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Original Article

Upregulation of RAC3 in bladder cancer predicts adverse clinical outcome and increased tumor immune response

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Abstract: The relationship between RAC3 expression and clinical outcome in bladder cancer (BLCA) was uncertain. In this study, the expression level of RAC3 in BLCA and its clinical outcome were analyzed through various independent public databases. The mRNA expression level of RAC3 in BLCA and normal bladder was evaluated from the Gene Expression Omnibus (GEO), Oncomine, and The Cancer Genome Atlas (TCGA) database. The protein expression of RAC3 in BLCA and normal bladder was investigated from immunohistochemical images through the Human Protein Atlas (HPA) database. Next, gene tumor immune analyses were performed. Furthermore, gene set enrichment analysis (GESA) by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes enrichment (KEGG) for RAC3 and its co-expressed genes were performed. Then, GESA was also performed to validate the KEGG pathways by the different expression of RAC3 in BLCA. The results indicated that, compared with normal bladder, the mRNA and protein expression of RAC3 in BLCA were both significantly higher than those of normal bladder tissues ($P<0.05$). The tumor immune analyses indicated RAC3 was associated with microsatellite instability, tumor mutational burden, tumor immune microenvironment, and immune cell infiltration level evaluation ($P<0.05$). The survival analysis result demonstrated that upregulation of RAC3 was associated with adverse survival in BLCA ($P<0.05$). Taken together, these findings suggest that RAC3 may be associated with adverse clinical outcome and increased tumor immune response in BLCA, and may be a prognostic and immunotherapy marker for BLCA.

Keywords: RAC3, bladder cancer, clinical, tumor immune, prognosis

Introduction

Bladder cancer (BLCA) is the most common type of cancer in the urinary system [1], with high morbidity and mortality rates, and affected about 3.4 million people worldwide in 2015 [2]. The worldwide age-standardized incidence rate (per 100,000 person/years) of BLCA was 9.0 for men and 2.2 for women, and the mortality rate (per 100,000 person/years) was 3.2 for men vs. 0.9 for women in 2018 [3]. BLCA can divided into high- and low-grade according to histology [4]. Low-grade BLCA rarely invades the bladder muscle, relapses, or metastasizes; the majority of BLCA-related deaths are due to high-grade disease [4]. Although the early diagnosis and treatment of BLCA can significantly improve the patient survival and prognosis status, BLCA-associated mortality remains high. Evidence indicates that immunotherapy is effective in the treatment of BLCA [5-7]. Therefore, it is crucial to explore novel clinical and therapeutic biomarkers to improve the treatment of BLCA.

Ras-related C3 botulinum toxin substrate 3 (RAC3) belongs to the Rho subfamily of Ras proteins [8], is a member of Rho-GTPase family, and adopts an active state bound to GTP and an inactive state bound to GDP [9]. Rac is a subset of Rho-GTPase. Rho and Rac-GTPases are related to human carcinogenesis, cancer cell proliferation, migration, and aggressiveness [9-11]. RAC3 is highly expressed in a wide range of tissues [8]. Previous studies indicate that RAC3 promotes cell proliferation

and cell aggressiveness in lung cancer [9], breast cancer [12], prostate cancer [13], esophagus [11] and ovarian cancer [14]. However, the role of RAC3 in the carcinogenesis of BLCA remains unclear.

In this study, the expression of RAC3 and its clinical outcomes in BLCA were investigated through various public independent bioinformatics datasets. In addition, the relationship between the tumor immune analyses and mRNA expression of RAC3 in BLCA were analyzed. Furthermore, gene set enrichment analysis (GSEA) was performed for genes co-expressed with RAC3 in BLCA, and finally, GSEA was performed to validate the differential expression of RAC3 in BLCA.

Materials and methods

Data acquisition and processing

All data were acquired and processed from Oncomine [15], Gene Expression Omnibus (GEO) [16], The Cancer Genome Atlas (TCGA) [17], Genotype-Tissue Expression (GTEx) [18], and Human Protein Atlas (HPA) [19] databases. The GEO accession GSE13507 [20] and Oncomine dataset Lee Bladder [21] were downloaded to analyze the mRNA expression of RAC3. The datasets from TCGA and GTEx databases were downloaded using R package 'TCGAbiolinks' v2.14.1 [22]. In the Oncomine database, the threshold settings were $P<0.05$, |fold change| >1.5, and gene rank in the top 10%.

Analysis of expression of RAC3 in BLCA and normal bladder

The mRNA expression of RAC3 between BLCA and normal bladder were obtained from the GEO, Oncomine and TCGA databases, respectively. The protein expression of RAC3 between BLCA and normal bladder tissue were validated by immunohistochemical images retrieved from the HPA database.

Tumor immune analyses

TCGA-BLCA dataset was further used for the evaluations of microsatellite instability (MSI), tumor mutational burden (TMB), tumor immune microenvironment, and immune cell infiltrate score. Microsatellite Analysis for Normal Tumor InStability (MANTIS) tool [23] was used for the estimation of MSI. The estimation of TMB was performed using the R package 'maftools' v2.2.10 [24]. Estimation of STromal and Immune cells in MAlignant Tumors using Expression data (ESTIMATE) algorithm [25] was used for tumor immune microenvironment evaluation of RAC3 expression in BLCA, Tumor IMmune Estimation Resource (TIMER) algorithm [26] was used for exploring the correlation between RAC3 expression and abundance of immune cells infiltration level in BLCA. Spearman's Rho method was used in all rank correlation coefficient analyses.

Survival analysis

Kaplan-Meier survival analysis was done to show a relationship between RAC3 expression and survival prognosis in BLCA using data from the GEO accession GSE13507 dataset, and then the TCGA-BLCA dataset was used for further verification.

Gene set enrichment analysis

The Spearman correlation coefficients between RAC3 and all other mRNA genes in TCGA-BLCA dataset were batch calculated by cor.test () function of R language. After sorting the Spearman correlation coefficient, the R package 'clusterProfiler' v3.14.3 [27] was carried out for co-expression Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway gene set enrichment analysis (GSEA). Finally, GSEA for KEGG signaling pathway verification was investigated according to the high- and low expression value of RAC3 in the TCGA-BLCA dataset using R package 'clusterProfiler' again. The |normalized enrichment score| >1 and $P<0.05$ were considered significant differences.

Statistical analysis

All statistical analyses were carried out using R software v4.02. The box plot and dot plot were constructed using the R package 'ggstatsplot' v0.50. Categorical variables were compared using Chi-square analysis or Fisher's exact test. Continuous data were compared using independent t-tests or one-way ANOVA. Kaplan-Meier analysis and Cox proportional hazard models were adopted for survival analysis using R package 'survminer' v0.4.7 and 'survival' v3.2.3. Multivariate Cox proportional hazards regression model was applied

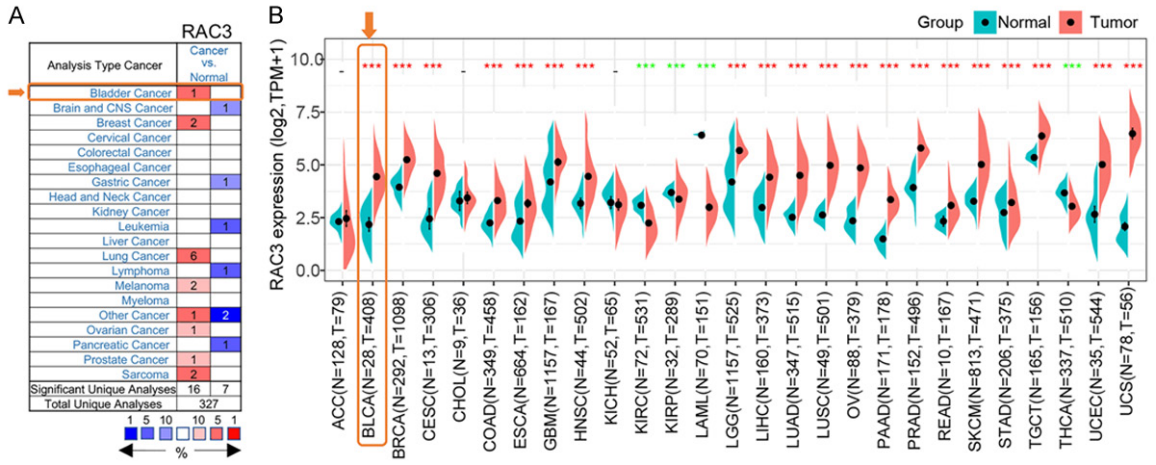

Figure 1. mRNA expression of RAC3 in various cancers. A. Expression of RAC3 in cancer vs. healthy tissues from the Oncomine database. The cell color indicates best gene rank percentile for the analysis within the cell. Red color represents up-regulation and blue color represents down-regulation. B. Expression of RAC3 in various types of tumor vs. healthy tissues from the TCGA and GETx database. Boxes represent the median, 25th and 75th percentiles, and each dot represents expression of samples.

to adjust for covariate effects. Missing data were coded and excluded from the analysis. Statistical significance was considered as $p<0.05$.

Results

Higher RAC3 mRNA expression in bladder cancer was identified by public databases

In the Oncomine database, the comparison between each type of cancer and normal tissues identified an upregulation of RAC3 expression in bladder cancer, breast cancer, lung cancer, melanoma, ovarian cancer, prostate cancer, sarcoma and uterine corpus leiomyoma, and a downregulation of RAC3 expression in brain and CNS cancer, gastric cancer, leukemia, lymphoma, pancreatic cancer, and skin squamous cell carcinoma (**Figure 1A**). In TCGA combined GTEx database, compared with matched normal tissues, among various cancers, only 4 cancer types showed significantly lower RAC3 expression, and the remaining 20 cancer types showed significantly higher RAC3 expression (**Figure 1B**). Furthermore, both results demonstrated a significant upregulation of RAC3 in bladder cancer.

BLCA exhibited higher level of RAC3 mRNA expression

Three independent datasets from the GEO, Oncomine and TGCA databases were analyzed for the mRNA expression level of RAC3 in BLCA and normal bladder samples. All the results demonstrated a significant upregulation of RAC3 in BLCA compared with normal bladder tissues (**Figure 2A**). We also compared the different histologic grades of bladder cancer in the three independent databases. The results showed that the higher the histologic grade of bladder cancer, the more significantly up-regulated the expression of RAC3 (**Figure 2B**).

BLCA exhibited a higher level of RAC3 protein expression

To validate the protein expression of RAC3 in BLCA, we investigated the immunohistochemical data of RAC3 expression in BLCA and normal bladder tissue from the HPA database, and the results showed that normal bladder tissue was low staining, the low-grade urothelial carcinoma tissue had medium staining, and high-grade urothelial carcinoma tissue had high staining. This revealed that RAC3 was upregulated in BLCA and positively associated with histologic grade (**Figure 3**).

Higher RAC3 expression predicted worse outcome in BLCA patients

To further investigate the relationship between RAC3 expression and clinicopathologic features of bladder cancer, we retrieved the clinicopathologic data of bladder cancer patients from the TCGA database. According to the mean expression of RAC3, the BLCA patients were divided into high and low expres-

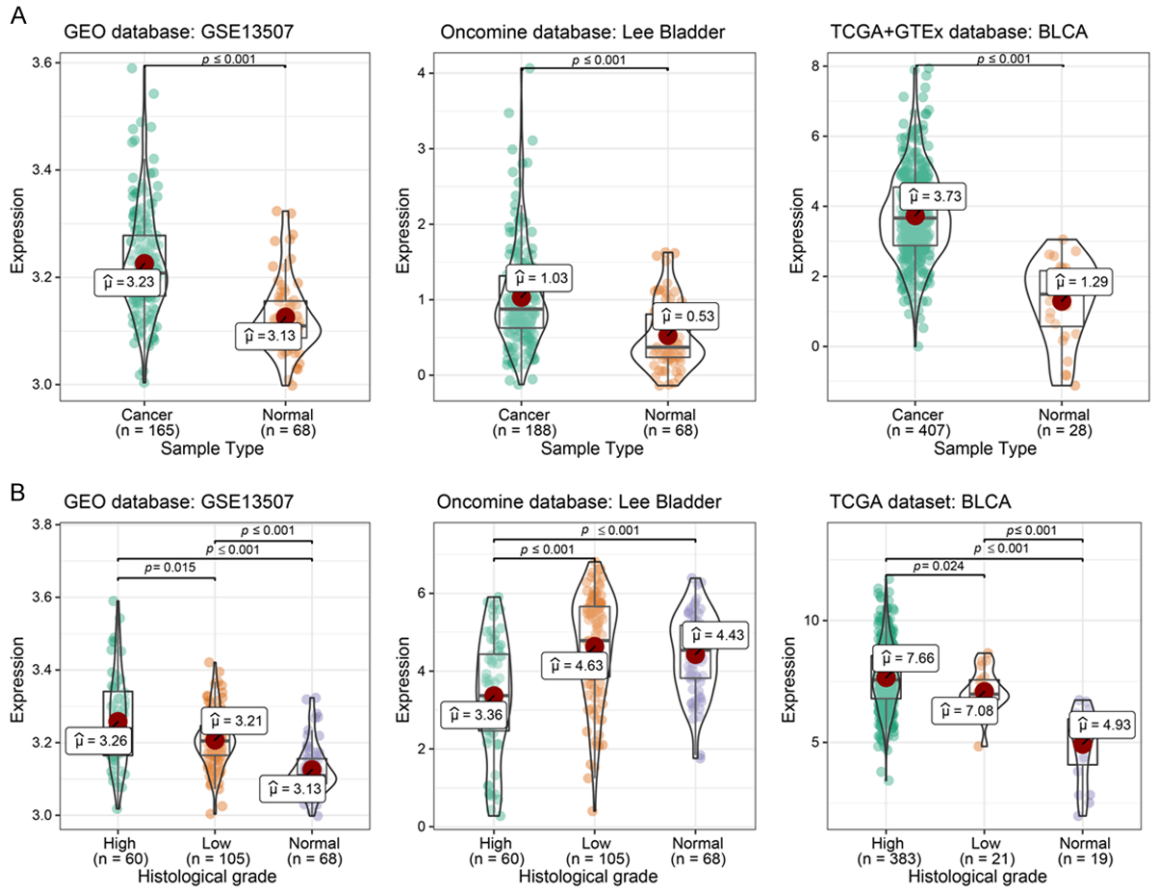

Figure 2. mRNA expression of RAC3 in patients with BLCA. A. mRNA expression of RAC3 in BLCA compared with normal bladder tissues from the GEO database GSE13507 dataset, Oncomine database Lee Bladder dataset and TCGA combined with GTEx databases bladder datasets, respectively. B. mRNA expression of RAC3 in different grades of BLCA compared with normal bladder tissues from the GEO database GSE13507 dataset, Oncomine database, Lee Bladder dataset, and TCGA database BLCA dataset, respectively.

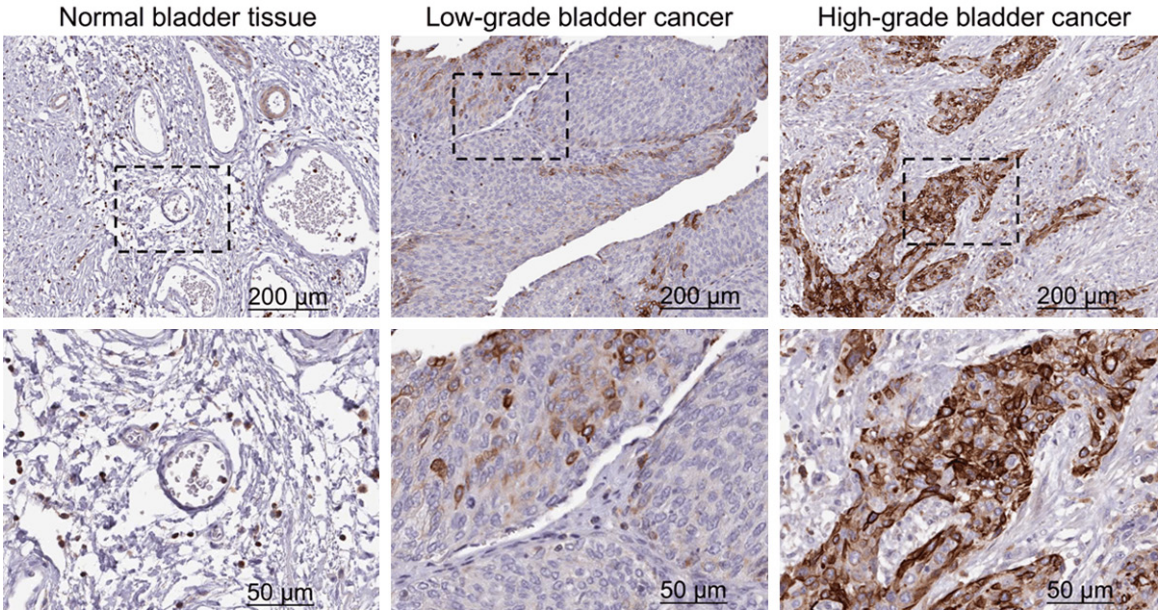

Figure 3. Protein expression of RAC3 in normal bladder, low-grade bladder cancer, and high-grade bladder cancer tissue by immunohistochemistry from the HPA database. Normal bladder tissue showed low staining, low-grade bladder cancer tissue showed medium staining, and high-grade bladder cancer tissue showed high staining. Bar of the upper figures = 200 μm. Bar of the lower figures = 50 μm.

Table 1. Relationship between different expression groups of RAC3 and clinical characteristics in bladder cancer

Finding	High expression (n = 192)		Low expression (n = 215)		*p*-value
	n	%	n	%	
Gender					0.65151
Female	52	27.08%	51	23.72%	
Male	140	72.92%	161	74.88%	
Age, years					0.62062
<60	39	20.31%	48	29.81%	
≥60	153	79.69%	167	77.67%	
BMI, kg/m^2					0.78845
<25	72	47.06%	77	35.81%	
≥25	98	51.04%	111	51.63%	
NA	22	11.46%	27	12.56%	
Smoking history					0.01445
Yes	67	34.90%	52	24.19%	
No	118	61.46%	157	73.02%	
NA	7	3.65%	6	11.54%	
Grade					0.02763
High	187	97.40%	199	92.56%	
Low	5	2.60%	16	7.44%	
Subtype					0.09985
Papillary	54	28.88%	78	36.28%	
Non-Papillary	134	69.79%	136	63.26%	
NA	4	2.08%	1	0.47%	
Lymph node metastasis					0.03398
No	104	54.17%	132	61.40%	
Yes	77	40.10%	62	28.84%	
NA	11	5.73%	21	9.77%	
Distant metastasis					0.00752
No	80	41.67%	116	53.95%	
Yes	9	4.69%	2	0.93%	
NA	103	53.65%	97	45.12%	
Cancer stage					0.04412
Stage I+II	54	28.13%	79	36.74%	
Stage III+IV	138	71.88%	136	63.26%	

BMI, Body mass index; NA, not available.

sion groups. Compared with the lower expression group of RAC3, the higher expression group of RAC3 had significant differences in smoking history, grade, lymph node metastasis, distant metastasis status, and cancer stage ($P<0.05$, **Table 1**). Upregulation of RAC3 in BLCA correlated with heavier smoking history, higher grade, higher stage, and more tendency to lymph node and distant metastasis.

RAC3 expression was associated with tumor immune response in BLCA

We next conducted a comprehensive analysis of tumor immunity using the TCGA-BLCA dataset. In the association analysis of tumor immune cell infiltration level and RAC3 expression, based on the TIMER algorithm, we found that RAC3 expression was correlated with mac-

rophages and neutrophils, among which, there was a significant negative correlation with neutrophils, and a positive association with macrophages in tumor cells (**Figure 4A**). Next, in the tumor immune microenvironment score analysis, based on the ESTIMATE algorithm, we found that RAC3 expression was significantly negatively correlated with ESTIMATE score and immune score (**Figure 4B**). Finally, we found that RAC3 expression were both significantly positively correlated with TMB and MSI (**Figure 4C**, **4D**).

Higher RAC3 expression predicted adverse survival prognosis in BLCA

We first performed a Kaplan-Meier survival analysis on patients with BLCA using the GES13507 dataset. Results showed that high expression of RAC3 was significantly negatively correlated with disease-specific survival (DSS) and overall survival (OS) (**Figure 5A**, **5B**). Then, TCGA-BLCA dataset was used to verify these results. At first, multiple risk factors for BLCA were analyzed by univariate and multivariate Cox hazard models. The results of univariate analysis showed that the survival prognosis of BLCA is correlated with age, lymph node metastasis, distant metastasis, tumor stage, and RAC3 expression. Also the high expression of RAC3 had a significant association. RAC3 expression was also correlated with survival prognosis by multivariate analysis ($P<0.05$, **Table 2**). Next, Kaplan-Meier survival analysis was performed to verify the prognostic relationship between the different expression levels of RAC3. Similarly, the results showed that high expression of RAC3 was not only negatively correlated with DSS and OS, but also significantly negatively correlated with disease-free interval (DFI) and progression-free interval (PFI) (**Figure 5C-F**). These results all indicated an adverse prognostic relevance of RAC3 expression in BLCA.

GSEA for RAC3 expression in BLCA

GO functional and KEGG pathway GSEA were performed with RAC3, and the co-expressed mRNA genes. GO functional GSEA for RAC3 and its co-expressed genes was predominantly associated with 'Spliceosomal tri-snRNP complex', 'Precatalytic spliceosome', 'Mitotic spindle assembly checkpoint', 'Spindle checkpoint', 'Mitochondrial translational termination', 'Nuclear DNA replication', 'DNA replication initiation', 'DNA unwinding involved in DNA replication', 'Negative regulation of sister chromatid segregation' and 'Cell cycle DNA replication' (**Figure 6A**). Furthermore, the KEGG pathways GSEA for RAC3 and its co-expressed genes demonstrated their association with 'DNA replication', 'Ribosome biogenesis in eukaryotes', 'Base excision repair', 'RNA transport', 'Spliceosome', 'Proteasome', 'Mismatch repair', 'Homologous recombination' and 'Cell cycle' (**Figure 6B**).

Finally, in order to verify the signal pathway, we divided the expression of RAC3 in the TCGA-BLCA dataset into high and low groups according to the mean expression value, and then KEGG pathway GSEA was conducted. The results demonstrated an association with 'Mannose type O-glycan biosynthesis', 'DNA replication', 'Homologous recombination', 'Mismatch repair', 'Base excision repair', 'Maturity onset diabetes of the young', 'Fanconi anemia pathway', 'Cysteine and methionine metabolism', 'Nicotine addiction', and 'Cell cycle' (**Figure 6C**).

Discussion

About 75% of primary BLCA are superficial tumors [28], which can be easily treated by transurethral resection of bladder tumor (TURBT). However, among these treated patients, about 70% still develop recurrence after a certain period of time [29]. ~30% progress to high-grade and high-stage disease [29]. Recurrence and progression of BLCA to a higher disease stage have a less favorable outcome [30]. Although many new tumor markers have been proposed, all of these markers have certain limitations in predicting the prognosis and treatment of BLCA, especially for tumor immunotherapy. New biomarkers are urgently needed to predict the prognosis of bladder cancer and evaluate the efficacy of immunotherapy. New biomarkers are urgently needed to predict the prognosis and evaluate the efficacy of immunotherapy in patients with BLCA.

The expression of RAC3 and its clinicopathologic characteristics in BLCA were not previously investigated. In this study, comprehensive bioinformatics analysis of multiple independent databases was performed. The results of mRNA expression demonstrated that

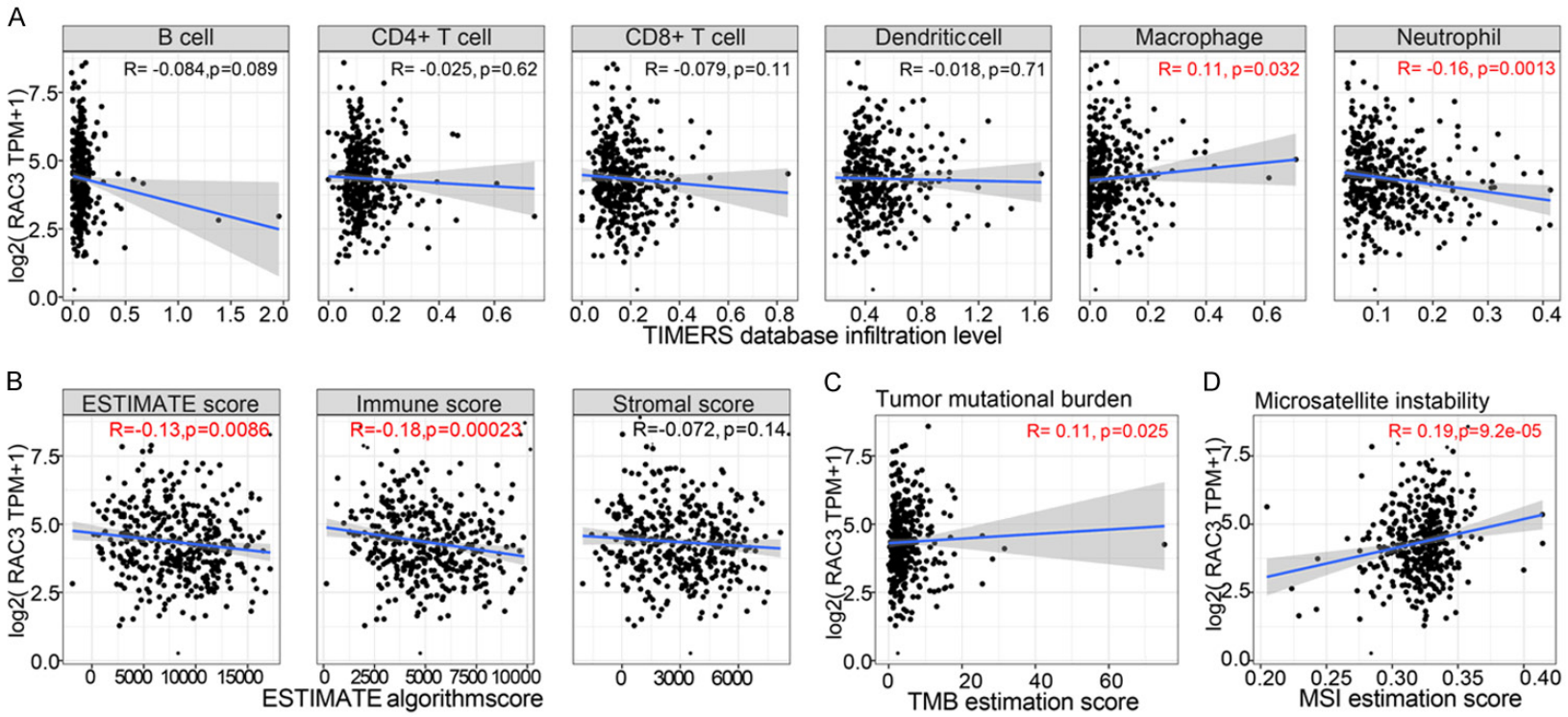

Figure 4. Tumor immune analysis in RAC3 expression from the TCGA-BLCA dataset. A. Correlation between RAC3 expression and abundance of immune cells' infiltration level in BLCA using the TIMER algorithm. B. Correlation between RAC3 expression and tumor immune microenvironment in BLCA using the ESTIMATE algorithm. C. Correlation between RAC3 expression and tumor mutational burden. D. Correlation between RAC3 expression and microsatellite instability.

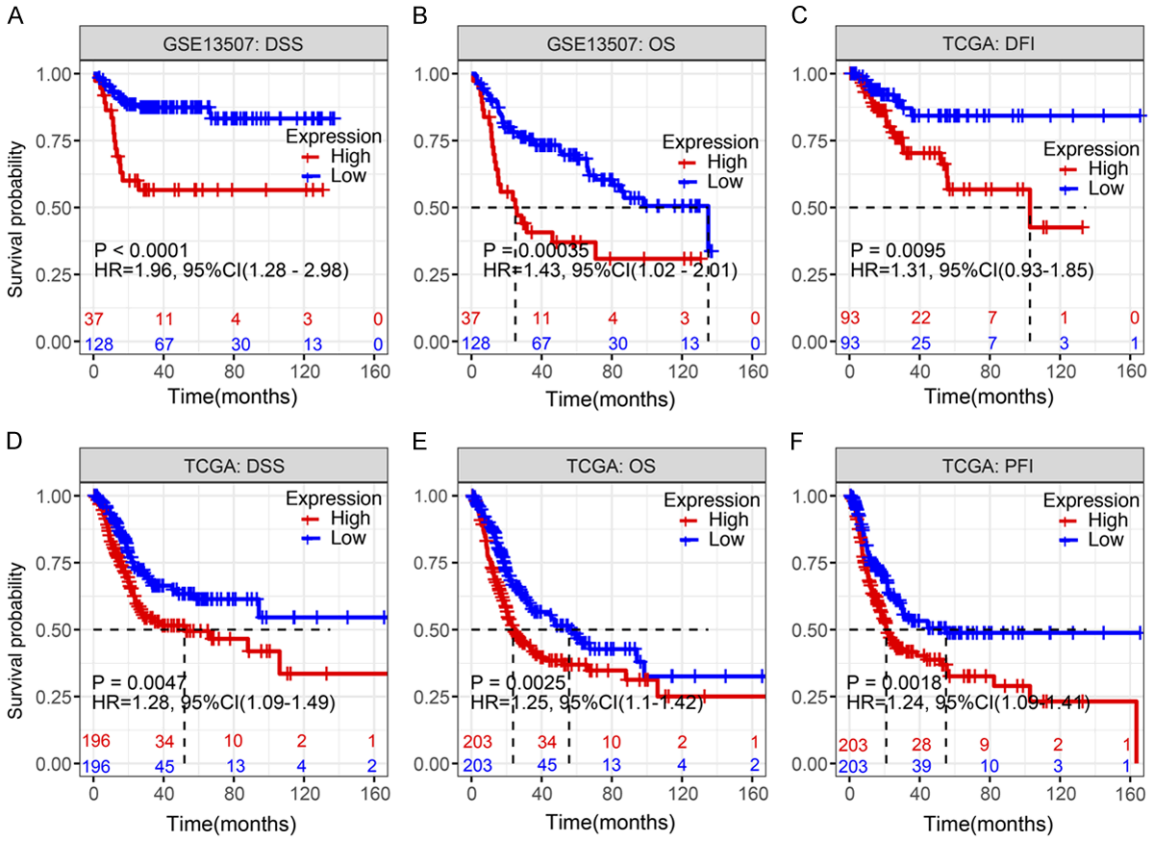

Figure 5. Assessment of the survival analysis according to RAC3 expression in patients with BLCA. Kaplan-Meier survival plot from the GEO database, gene expression data and (A) disease specific survival, and (B) overall survival information were downloaded from the GSE13507 dataset. Kaplan-Meier survival plots from TCGA database, gene expression data, and (C) disease free interval, (D) disease specific survival, (E) overall survival, and (F) progression free interval information were downloaded from the BLCA dataset.

Table 2. Univariate and stepwise multivariate Cox hazard analysis of risk factors for survival prognosis in BLCA patients

Characteristic	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
RAC3 expression (value)	1.21	1.07-1.36	0.002	1.13	0.83-1.55	0.033
RAC3 expression (high vs. low)	1.39	1.03-1.87	0.021	1.25	0.56-2.8	0.041
Age (years)	1.03	1.02-1.05	<0.001	1.03	1-1.05	0.046
BMI (kg/m^2)	0.99	0.96-1.02	0.465	-	-	-
Gender (male vs. female)	1.12	0.81-1.56	0.489	-	-	-
Grade (high vs. low)	0.35	0.09-1.41	0.138	-	-	-
Subtype (non-papillary vs. papillary)	0.66	0.47-0.95	0.023	0.69	0.39-1.25	0.221
Lymph node metastasis (yes vs. no)	2.33	1.7-3.19	<0.001	1.17	0.43-3.17	0.753
Distant metastasis (yes vs. no)	3.31	1.58-6.93	0.001	1.95	0.79-4.82	0.147
Cancer Stage (III+IV vs. I+II)	1.73	1.42-2.09	<0.001	1.46	0.76-2.81	0.256

The group before vs. represents the reference group.

RAC3 was upregulated in different types of cancer by Oncomine and TCGA database, both including BLCA, and the results of mRNA and protein expression in BLCA tissues both demonstrated RAC3 were higher compared with normal bladder tissues, respectively. In addi-

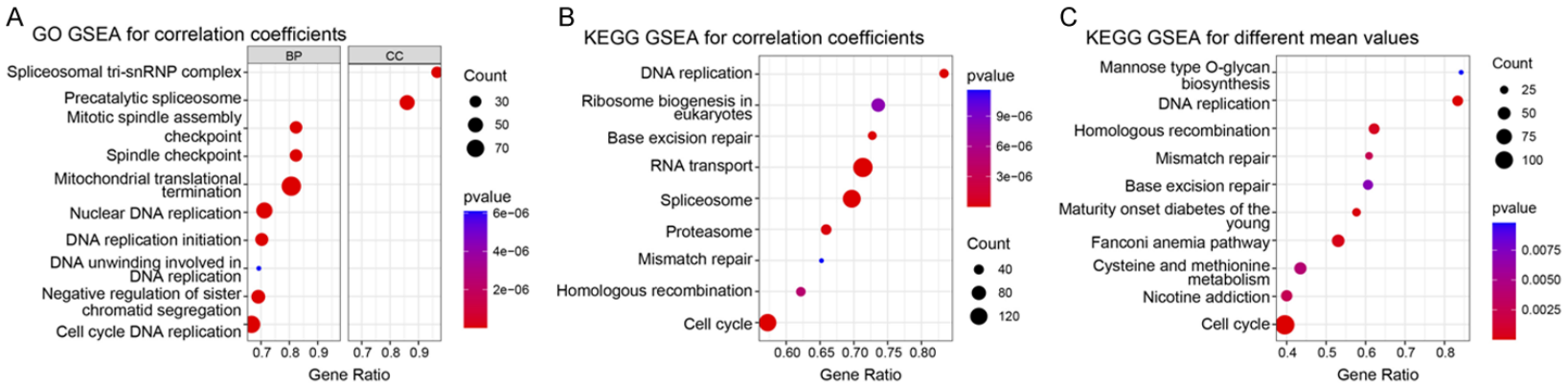

Figure 6. Gene set enrichment analysis of RAC3 expression in BLCA. A. Dot plots of GO gene set enrichment analysis for genes co-expressed with RAC3 in BLCA. B. Dot plots of KEGG gene set enrichment analysis for genes co-expressed with RAC3 in BLCA. C. Dot plot of KEGG gene set enrichment analysis for high expression of RAC3 in BLCA. Size of the dot represents the gene set count, the color represents the *p*-value.

tion, RAC3 upregulation also was positively correlated with the histologic grade. Next, a clinicopathologic analysis using data from the TCGA-BLCA dataset was conducted. The results suggest that high expression of RAC3 correlated with high grade, high stage, lymph node metastasis, and distant metastasis, Then, we explored the association of survival analysis between the different RAC3 expression levels in BLCA. Firstly, the Kaplan-Meier analysis result of OS and DSS using data from the GEO accession GSE13507 dataset suggested that higher RAC3 expression gave a poorer prognosis in BLCA. At the same time, Kaplan-Meier analysis using the data from TCGA-BLCA dataset was preformed to verify the result again. The results also showed higher RAC3 expression was significantly related to adverse prognosis. Taken together, these findings demonstrated that RAC3 may be considered as a proto-oncogene in BLCA, and may therefore accelerate the progression to high grade and high stage of BLCA. These results also highlight the role of RAC3 as a therapeutic target for BLCA. However, the mechanism of RAC3 in disease progression and prognosis of patients with BLCA needs further investigation.

The small G proteins, Rac, are members of the Rho-GTPase subfamily of the Ras superfamily [8]. Rac family members participate in specialized cellular functions. Rac binds to and hydrolyzes GTP, and thus possesses the unique ability to cycle between an inactive GDP-bound state and an active GTP-bound state [9]. RAC3 is overexpressed in different types of human tumors including brain tumors [31], lung cancer [9], breast cancer [12], prostate cancer [13], esophageal [11], and ovarian cancer [14]. Although some studies demonstrated that RAC3 overexpression may been an indicator of poor prognosis in breast cancer [12] and lung cancer [9], there is currently a lack of research on BLCA. At present, only one article [6] on the identification of validation prognostic signatures of BLCA based on the immune related genes has been reported, and the expression profile and functional role of RAC3 in BLCA remain unknown.

The immune system has a key role to play in controlling cancer initiation and progression [32, 33]. Recent evidence [32-34] indicates the immunosuppressive nature of the tumor microenvironment. Immune cells adapt to the metabolic needs of cancer cells in a dynamic manner, thereby promoting tumorigenesis and resistance to treatment [32]. Growing evidence [32-34] also suggests that the innate immune cells (macrophages, neutrophils, dendritic cells, innate lymphoid cells, myeloid-derived suppressor cells, and natural killer cells) as well as adaptive immune cells (T cells and B cells) contribute to tumor progression when present in the tumor microenvironment. In the present study, we identified two types of immune cell infiltration associated with RAC3 expression in BLCA using data from the TCGA database, based on the TIMER algorithm, of which neutrophils were significantly negatively associated with RAC3 expression in BLCA, suggesting that in BLCA cells, reducing neutrophil infiltration may promote the cancer progression of BLCA. Next, ESTIMATE algorithm was performed to predict the presence of infiltrating stromal and immune cells in tumor tissues using TCGA-BLCA data. ESTIMATE algorithm is based on single sample GSEA and generates three scores. We found that the ESTIMATE score and immune score were significantly negatively correlated with RAC3 expression in BLCA, which means that tumor purity and immune cell infiltration in tumor tissue were significantly negatively correlated with the expression of RAC3 in BLCA. The stromal score did not show a significant association, which suggests that the existence of RAC3 expression in tumor tissue has not been captured during TCGA samples. However, more research is needed to validate this.

In recent years, immunotherapies as treatments for skin, bladder, lung, prostate, and kidney cancers have shown broad prospects, with extremely durable responses for some patients [35, 36]. Evidence [37-39] had shown MSI and high TMB as emerging biomarkers of sensitivity to immune checkpoint inhibitors and significantly associated with response to PD-1 and PD-L1 blockade immunotherapy. High TMB enhances tumor immunogenicity through increased numbers of tumor neoantigens that may promote an immune response [39]. It was reported [40-42] that MSI may be an independent prognostic marker for assessing risk of recurrence in BLCA. Warrick *et al* [43] found that intratumoral molecular hetero-

geneity and high somatic mutation burden could be related to therapeutic response in patients with BLCA. Diogo *et al* [44] found that high TMB was associated with a benefit from immunotherapy with bacillus Calmette-Guérin (BCG) for non-muscle invasive bladder cancer. In the present study, the high expression of RAC3 showed an association with high TMB and MSI, suggesting that upregulation of RAC3 may indicate a stronger immune response in BLCA. Therefore, we speculated that although upregulation of RAC3 predicts poor clinical outcome, it suggests sensitivity to immunotherapy, which provides a new target for clinical immunotherapy.

In this study, the gene set enrichment analysis of biologic function and signaling pathway of RAC3 in BLCA was comprehensively investigated. First we used the TCGA-BLCA dataset to batch calculate the Spearman correlation coefficients for RAC3 and its co-expressed genes, then ranked the correlation coefficients. Next, GSEA method was used to identify the functional and signaling pathways. Subsequently, GSEA between high- and low expression of RAC3 in TCGA-BLCA dataset was performed to validate the signal pathway again. All functional and signaling pathway enrichment analyses found DNA replication and cell cycle involvement, suggesting that upregulation of RAC3 may be involved in the oncogenesis of BLCA through these two processes. Oncogenesis is a multistep process by which normal cells progressively evolve to a neoplastic state, while genome instability produces genetic diversity and accelerates the acquisition of tumorigenic abilities. DNA replication is afundamental biologic process in which dysregulation leads to genome instability [45]. Complete and accurate DNA replication is necessary for proliferation and genome stability [46]. Genome instability is a cancer hallmark, and endows genetic diversity during tumorigenesis [45, 46]. Numerous studies [45, 47, 48] have shown that most cancers can overcome the stresses due to the disturbance of DNA replication. In addition, the mammalian cell cycle is a well-organized and complex regulation process, which is usually divided into G0/G1, S, G2, and M phases, and is mainly controlled by different cyclin-dependent kinases (CDKs) and their functional cyclin partners. Before replicating DNA during the reproductive cycle, cells enter G1 phase, during which they interpret a plethora of signals that affect cell fate and cell division. Errors in this process can cause cancer [49]. At the same time, the abnormal function of cell cycle regulators results in uncontrolled cell proliferation, which makes it an attractive target for cancer treatment [50].

In summary, this study comprehensively analyzed the expression profile, clinicopathologic characteristics, prognosis, and tumor immunoassay of RAC3 in BLCA. RAC3 was significantly upregulated in BLCA and correlated with prognosis. Tumor immunoassay indicated that RAC3 had a good immune response to BLCA. This study provides a new direction to explore the clinical prognosis and tumor immunotherapy biomarkers for BLCA.

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Disclosure of conflict of interest

None.

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