parameters on survival were analyzed by univariate and multivariate Cox regression. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Acquisition of DEmRNA Related to Immunity

We identified 2942 DEmRNAs (1652 up-regulated and 1290 down-regulated) between BLCA and normal bladder specimens. The map of volcanic distribution is shown in **Figure 1(a)**. Immune-related genes were downloaded from the ImmPort database. We used Venn overlap analysis to obtain overlapping target genes between immune-related genes and DEmRNA related to the prognosis of BLCA patients. The results showed that 75 genes such as PAEP, PTGFR,

INHBA, EREG, AHNAK, BPIFA2, NFATC1, NR0B2, GRP and PDGFRA were overlapping target genes (**Figure 1(b)**). Through comprehensive comparison, we finally chose PAEP as the target gene. A heat map of PAEP and its co-expressed mRNA is shown in **Figure 1(c)**. The first five genes were positively correlated with the expression of PAEP, and the last five genes were negatively correlated with the expression of PAEP.

3.2. The High Expression of PAEP Is Closely Related to the Poor Prognosis of BLCA

The expression of PAEP in pan-cancer in the UCSC XENA database is shown in **Figure 2(a)**. We visualized the expression of PAEP from the TCGA database, as shown in **Figure 2(b)** and **Figure 2(c)**. Compared with normal bladder tissues, the expression level of PAEP in BLCA was higher. PAEP can be used as a potential diagnostic biomarker with an AUC of 0.780. (**Figure 2(d)**). Based on survival analysis, BLCA patients with high PAEP expression had poor overall survival, as shown in **Figure 2(e)**.

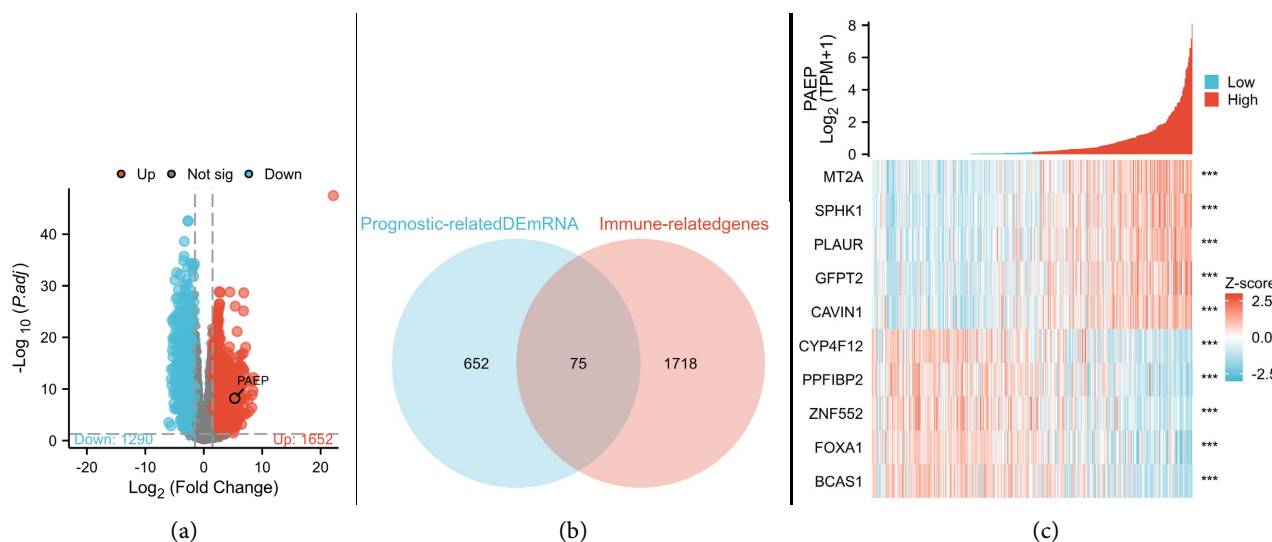
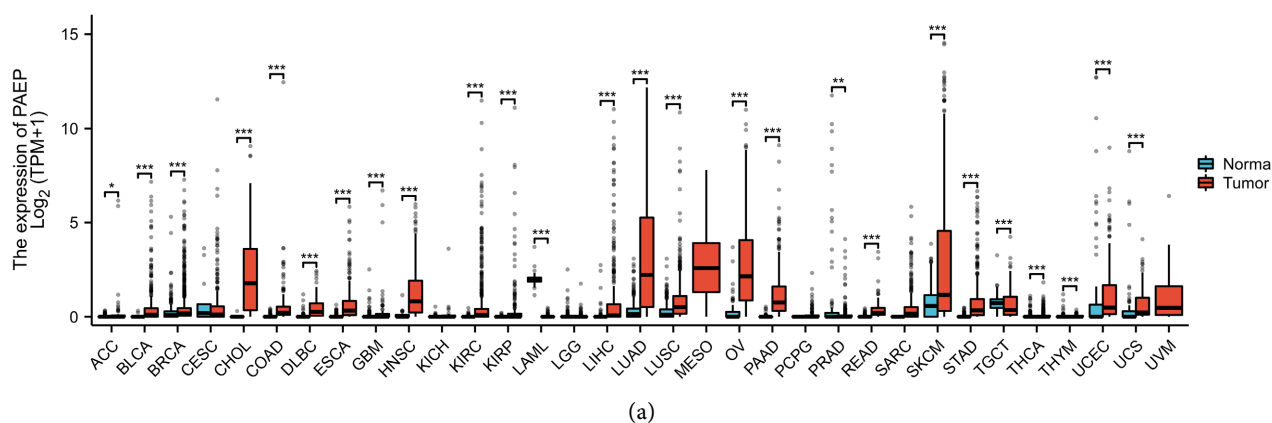


Figure 1. (a) Clinical differential molecules of bladder cancer, (b) Prognostic-related DE mRNA AND Immune-related genes, (c) PAEP and co-expressed gene correlation heat map (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$).



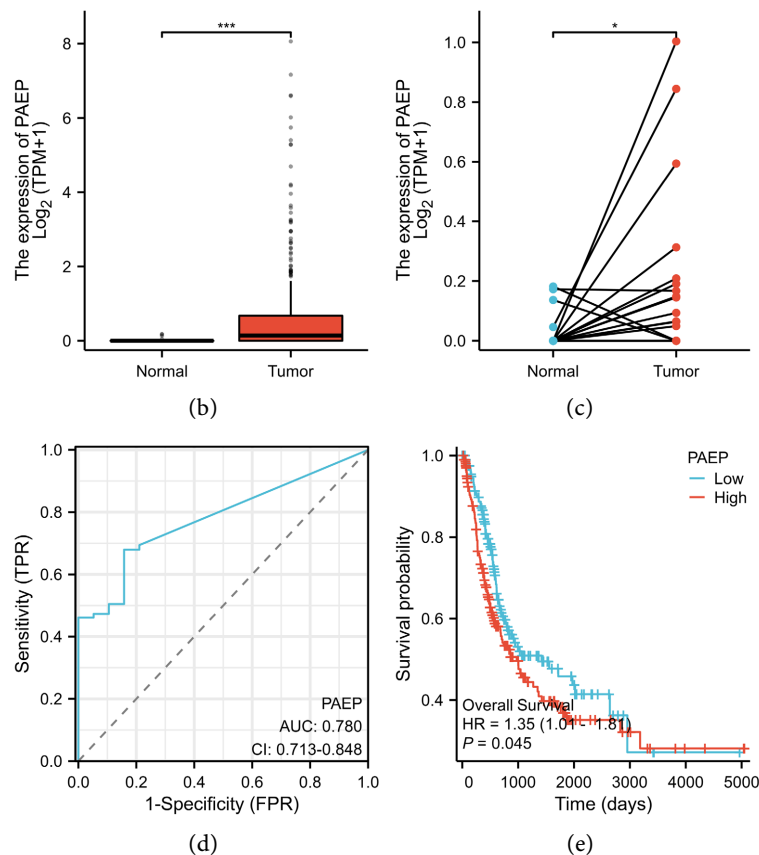


Figure 2. (a) The expression of PAEP in pan-carcinoma (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$), (b) The grouped sample comparison diagram of PAEP in TCGA database (normal vs tumor) (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$), (c) The paired sample comparison diagram of PAEP in TCGA database (normal vs tumor) (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$), (d) the ROC curve of PAEP, (e) Overall survival curve of PAEP from TCGA database.

3.3. The Correlation between PAEP Expression and Clinicopathological Parameters

The correlation between PAEP expression and clinicopathological factors was analyzed by Chi-square test. For example, the Chi-square test in **Table 1** showed that PAEP was associated with Pathologic T stage ($P = 0.009$), Pathologic stage ($P < 0.001$), Histologic grade ($P < 0.001$) and Subtype ($P < 0.001$) in BLCA patients.

As shown in **Figure 3(a)**, based on Kruskal-Wallis test and Dunn's test, PAEP expression was correlated with the T stage of BLCA patients. The overall survival rate of patients with different BLCA subgroups with high or low expression of PAEP is shown in **Figures 3(b)-(h)**.

The results indicated that Pathologic T stage: T2 (HR = 2.29 (1.16 - 4.53), $P = 0.017$), Pathologic N stage: N0 (HR = 1.74 (1.10 - 2.74), $P = 0.018$), Pathologic stage: Stage II (HR = 2.01 (1.03 - 3.92), $P = 0.042$), Gender: Female (HR = 1.79 (1.02 - 3.13), $P = 0.042$), Histologic grade: High grade (HR = 1.35 (1.00 - 1.81), $P = 0.048$), Lymphovascular invasion: No (HR = 2.41 (1.32 - 4.40), $P = 0.004$), Subtype: Papillary (HR = 2.39 (1.31 - 4.39), $P = 0.005$) were associated with poor overall survival with increased ULBP2 expression.

Table 1. PAEP expression associated with clinicopathological characteristics (chi-square test) (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$).

Characteristics	Low expression of PAEP	High expression of PAEP	P value
n	206	206	
Age, n (%)			0.112
≤70	124 (30.1%)	108 (26.2%)	
>70	82 (19.9%)	98 (23.8%)	
Gender, n (%)			0.823
Female	53 (12.9%)	55 (13.3%)	
Male	153 (37.1%)	151 (36.7%)	
Pathologic T stage, n (%)			0.009
T1	3 (0.8%)	2 (0.5%)	
T2	69 (18.3%)	49 (13%)	
T3	78 (20.6%)	118 (31.2%)	
T4	32 (8.5%)	27 (7.1%)	
Pathologic N stage, n (%)			0.448
N0	111 (30.2%)	127 (34.5%)	
N1	21 (5.7%)	25 (6.8%)	
N2	42 (11.4%)	35 (9.5%)	
N3	2 (0.5%)	5 (1.4%)	
Pathologic M stage, n (%)			0.408
M0	126 (59.4%)	75 (35.4%)	
M1	5 (2.4%)	6 (2.8%)	
Pathologic stage, n (%)			<0.001
Stage I	3 (0.7%)	1 (0.2%)	
Stage II	81 (19.8%)	48 (11.7%)	
Stage III	54 (13.2%)	88 (21.5%)	
Stage IV	68 (16.6%)	67 (16.3%)	
Histologic grade, n (%)			<0.001
High grade	185 (45.2%)	203 (49.6%)	
Low grade	19 (4.6%)	2 (0.5%)	
Lymphovascular invasion, n (%)			0.861
No	64 (22.8%)	65 (23.1%)	
Yes	77 (27.4%)	75 (26.7%)	
Subtype, n (%)			<0.001
Non-Papillary	117 (28.7%)	156 (38.3%)	
Papillary	87 (21.4%)	47 (11.5%)	

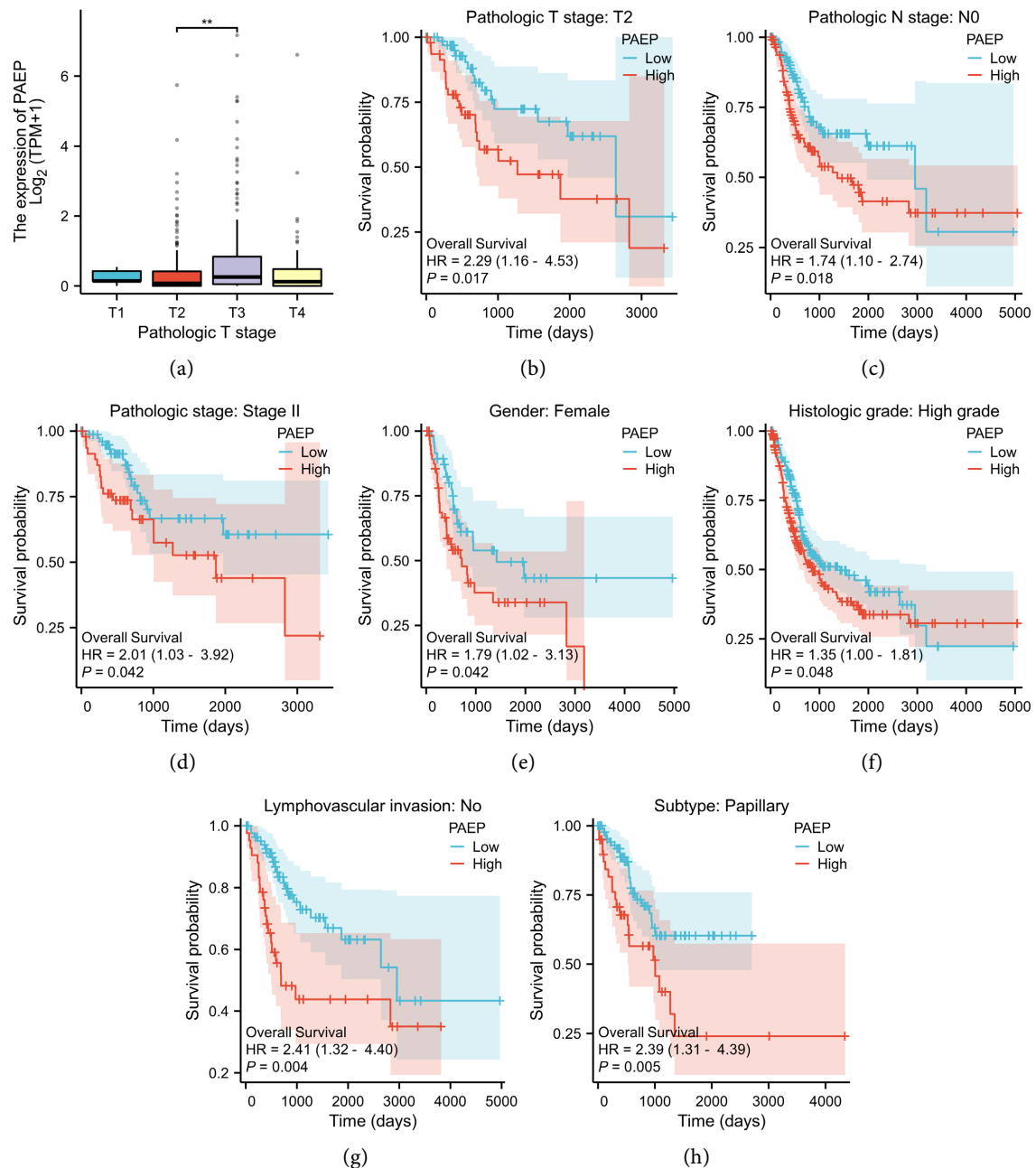


Figure 3. (a) PAEP expression was correlated with T stage in BLCA patients, (b) Prognostic comparison between high and low PAEP groups in Pathologic T stage (T2), (c) Comparison of the prognosis between high and low PAEP groups in the Pathologic N stage, (d) Comparison of the prognosis between high and low PAEP groups in the Pathologic stage: Stage II subgroup, (e) Prognostic comparison between high and low PAEP groups in the Gender: Female subgroup, (f) The prognosis between high and low PAEP groups in the comparison subgroup (Histologic grade: High grade, (g) Compare the prognosis between high and low PAEP groups in Lymphovascular invasion (No), (h) Comparison of the prognosis between the high and low PAEP groups in the Subtype: Papillary subgroup. (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$).

In order to study the effect of PAEP expression and clinicopathological parameters on survival, we used univariate and multivariate Cox regression analysis. Among the variables with $P < 0.05$ in the univariate Cox regression model, Age, Pathologic T stage, Pathologic N stage, Pathologic M stage, Pathologic stage,

Lymphovascular invasion, Subtype, and PAEP expression were all significant. Then, the multivariate cox regression model includes these variables and PAEP. In this study. Age ($P = 0.018$), Pathologic T stage ($P = < 0.001$), Pathologic N stage ($P \leq 0.001$), Pathologic M stage ($P = 0.002$), Pathologic stage ($P \leq 0.001$), Lymphovascular invasion ($P \leq 0.001$), Subtype ($P = 0.036$), PAEP ($P = 0.045$) independently affected the overall survival of BLCA patients. As shown in **Table 2**.

Table 2. Univariate and multivariate analyses of clinicopathological parameters in patients with BLCA.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	411				
≤70	231	Reference		Reference	
>70	180	1.424 (1.064 - 1.906)	0.018	1.158 (0.623 - 2.153)	0.643
Gender	411				
Female	108	Reference			
Male	303	0.868 (0.629 - 1.198)	0.390		
Pathologic T stage	377				
T1&T2	123	Reference		Reference	
T3&T4	254	2.157 (1.485 - 3.132)	<0.001	2.126 (0.474 - 9.536)	0.325
Pathologic N stage	367				
N0	238	Reference		Reference	
N1&N2&N3	129	2.250 (1.649 - 3.072)	<0.001	1.528 (0.686 - 3.406)	0.300
Pathologic M stage	212				
M0	201	Reference		Reference	
M1	11	3.112 (1.491 - 6.493)	0.002	1.093 (0.309 - 3.872)	0.890
Pathologic stage	409				
Stage I&Stage II	133	Reference		Reference	
Stage III&Stage IV	276	2.267 (1.567 - 3.281)	<0.001	0.670 (0.111 - 4.035)	0.662
Histologic grade	408				
High grade	387	Reference		Reference	
Low grade	21	0.338 (0.084 - 1.365)	0.128	0.000 (0.000 - Inf)	0.997
Lymphovascular invasion	280				
No	129	Reference		Reference	
Yes	151	2.247 (1.547 - 3.263)	<0.001	1.963 (0.896 - 4.300)	0.092
Subtype	406				
Non-Papillary	273	Reference		Reference	
Papillary	133	0.690 (0.487 - 0.976)	0.036	1.241 (0.595 - 2.588)	0.565
PAEP	411				
Low	206	Reference		Reference	
High	205	1.351 (1.007 - 1.812)	0.045	1.317 (0.684 - 2.536)	0.410

3.4. Functional Enrichment Analysis

GO includes three components: molecular function (MF), cellular component (CC) and biological process (BP). GO enrichment revealed 15 functions of PAEP related to immunity. KEGG analysis showed that PAEP and its co-expressed mRNA were enriched in 10 signaling pathways related to tumorigenesis and immunosuppression. The results are shown in **Figure 4(a)** and **Figure 4(b)**.

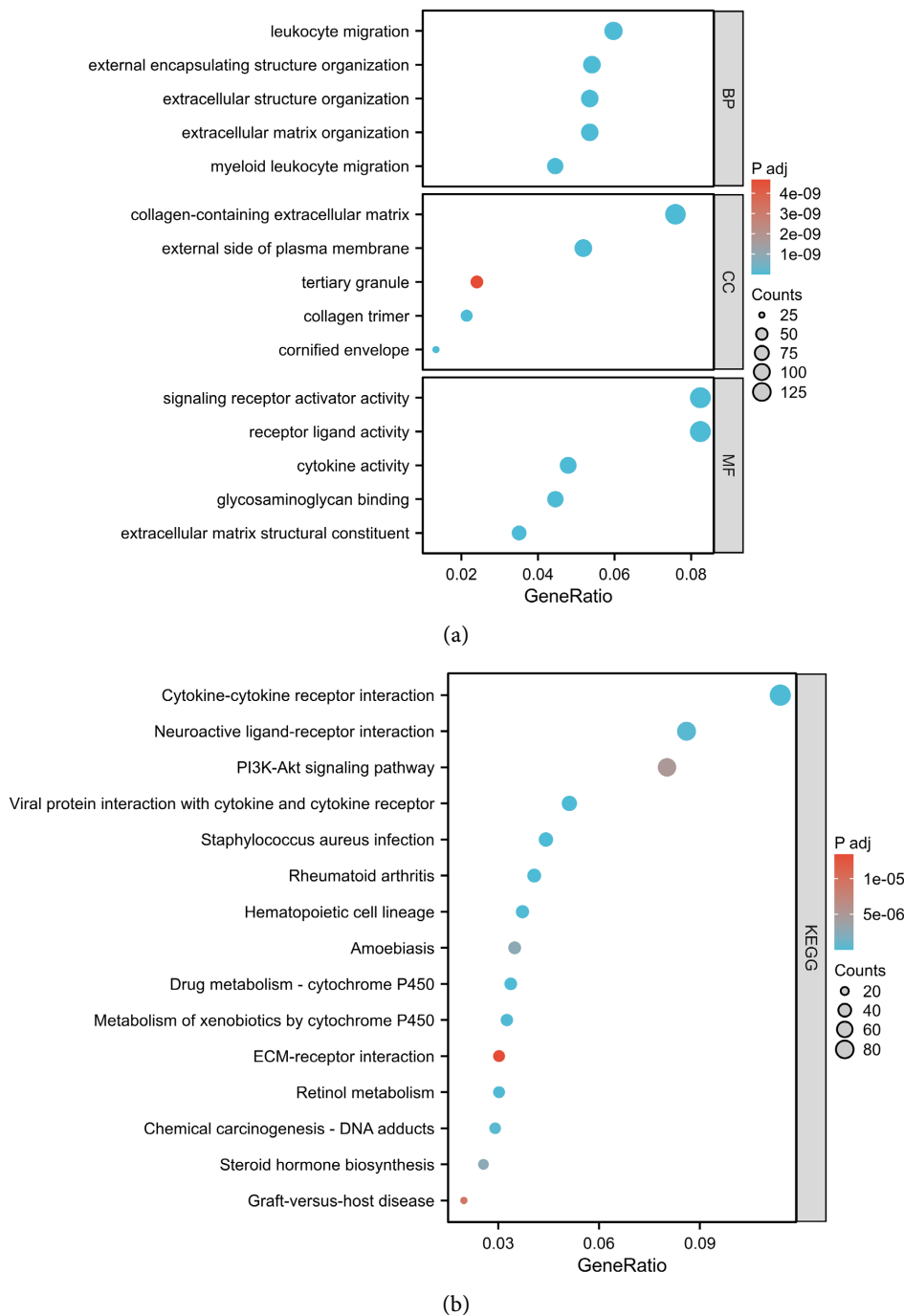
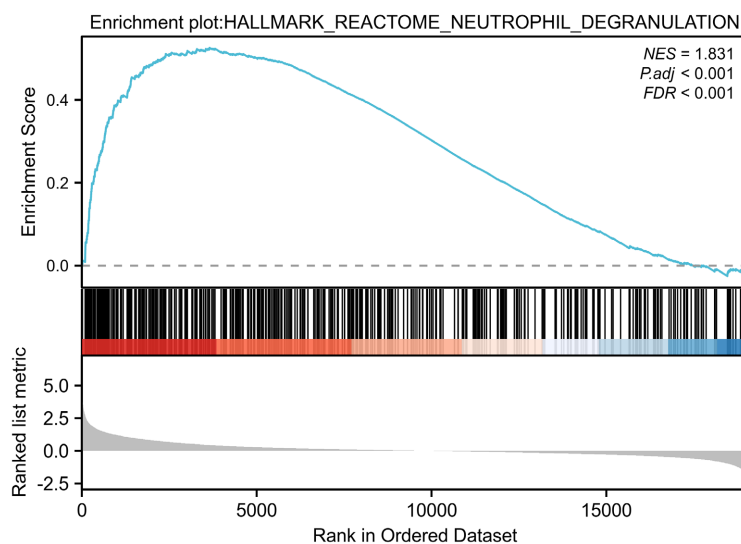


Figure 4. (a) PAEP and its co-expression mRNA-related GO functional enrichment analysis, (b) PAEP and its co-expression mRNA-related KEGG pathway.

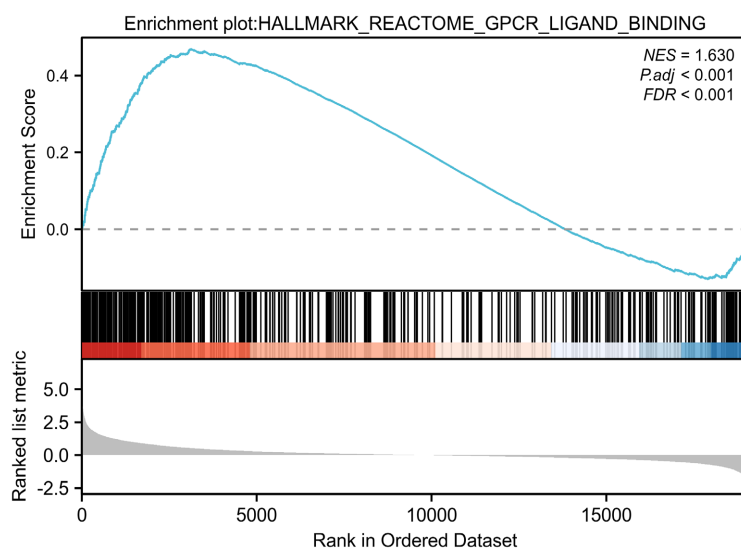
In BLCA patients with high PAEP expression, GSEA analysis showed that the up-regulated signature gene set was mainly enriched in pathways related to tumorigenesis and immune response, including (a) Cytokine-Cytokine Receptor Interaction, (b) Antigen Processing And Presentation, (c) JAK-STATt Signaling Pathway, (d) Natural Killer Cell Mediated Cytotoxin, (e) MET Activates PTK2 Signaling, (f) PI3K-AKT Signaling In Cancer, (g) T Cell Receptor Signaling Pathway, (h) Cancer Immunotherapy By PD1 Blockade, (i) MAPK Signaling Pathway, (j) PI3KAKT Signaling Pathway. The results are shown in **Figures 5(a)-(j)**.

3.5. The Correlation between PAEP Expression and Tumor Immunity

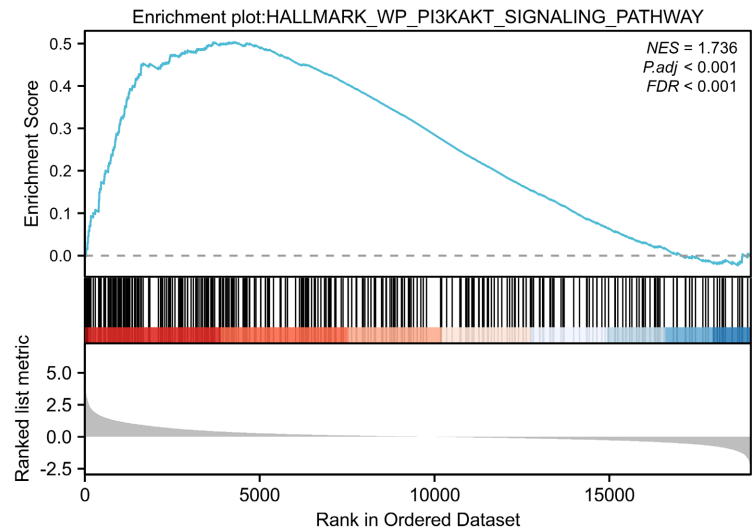
As shown in **Figure 6(a)**, the ss GSEA algorithm was used to evaluate the



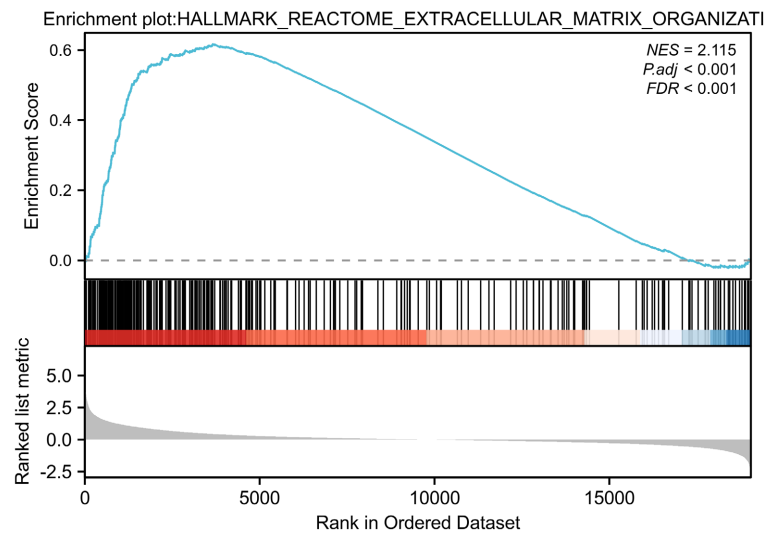
(a)



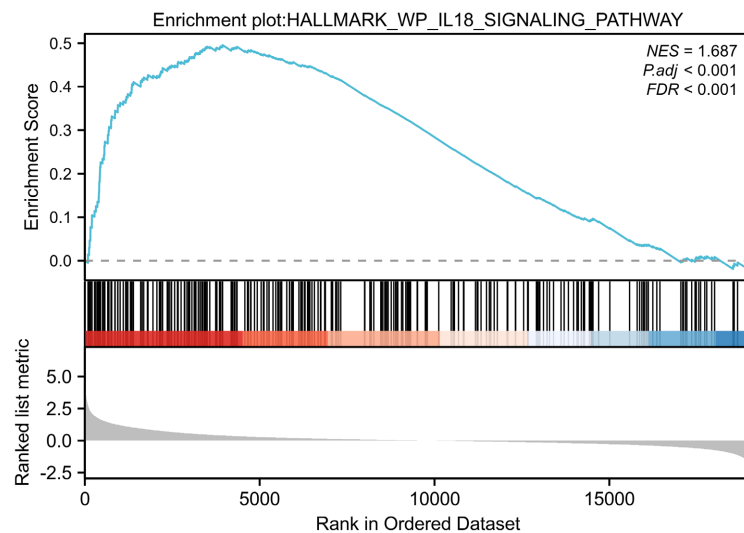
(b)



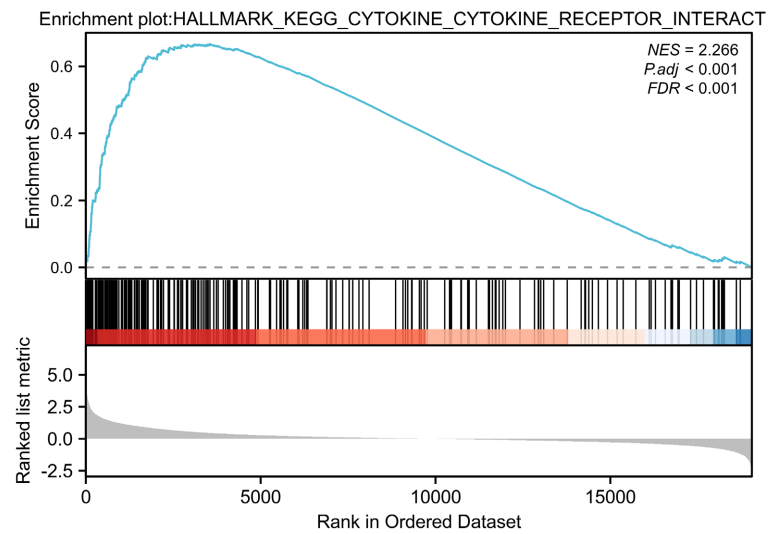
(c)



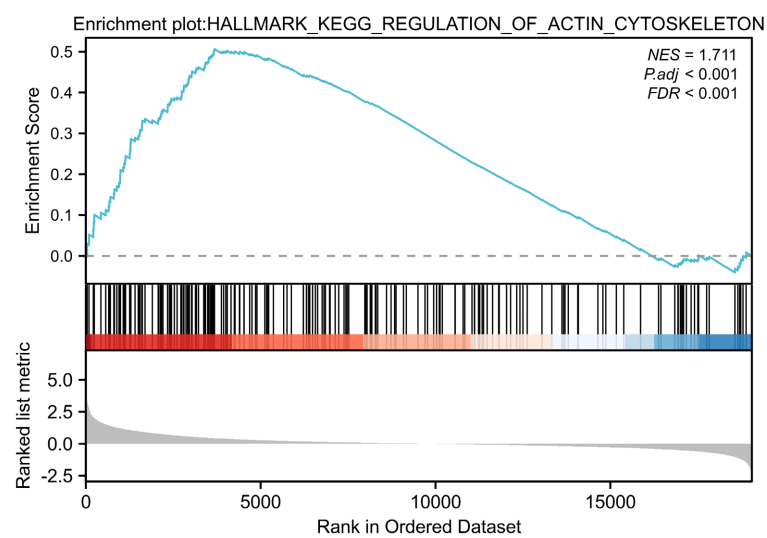
(d)



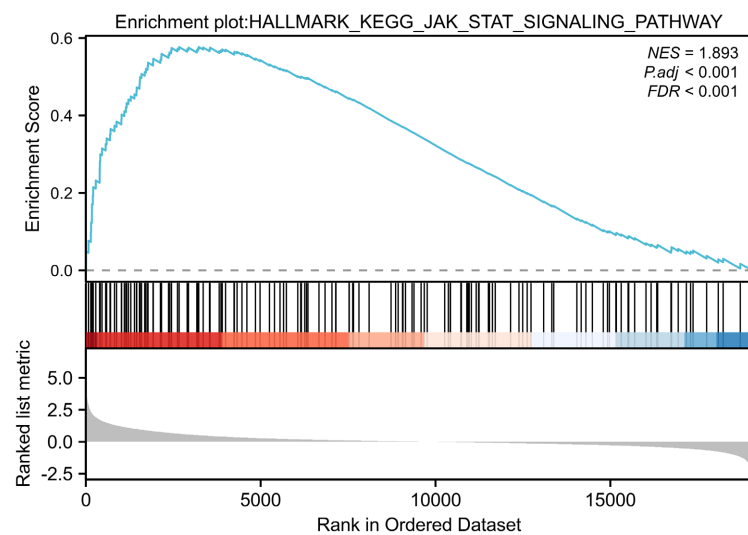
(e)



(f)



(g)



(h)

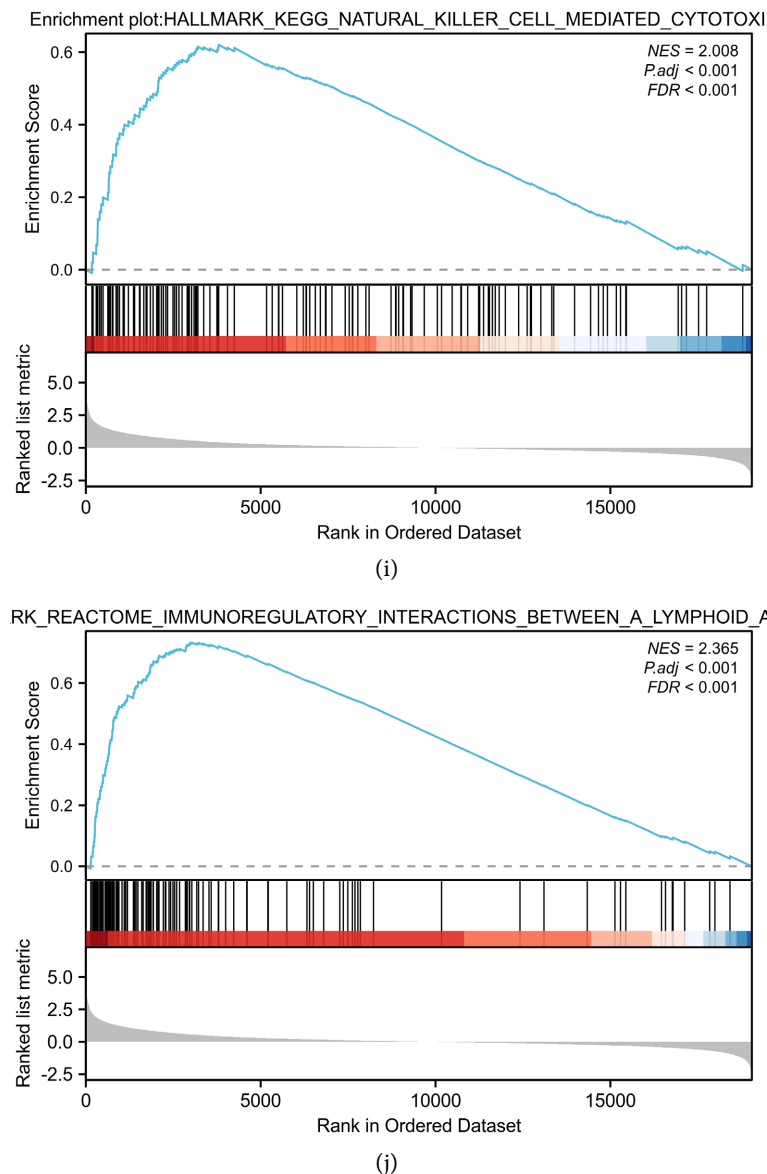
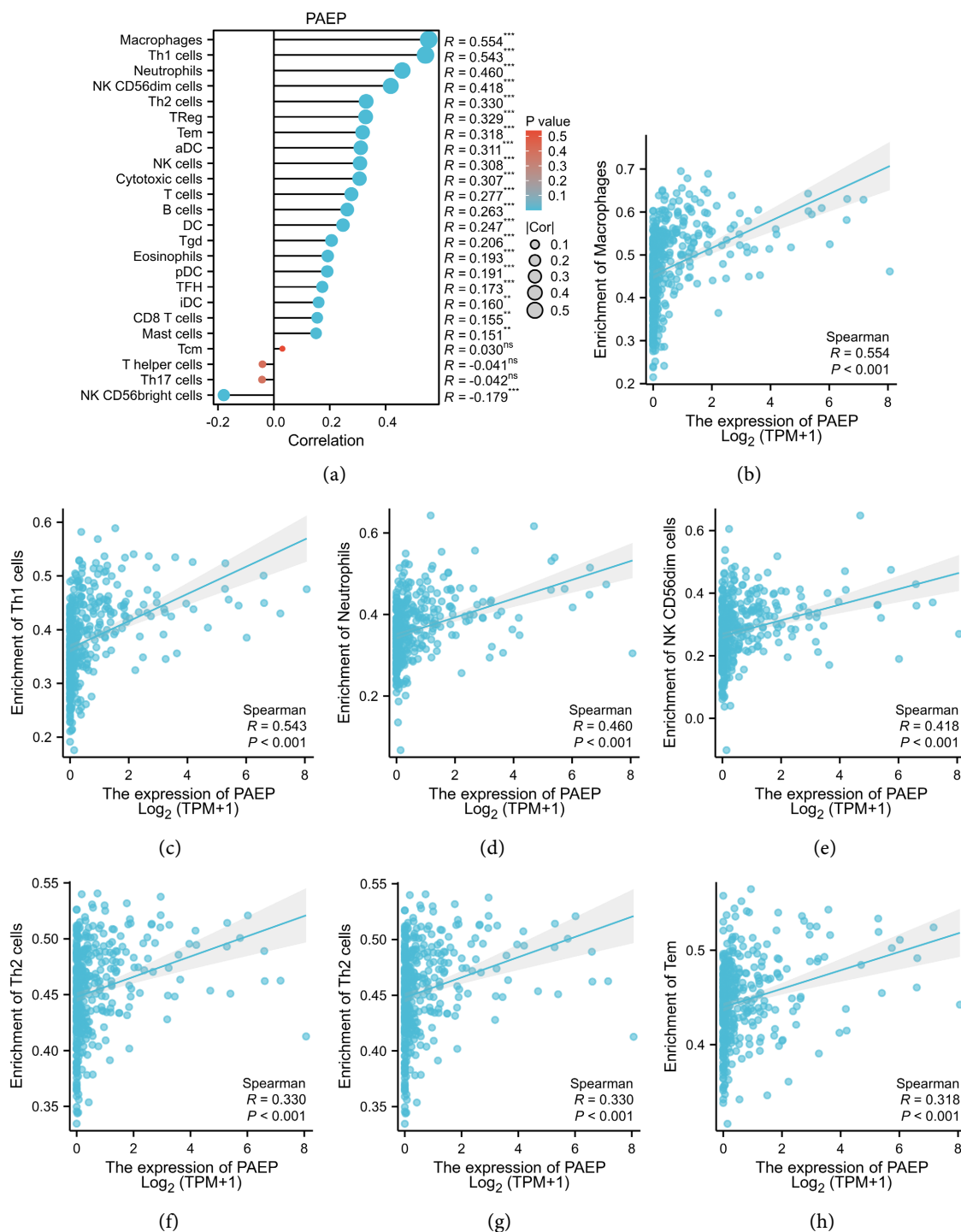


Figure 5. In BLCA patients with high PAEP expression, GSEA analysis showed that the up-regulated signature gene set was mainly enriched in pathways related to tumorigenesis and immune response, including (a) Cytokine-Cytokine Receptor Interaction, (b) Antigen Processing And Presentation, (c) JAK-STATt Signaling Pathway, (d) Natural Killer Cell-Mediated Cytotoxin, (e) MET Activates PTK2 Signaling, (f) PI3K-AKT Signaling In Cancer, (g) T Cell Receptor Signaling Pathway, (h) Cancer Immunotherapy By PD1 Blockade, (i) MAPK Signaling Pathway, (j) PI3KAKT Signaling Pathway.

relationship between the relative abundance of 24 immune cells in BLCA and the expression of PAEP. As shown in **Figures 6(b)-(m)**, different types of immune cells were associated with PAEP expression, including Macrophages ($P < 0.001$, $r = 0.554$), Th1 cells ($P < 0.001$, $r = 0.543$), Neutrophils ($P < 0.001$, $r = 0.460$), NK CD56dim cells ($P < 0.001$, $r = 0.418$), Th2 cells ($P < 0.001$, $r = 0.330$), Treg cells ($P = 0.001$, $r = 0.329$), Tem cells ($P < 0.001$, $r = 0.318$), aDC cells ($P < 0.001$, $r = 0.311$), NK cells ($P = 0.001$, $r = 0.308$), Cytotoxic cells ($P < 0.001$, $r = 0.307$), T cells ($P < 0.001$, $r = 0.277$), B cells ($P < 0.001$, $r = 0.263$)

Wilcoxon rank sum test was used to detect the enrichment of immune cells in

PAEP high and low expression groups. The results showed that compared with the PAEP low expression group, the PAEP high expression group had higher enrichment of (a) Macrophages, (b) Th1 cells, (c) Neutrophils, (d) NK CD56dim cells, (e) Th2 cells, (f) Treg cells, (g) Tem cells, (h) aDC cells, (i) NK cells, (j) Cytotoxic cells, (k) T cells, and (l) B cells (m) DC cells, (n) Tgd, (o) Eosinophils, (p) pDC cells, (q) TFH cells, (r) CD8 T cells, (s) iDC cells, and (t) Mast cells. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ (Figures 7(a)-(t)).



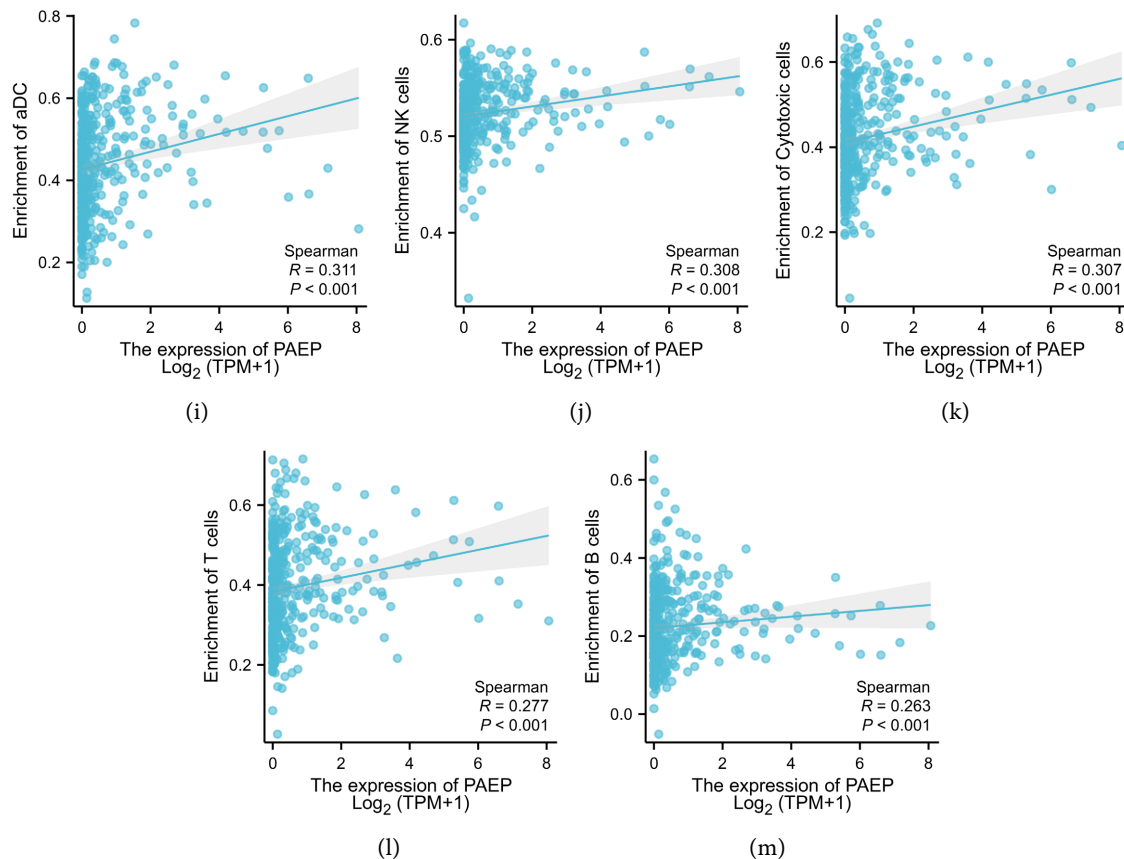


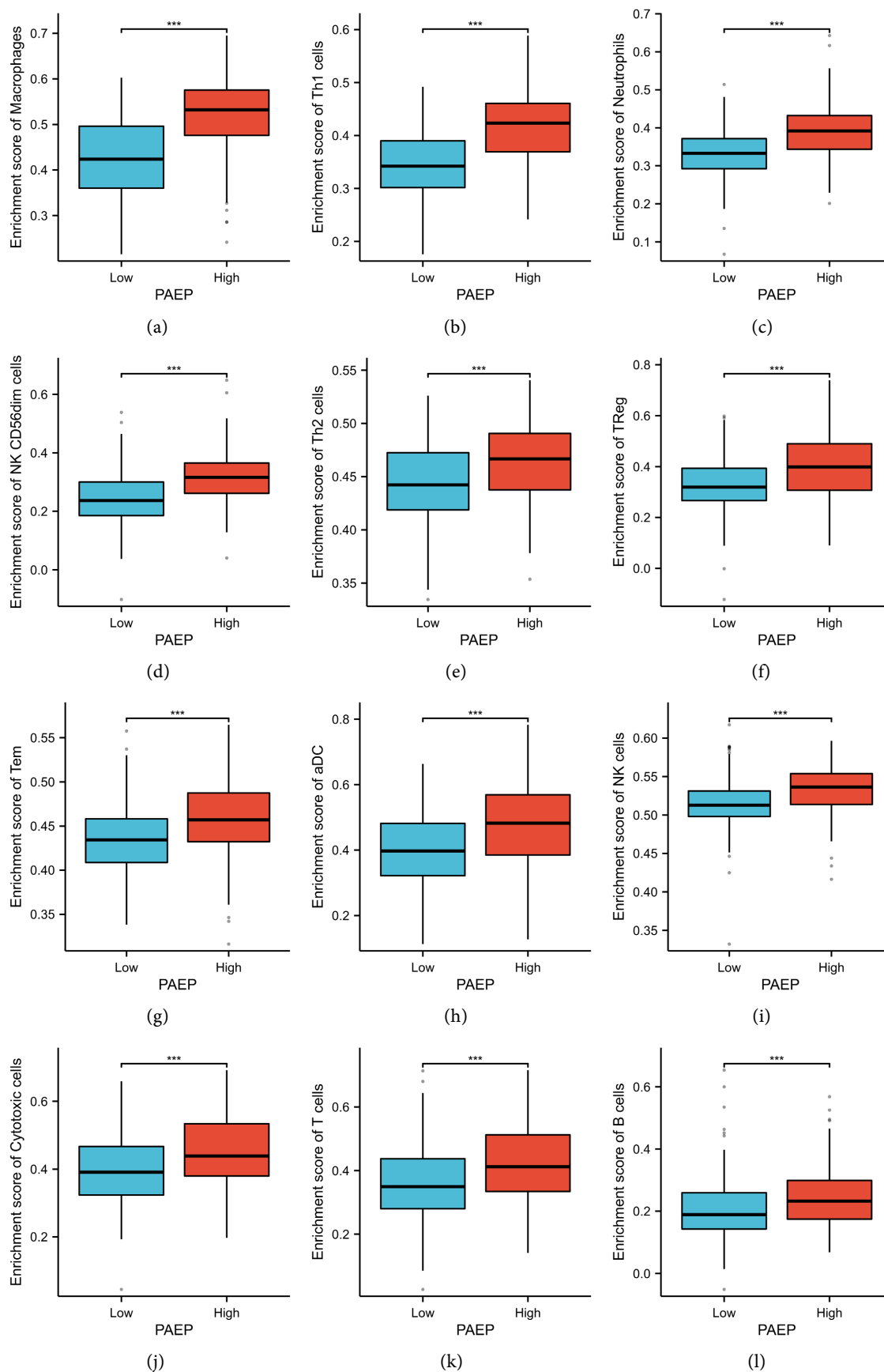
Figure 6. (a) In the bar graph, PAEP expression was correlated with 24 immune infiltration cells. The horizontal axis represents correlations, and the vertical axis represents immune cells. PAEP expression was positively correlated with (b) Macrophages, (c) Th1 cells, (d) Neutrophils, (e) NK CD56dim cells, (f) Th2 cells, (g) Treg cells, (h) Tem cells, (i) aDC cells, (j) NK cells, (k) Cytotoxic cells, (l) T cells, and (m) B cells.

Spearman correlation analysis showed that PAEP expression was positively correlated with PD-1 (PDCD1) ($P < 0.001$, $r = 0.345$), PD-L1 (CD274) ($P < 0.001$, $r = 0.460$) CTLA4 ($P < 0.001$, $r = 0.393$) (Figures 8(a)-(c)).

4. Discussion

For BLCA, molecular targeted therapy and immunotherapy have played a certain therapeutic role in recent years [6] [46] [47]. For example, the development of immune checkpoint inhibitors has shown clinical efficacy [48] [49], The use of immune checkpoint inhibitor therapy has a positive therapeutic effect on patients with bladder cancer. These therapies have been shown to cure some bladder cancer patients and significantly reduce adverse events [48]. However, not all patients with bladder cancer can benefit from the treatment of immune checkpoint inhibitors [50] [51], Therefore, it is necessary to further search for immune-related genes to improve the diagnosis and prognosis of BLCA patients.

We obtained clinical data and RNA data of BLCA patients from the TCGA database, and downloaded immune-related genes from the ImmPort database [52]. Then, DEMRNA in BLCA was obtained by bioinformatics method. Through



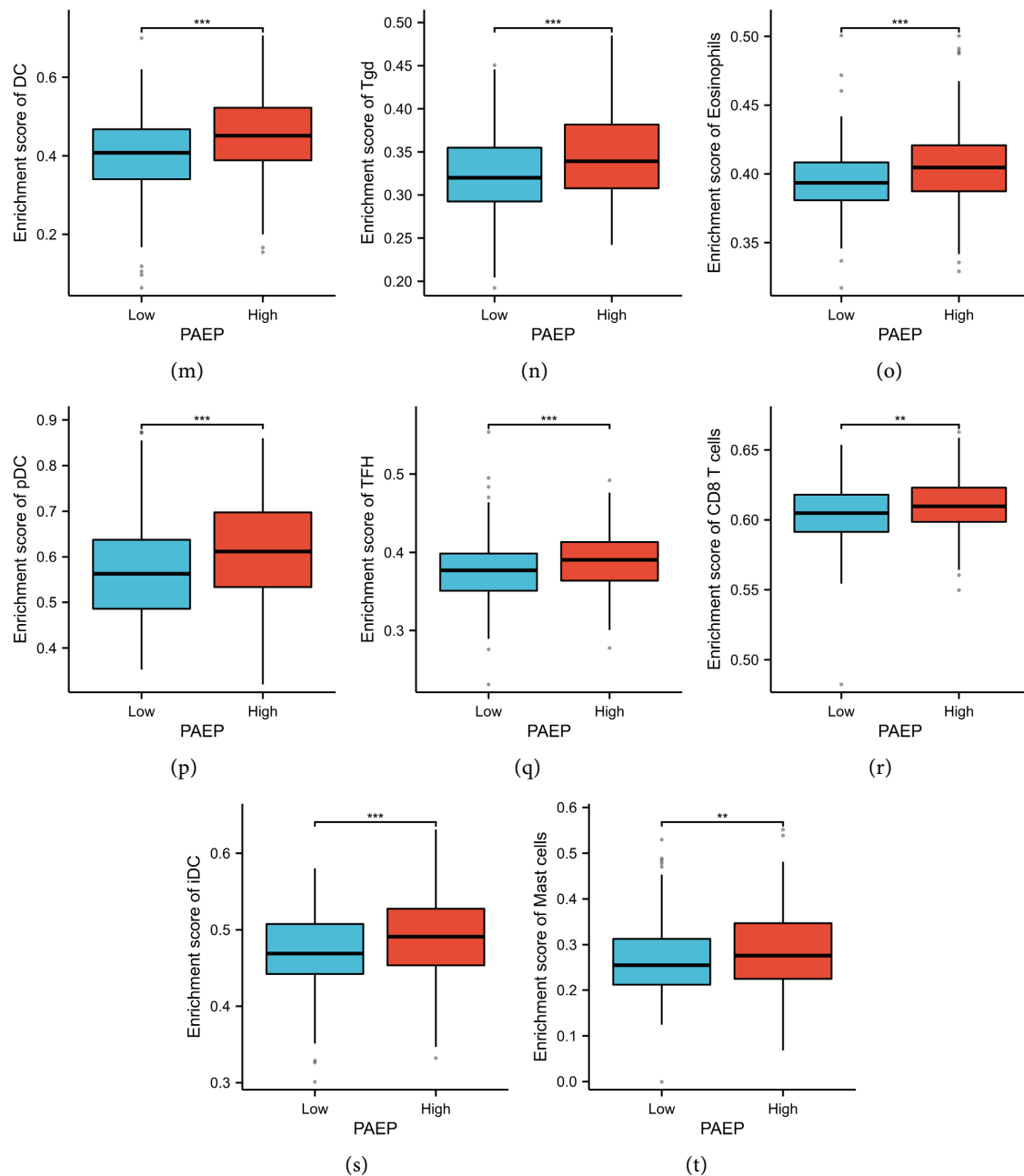


Figure 7. Wilcoxon rank sum test was used to detect the enrichment of immune cells in PAEP high and low expression groups. The results showed that compared with the PAEP low expression group, the PAEP high expression group had higher enrichment of (a) Macrophages, (b) Th1 cells, (c) Neutrophils, (d) NK CD56dim cells, (e) Th2 cells, (f) Treg cells, (g) Tem cells, (h) aDC cells, (i) NK cells, (j) Cytotoxic cells, (k) T cells, and (l) B cells (m) DC cells, (n) Tgd, (o) Eosinophils, (p) pDC cells, (q) TFH cells, (r) CD8 T cells, (s) iDC cells, and (t) Mast cells. (a)-(t) (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$).

Venn diagram analysis, we finally selected PAEP as the target gene. Studies have found that the expression level of PAEP is significantly increased in human malignant melanoma and inhibits the activation, proliferation and cytotoxicity of T lymphocytes, which may be caused by the induction of immune tolerance by malignant melanoma cells in the tumor microenvironment [53]. The expression level of PAEP is highly expressed in non-small cell lung cancer and plays an

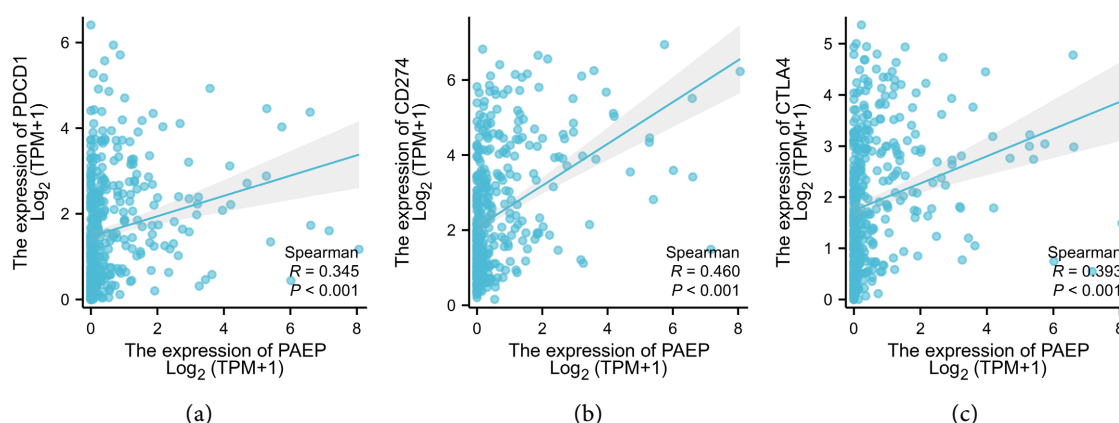


Figure 8. (a)-(c) PAEP and tumor immune checkpoint expression. There was a significant correlation between (a) PD1, (b) PD-L1, (c) CTLA4.

immunosuppressive role in non-small cell lung cancer [54]. PAEP is highly expressed in ovarian cancer and is a potential biomarker [29]. However, there are few studies related to PAEP in BLCA. In our study, according to the UCSC XENA database and the TCGA database, bladder cancer tissues have higher PAEP expression levels than normal bladder samples. The up-regulated expression of PAEP in BLCA was associated with Pathologic T stage ($P = 0.009$), Pathologic stage ($P < 0.001$), Histologic grade ($P < 0.001$) and Subtype ($P < 0.001$). PAEP has a high diagnostic rate ($AUC = 0.780$). In addition, according to univariate and multivariate Cox regression analysis, we found that PAEP was an independent prognostic factor ($P = 0.045$), suggesting that BLCA patients may benefit from the use of PAEP as a biomarker for diagnosis and prognosis.

Through GO, KEGG and GSEA pathway enrichment analysis, it was found that PAEP and its co-expressed mRNA were enriched in some signaling pathways, such as Cytokine-cytokine receptor interaction. Research has indicated Cytokine-cytokine receptor interaction is an important way to contain a variety of cytokines and their receptors. The binding of cytokines and their receptors has effects on cells, such as cell growth, proliferation and differentiation, and regulates collective immune responses [55]. Study has shown that the PI3K/AKT pathway plays a key role in promotion of the EMT process during Colorectal cancer progression by upregulation of mesenchymal markers and EMT specific transcription factors that result in Colorectal cancer metastasis [56]. It has been found that PI3K/AKT pathway regulates EMT-mediated metastasis in bladder cancer [57] [58]. IL-17 is a pro-inflammatory cytokine that plays a key regulatory role in the anti-tumor immune response. Th17, as a subtype of CD4 + cells, releases cytokine IL-17, which plays a key role in the innate and adaptive immune system [59].

Activation of PI3K/Akt signaling mediates drug resistance and reduces radio-sensitivity of tumor cells. Anti-tumor compounds suppress PI3K/Akt signaling in impairing tumor progression [60]. To improve response rates, combination therapies are being investigated, including combining anti-PD(L)1 drugs

with other immunotherapies, targeted therapies, or chemotherapy. Additionally, biomarkers are being studied to identify which patients are most likely to benefit from anti-PD(L)1 therapy. Overall, the development of anti-PD(L)1 drugs has revolutionized the treatment of metastatic urothelial cancer, but there is still much research needed to optimize their efficacy and improve outcomes for all patients [61] [62]. NK cells could alleviate the side effects of cisplatin treatment and enhance antitumor activity. The combination of NK cells and cisplatin thus provides a promising option for chemoimmunotherapy for bladder cancer [63]. NK cells from NMIBC patients displayed a low density on NK cytotoxicity receptors, adhesion molecules and a more immature phenotype, losing their ability to kill and drive differentiation of CSCs [64].

In the study, the correlation analysis revealed significant associations between PAEP and PD-L1 (CD274), PD-1 (PDCD1), CTLA4, and immune cells (such as Macrophages, Th1 cells, Neutrophils, NK CD56dim cells, Th2 cells, Treg cells, Tem cells, aDC cells, NK cells, Cytotoxic cells, T cells, B cells, and so on). Our results indicated that in the microenvironment of BLCA, PAEP was associated closely with immune infiltration and immunosuppression.

In summary, the results obtained at present are all from the public database, which are not verified at the cellular and molecular level in vitro. In vitro experiments will be carried out in the later stage to further understand its molecular mechanism.

5. Conclusions

In summary, our study shows that PAEP is associated with tumor immunity, It may be a biomarker related to the diagnosis and prognosis of bladder cancer patients, and a potential target for bladder cancer immunotherapy. In future studies, the function of PAEP will be further verified at the cellular level.

Highlight Box

Key findings

PAEP is a biomarker related to prognosis and immunity in bladder cancer

What is known and what is new?

To conclude, our study suggested that PAEP was associated with tumor immunity, and might be a biomarker associated with the diagnosis and prognosis of BLCA patients, and a potential target of BLCA immunotherapy.

What is the implication, and what should change now?

The results obtained at present are all from the public database, which are not verified at the cellular and molecular level in vitro. In vitro experiments will be carried out in the later stage to further understand its molecular mechanism.

- Report here about implications and actions needed.
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Contributions

1) Conception and design: Ning Tang, Shasha Gai; 2) Administrative support: Qun Huang; 3) Provision of study materials or patients: Ning Tang, Qun Huang; 4) Collection and assembly of data: Ning Tang, Shasha Gai; 5) Data analysis and interpretation: Ning Tang, Shasha Gai; 6) Manuscript writing: All authors; 7) Final approval of manuscript: All authors

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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