Discover Oncology

Analysis

RAP1GAP is a prognostic biomarker and correlates with immune infiltrates in bladder cancer

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Abstract

Background The role of RAP1GAP in tumor progression has garnered increasing attention; however, its prognostic value and immunological influence across various cancers remain uncertain. Our study presents a pan-cancer analysis to investigate its involvement in oncogenesis and immune regulation.

Methods Public databases were utilized to assess RAP1GAP expression across cancers. Cox regression analysis evaluated its prognostic value, while Pearson correlation examined associations with genomic heterogeneity, tumor stemness, immune cell infiltration, and immune checkpoints. Immunohistochemical staining of bladder cancer and adjacent tissues assessed RAP1GAP expression and clinical correlations.

Results RAP1GAP expression is differentially expressed in a variety of tumor types and predicts a better or worse prognosis for tumor patients. It was strongly linked to genomic heterogeneity and tumor stemness in multiple cancers. Immunohistochemistry showed increased RAP1GAP expression in bladder cancer. Immune cell analysis revealed high RAP1GAP expression was associated with greater infiltration of plasma cells, naive $CD4^+$T cells, Tregs, and eosinophils, while low expression correlated with increased $CD8^+$T cells, activated memory $CD4^+$T cells, and M1 macrophages.

Conclusion RAP1GAP is a potential prognostic biomarker and immune regulator, with promising implications as an immunotherapeutic target for bladder cancer.

Keywords RAP1GAP · Prognostic biomarker · Immune cell · Bladder cancer

Abbreviations

TCGA	The Cancer Genome Atlas
IHC	Immunohistochemical
RAP1GAP	RAP1 GTPase activating protein
OS	Overall survival
DSS	Disease-specific survival
DFI	Disease-free interval
PFI	Progression-free interval
TMB	Tumor mutation burden
MSI	Microsatellite instability

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DNAss	DNA methylation-based stem scores
RNAss	RNA expression-based stem scores
GDSC	Drug sensitivity in cancer
CTRP	Cancer therapeutics response portal
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
GSEA	Gene set enrichment analysis
TCIA	Cancer immunome atlas
ACC	Adrenocortical carcinoma
BLCA	Bladder urothelial carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
COADREAD	Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
ESCA	Esophageal carcinoma
FPPP	FFPE pilot phase ii
GBM	Glioblastoma multiforme
GBMLGG	Glioma
HNSC	Head and neck squamous cell carcinoma
KICH	Kidney chromophobe
KIPAN	Pan-kidney cohort (KICH + KIRC + KIRP)
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute myeloid leukemia
LGG	Brain lower grade glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
STAD	Stomach adenocarcinoma
SKCM	Skin cutaneous melanoma
STES	Stomach and esophageal carcinoma
TGCT	Testicular germ cell tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine corpus endometrial carcinoma
UCS	Uterine carcinosarcoma
UVM	Uveal melanoma
OS	Osteosarcoma
ALL	Acute lymphoblastic leukemia
NB	Neuroblastoma
WT	High-risk Wilms tumor

1 Introduction

Advances in traditional surgery, chemotherapy, and radiotherapy, alongside the emergence of targeted therapies and immunotherapies, have brought renewed hope to cancer patients [1, 2]. Among these, immune checkpoint inhibitors have achieved groundbreaking clinical success across various solid tumors [3, 4]. However, despite these advancements, significant challenges persist. Many patients respond poorly to current treatments or develop resistance, limiting overall efficacy [5–7]. While targeted therapies offer precision, their effectiveness is constrained by tumor heterogeneity and individual variability [8, 9]. Similarly, immunotherapy benefits only a subset of patients, as many tumors either fail to respond or evade immune detection through escape mechanisms [10]. Therefore, exploring new therapeutic targets and gaining deeper insights into the fundamental molecular mechanisms within the tumor microenvironment (TME) remain essential research priorities.

RAP1GAP (RAP1 GTPase Activating Protein), a negative regulator of RAP1, is essential for cell adhesion, migration, and polarity maintenance [11]. Recent studies have increasingly explored its role in cancer, particularly its impact on tumor progression [12, 13]. However, its function within the TME remains unclear. Evidence suggests RAP1GAP may contribute to immune evasion and tumor progression by modulating immune cell activity and tumor-associated stromal function [14–16]. Despite these findings, the precise mechanisms by which RAP1GAP interacts with immune cells—especially its coordination with other molecules in the TME to regulate immune responses—remain largely unexplored.

Although cancer treatment has advanced significantly, identifying new therapeutic targets and elucidating underlying mechanisms remain critical. This study underscores the need for further investigation into RAP1GAP's role as a multifunctional regulator in cancer, focusing particularly on bladder cancer.

2 Materials and methods

2.1 Data collection and analysis

The standardized cancer dataset, comprising RNA sequencing data and clinical information for 34 tumor types, was obtained from the UCSC website [17]. The Sangerbox platform integrates this dataset and facilitates subsequent bioinformatics analysis [18]. Immunofluorescence data on RAP1GAP expression in A-431 and U-251MG cell lines were sourced from the HPA database [19]. Expression data of RAP1GAP across various tumor samples were extracted for further analysis. Unmatched Wilcoxon rank sum and signed rank tests were used to assess expression differences. Based on previous studies, we examined the association between RAP1GAP expression and prognosis, including overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI). Prognostic significance was determined using the log-rank test [20].

2.2 Genomic heterogeneity and tumor stemness analysis

We obtained single nucleotide variation data for all tumor types from the GDC website and used the R package "maftools" to calculate tumor mutation burden (TMB) [21]. The correlation between microsatellite instability (MSI) scores and RAP1GAP expression was analyzed based on previous studies [22]. Additionally, we integrated stem index and gene expression data from prior research to assess the correlations between DNA methylation-based stem scores (DNAss) and RNA expression-based stem scores (RNAss) across tumor types [23].

2.3 Immune related and RNA-modification genes

We collected expression data for 150 immune regulatory genes across multiple tumor types and examined their association with RAP1GAP expression [18]. Similarly, we examined the relationship between RAP1GAP expression and 60 immune checkpoint-related genes [24]. Additionally, we extracted expression data for 44 RNA-modification genes and assessed their correlation with RAP1GAP expression across different tumor types [18].

Fig. 1 Correlation analysis between RAP1GAP expression and clinical features in pan-cancer. **A** Transcriptional expression analysis of RAP-1GAP in pan-cancer. **B** Immunofluorescence results revealed the protein expression of RAP1GAP in A-431 and U-251MG cell lines according to the Human Protein Atlas. **C** Prognostic analysis of RAP1GAP in pan-cancer. **D** Association between tumor mutation burden (TMB) and RAP1GAP expression in pan-cancer: THYM (R = 0.28), LUAD (R = − 0.20), STES (R = − 0.10), KIPAN (R = − 0.17), STAD (R = − 0.12) and KIRC (R = − 0.18). **E** Association between microsatellite instability (MSI) and RAP1GAP expression in pan-cancer: GBMLGG (R = 0.198), COAD (R = 0.322), COADREAD (R = 0.319), KIPAN (R = 0.248), UCEC (R = 0.198), THYM(R = 0.225), READ (R = 0.331), TGCT (R = 0.396), BRCA (R = − 0.084), STES (R = − 0.128), STAD (R = − 0.159), HNSC (R = − 0.099), THCA (R = − 0.095), and UCS (R = − 0.278). **F** Association between DNA methylation based (DNAss) and RAP1GAP expression in pan-cancer: STES (R = 0.123, KIPAN (R = 0.093), STAD (R = 0.183), HNSC (R = 0.099), THYM (R = 0.387), PCPG (R = 0.309), UVM (R = 0.233), BLCA (R = 0.177), CESC (R = − 0.201), LUAD (R = − 0.155), PRAD (R = − 0.205), UCEC (R = − 0.175), LIHC (R = − 0.273), THCA (R = − 0.356), TGCT (R = − 0.206), and DLBC (R = − 0.362). **G** Association between RNA methylation based (RNAss) and RAP1GAP expression in pan-cancer: GBM (R = 0.221), GBMLGG (R = 0.471), LGG (R = 0.481), KIRP (R = 0.333), KIPAN (R = 0.439), PRAD (R = 0.289), KIRC (R = 0.115), THCA(R = 0.385), PCPG (R = 0.375), ACC (R = 0.518), KICH (R = 0.308), CESC (R = − 0.134), LUAD (R = − 0.240), COAD (R = − 0.191), COADREAD (R = − 0.208), LIHC (R = − 0.131), READ (R = − 0.304), and TGCT (R = − 0.365). (H) Association between age and RAP1GAP expression in pan-cancer: LUAD (R = 0.135), THCA (R = 0.106), PCPG (R = 0.175), UCS (R = 0.293), GBMLGG (R = − 0.119), COAD (R = − 0.118), SARC (R = − 0.186), KIPAN (R = − 0.117), and MESO (R = − 0.273)

2.4 Gene mutation and drug sensitivity analysis

We integrated mutation data from all tumor samples and used the R package "maftools" to obtain protein domain information [21]. RAP1GAP mutation frequencies across tumor types were retrieved from the cBioPortal website [25]. We utilized the GSCALite platform to assess the correlation between RAP1GAP expression and drug sensitivity using data from the GDSC and CTRP databases [26].

2.5 Functional enrichment analysis

We analyzed the biological functions and signaling pathways of RAP1GAP-related genes using the R package "clusterProfiler" for Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, and Gene Set Enrichment Analysis (GSEA) [27].

2.6 Immunological value analysis

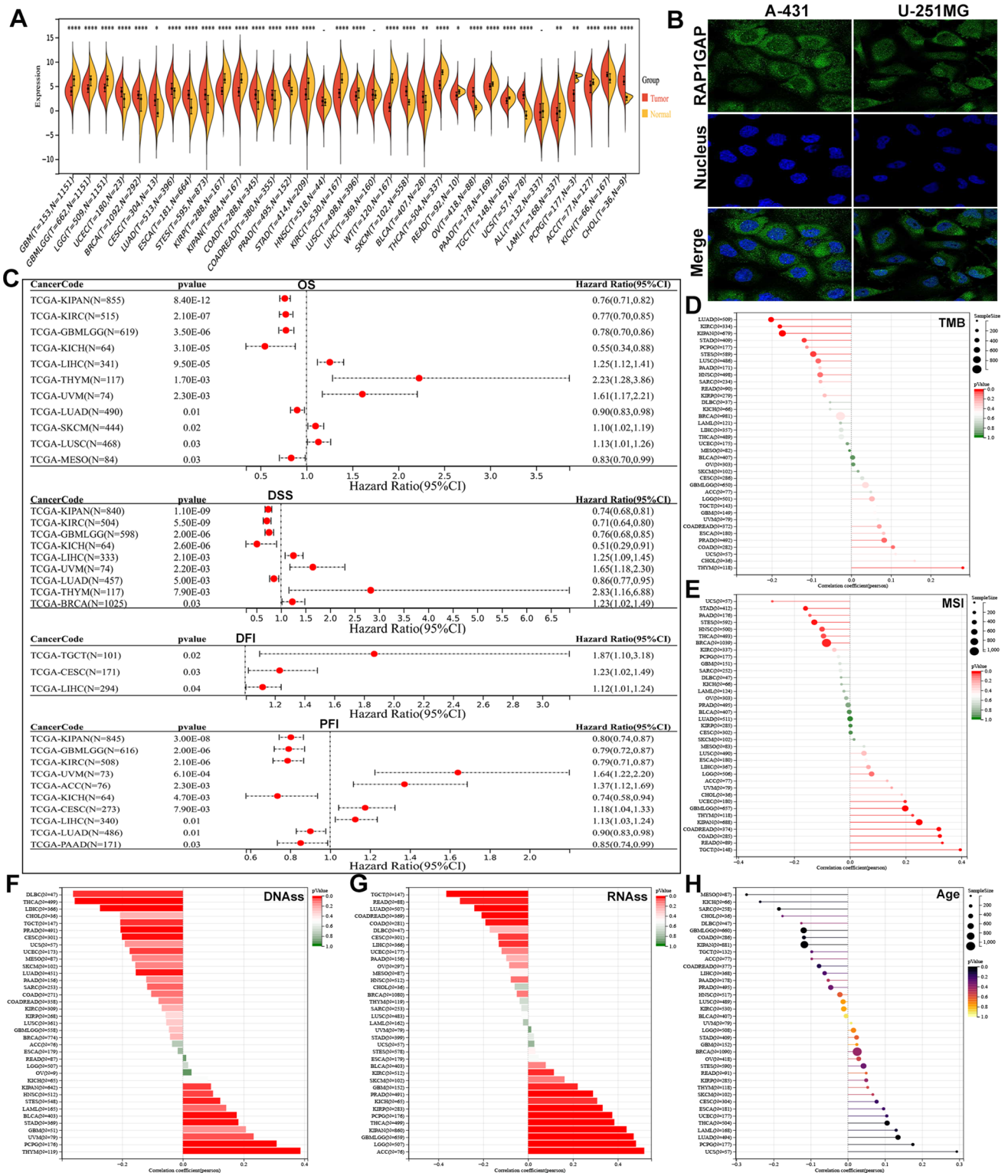
Using the CIBERSORT algorithm, we assessed immune cell infiltration in the TCGA-BLCA cohort and analyzed its correlation with RAP1GAP expression in bladder cancer patients [28]. Additionally, we predicted the clinical response to immunotherapy in high- and low-RAP1GAP expression groups based on the Cancer Immunome Atlas (TCIA) [29].

2.7 Clinical samples and immunohistochemistry (IHC) assay

We utilized a commercial bladder cancer tissue microarray (Cat. No. HBlaU050CS01, Shanghai Outdo Biotech, China) containing 36 tumor tissues and 10 adjacent normal tissues to assess RAP1GAP expression. IHC was performed using a kit from ZSBG-BIO (China). Following dewaxing, rehydration, and antigen retrieval, the tissues were incubated with a primary anti-RAP1GAP antibody (Affinity, USA). IHC scores were independently evaluated by two senior pathologists and classified as low (≤ 3) or high (> 3) expression group.

2.8 Statistical analysis

Data analysis was conducted using R (version 4.2.1) and relevant bioinformatics packages. Group comparisons were performed using the *t-test* or *Chi-square test*, with statistical significance set at $P < 0.05$.


A
Expression
Group
Tumor
Normal
B
A-431
U-251MG
RAP1GAP
Nucleus
Merge
C
CancerCode
pvalue
OS
Hazard Ratio(95%CI)
TCGA-KIPAN(N=855) 8.40E-12 0.76(0.71,0.82)
TCGA-KIRC(N=515) 2.10E-07 0.77(0.70,0.85)
TCGA-GBMLGG(N=619) 3.50E-06 0.78(0.70,0.86)
TCGA-KICH(N=64) 3.10E-05 0.55(0.34,0.88)
TCGA-LIHC(N=341) 9.50E-05 1.25(1.12,1.41)
TCGA-THYM(N=117) 1.70E-03 2.23(1.28,3.86)
TCGA-UVM(N=74) 2.30E-03 1.61(1.17,2.21)
TCGA-LUAD(N=490) 0.01 0.90(0.83,0.98)
TCGA-SKCM(N=444) 0.02 1.10(1.02,1.19)
TCGA-LUSC(N=468) 0.03 1.13(1.01,1.26)
TCGA-MESO(N=84) 0.03 0.83(0.70,0.99)
Hazard Ratio(95%CI)
DSS
TCGA-KIPAN(N=840) 1.10E-09 0.74(0.68,0.81)
TCGA-KIRC(N=504) 5.50E-09 0.71(0.64,0.80)
TCGA-GBMLGG(N=598) 2.00E-06 0.76(0.68,0.85)
TCGA-KICH(N=64) 2.60E-06 0.51(0.29,0.91)
TCGA-LIHC(N=333) 2.10E-03 1.25(1.09,1.45)
TCGA-UVM(N=74) 2.20E-03 1.65(1.18,2.30)
TCGA-LUAD(N=457) 5.00E-03 0.86(0.77,0.95)
TCGA-THYM(N=117) 7.90E-03 2.83(1.16,6.88)
TCGA-BRCA(N=1025) 0.03 1.23(1.02,1.49)
Hazard Ratio(95%CI)
DFI
TCGA-TGCT(N=101) 0.02 1.87(1.10,3.18)
TCGA-CESC(N=171) 0.03 1.23(1.02,1.49)
TCGA-LIHC(N=294) 0.04 1.12(1.01,1.24)
Hazard Ratio(95%CI)
PFI
TCGA-KIPAN(N=845) 3.00E-08 0.80(0.74,0.87)
TCGA-GBMLGG(N=616) 2.00E-06 0.79(0.72,0.87)
TCGA-KIRC(N=508) 2.10E-06 0.79(0.71,0.87)
TCGA-UVM(N=73) 6.10E-04 1.64(1.22,2.20)
TCGA-ACC(N=76) 2.30E-03 1.37(1.12,1.69)
TCGA-KICH(N=64) 4.70E-03 0.74(0.58,0.94)
TCGA-CESC(N=273) 7.90E-03 1.18(1.04,1.33)
TCGA-LIHC(N=340) 0.01 1.13(1.03,1.24)
TCGA-LUAD(N=486) 0.01 0.90(0.83,0.98)
TCGA-PAAD(N=171) 0.03 0.85(0.74,0.99)
Hazard Ratio(95%CI)
D
TMB
SampleSize
pValue
Correlation coefficient(pearson)
E
MSI
SampleSize
pValue
Correlation coefficient(pearson)
F
DNAss
pValue
Correlation coefficient(pearson)
G
RNAss
pValue
Correlation coefficient(pearson)
H
Age
SampleSize
pValue
Correlation coefficient(pearson)

3 Results

3.1 Association between RAP1GAP expression with clinical features in human cancer

Pan-cancer analysis revealed that RAP1GAP expression was upregulated in 15 tumor types and downregulated in 16 tumor types (Fig. 1A). Immunofluorescence analysis in A-431 and U-251MG cell lines showed RAP1GAP predominantly localized in the cytoplasm (Fig. 1B). We then examined the prognostic significance of RAP1GAP across different cancer types. OS analysis revealed that high RAP1GAP expression predicted poor prognosis in five tumor types, whereas low expression indicated worse outcomes in six tumor types (Fig. 1C). In the DSS analysis, elevated RAP1GAP indicated poor prognosis in four tumor types but better survival in five tumor types (Fig. 1C). For DFI, higher RAP1GAP expression predicted a worse prognosis (Fig. 1C). In the PFI analysis, increased RAP1GAP expression was linked to poor outcomes in four tumor types but favorable prognosis in six tumor types (Fig. 1C). RAP1GAP expression showed a significant positive correlation with TMB in THYM but a negative correlation in five tumor types (Fig. 1D). For MSI, RAP1GAP expression was positively associated in eight tumor types and negatively associated in six tumor types (Fig. 1E). Similarly, its expression correlated positively with DNAss in eight tumor types and negatively in another eight tumor types (Fig. 1F). A similar pattern was observed for RNAss, with positive correlations in eight tumor types and negative correlations in another eight tumor types (Fig. 1G). Additionally, RAP1GAP expression was significantly associated with age, showing a positive correlation in four tumor types and a negative correlation in five tumor types (Fig. 1H).

3.2 Association between RAP1GAP expression with immune regulation, checkpoints, and RNA modification in human cancer

RAP1GAP expression correlated positively with immunomodulatory genes in UVM, LUAD, LIHC, COAD, and COADREAD but negatively in THCA, KIPAN, KIRP, LGG, and BLCA (Fig. 2A). Among 60 immune checkpoint genes, RAP1GAP expression exhibited significant positive correlations in UVM, THYM, LIHC, and high-risk WT but was negatively correlated in THCA, KIPAN, LGG, and GBMLGG (Fig. 2B). For 44 RNA modification genes, RAP1GAP expression showed strong positive associations in LIHC, BRCA, UCEC, ACC, and WT, while a significant negative correlation was observed in KIPAN (Fig. 2C).

3.3 Association between RAP1GAP expression with mutant landscape and drug sensitivity in human cancer

We analyzed RAP1GAP mutation data from a pan-cancer dataset, mapping its mutation landscape and protein domains across different tumor types (Fig. 3A). Mutation frequency and types were retrieved from cBioPortal, revealing that "mRNA High" was the most prevalent alteration in nearly all tumors (Fig. 3B). Next, we examined RAP1GAP methylation levels across various cancers using the GSCALite database, identifying significant differences in 10 tumor types (Fig. 3C). We also assessed copy number variations (CNVs) and found that the hete. Amp CNV type was predominant in most cancers (Fig. 3D). Single nucleotide variation (SNV) analysis showed higher mutation frequencies in UCEC (20%) and SKCM (17%) (Fig. 3E). Additionally, we explored RAP1GAP-associated drug sensitivity in the GDSC and CTRP databases, where (5Z)-7-Oxozeaenol, Bleomycin (50 μM), IPA-3, 1S, 3R-RSL-3, etoposide, and austocystin D exhibited notable sensitivity correlations (Fig. 3F–G).

3.4 Expression patterns and experimental verification of RAP1GAP in bladder cancer

Analysis of the TCGA-BLCA cohort revealed significantly elevated RAP1GAP expression in bladder cancer (Fig. 4A, B). Moreover, promoter methylation levels of RAP1GAP were notably lower in tumor tissues (Fig. 4C). Receiver operating characteristic (ROC) analysis demonstrated the diagnostic potential of RAP1GAP in bladder cancer, with an AUC of 0.707 (Fig. 4D). Based on median expression levels, patients were classified into high- and low-expression groups, showing significant differences in clinical stage and N stage (Fig. 4E). IHC analysis of a commercial bladder cancer tissue microarray further confirmed RAP1GAP upregulation in tumor tissues (Fig. 4F–G). RAP1GAP expression showed no significant association with clinical features such as PD-L1 expression and CD8-positive cell infiltration (Fig. 4H; Table 1). Prognostic

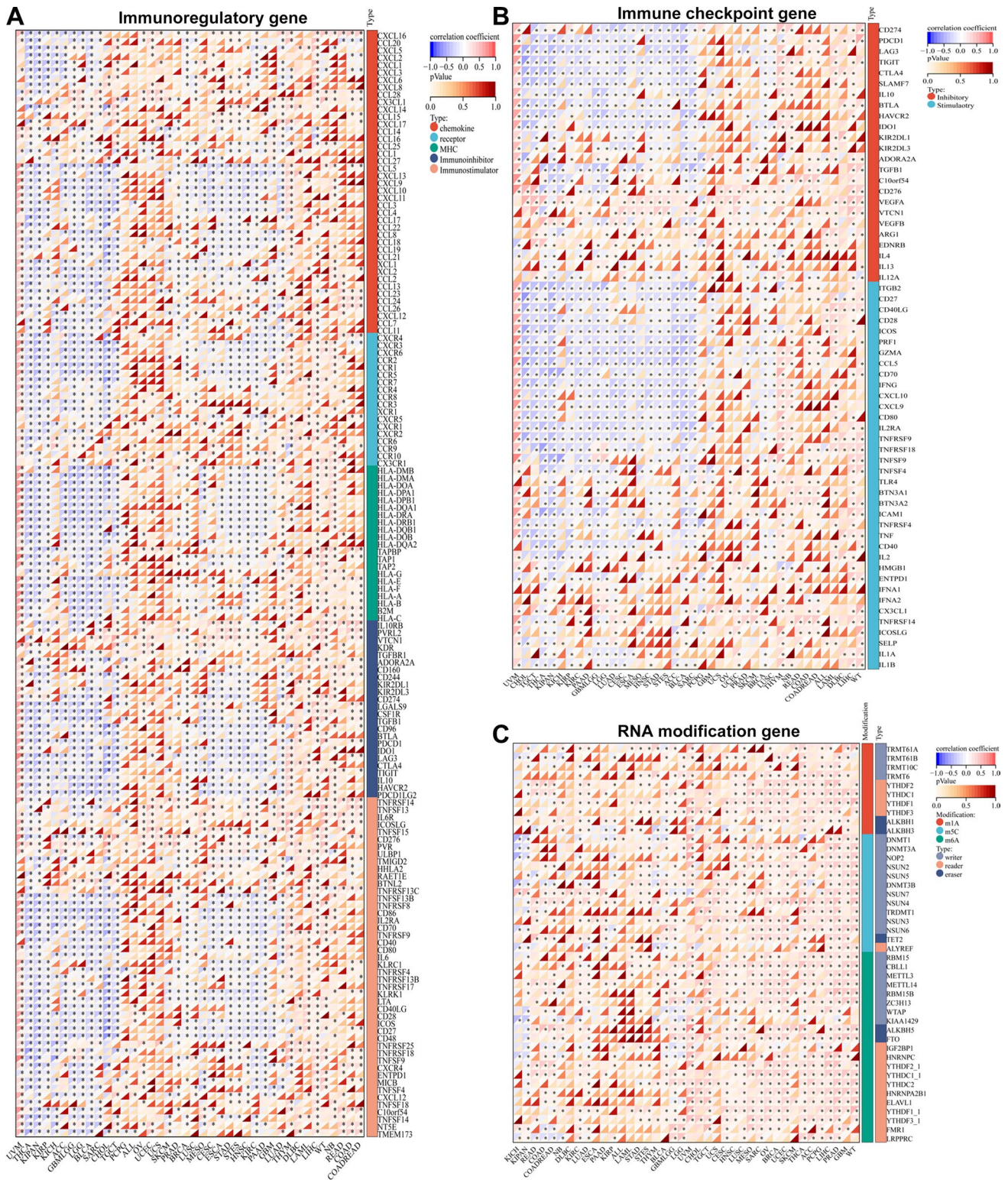


Fig. 2 Pearson analysis of RAP1GAP expression, regulatory genes, immune checkpoints, and RNA modification in pan-cancer. **A** The correlation of RAP1GAP expression with immunoregulatory gene in pan-cancer. **B** The correlation of RAP1GAP expression with immune checkpoint genes in pan-cancer. **C** The correlation of RAP1GAP expression with RNA modification in pan-cancer

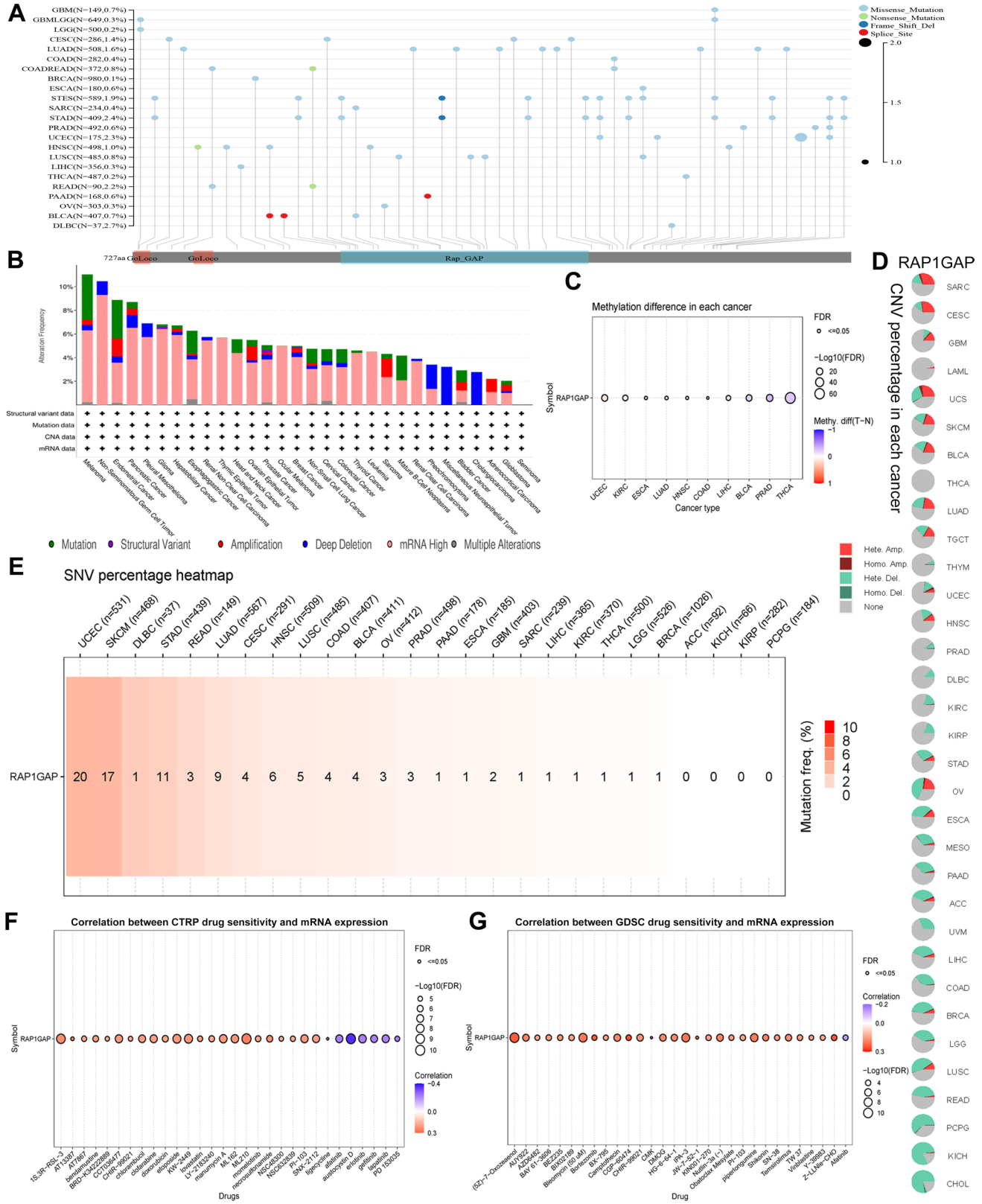


Fig. 3 Mutation analysis of RAP1GAP and its difference in pan-cancer. **A** RAP1GAP mutation landscape in different tumor types. **B** The mutation frequency of RAP1GAP varies among tumor types. **C** RAP1GAP methylation differences were analyzed in different tumor types. **D** Copy number variant (CNV) of RAP1GAP were analyzed in different tumor types. **E** Single nucleotide variants (SNVs) of RAP1GAP were analyzed in different tumor types. **F** The correlation between RAP1GAP expression and the sensitivity of CTRP drugs in pan-cancer. **G** The correlation between RAP1GAP expression and the sensitivity of GDSC drugs in pan-cancer

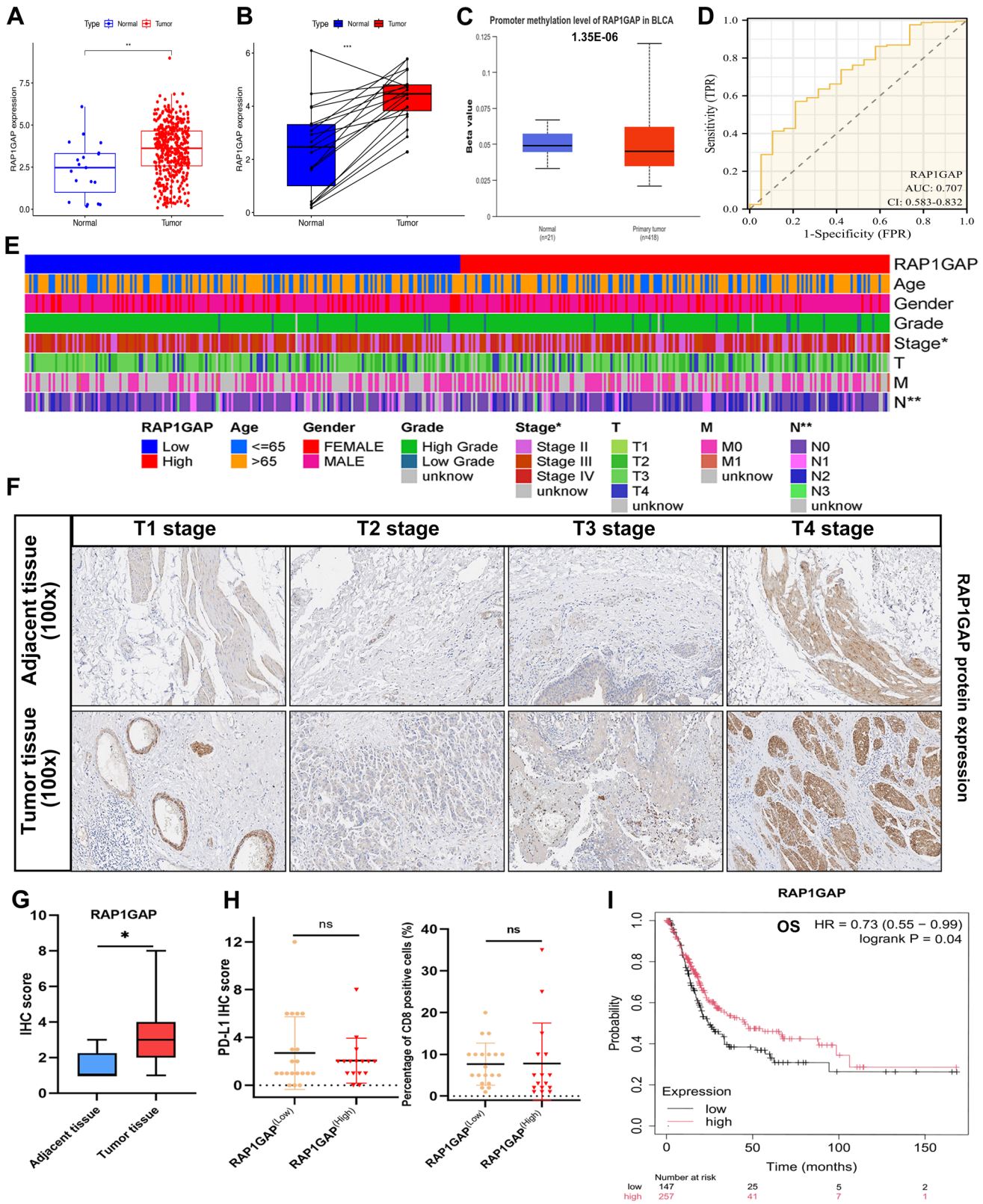


Fig. 4 Correlation analysis between RAP1GAP expression and clinical features in bladder cancer. **A** Unpaired and **B** paired analysis of RAP1GAP transcription expression in bladder cancer. **C** The promoter methylation levels of RAP1GAP in bladder cancer. **D** ROC curve analysis revealed the diagnostic value of RAP-1GAP expression in bladder cancer. **E** Association between RAP1GAP expression and clinical features in bladder cancer. **F** Representative pictures of immunohistochemistry staining of RAP1GAP in tumor and matched adjacent normal tissues with different T stage (magnification: 100×). **G** Immunohistochemistry score analysis of RAP1GAP staining in tumor and adjacent normal tissues. **H** Distribution of PD-L1 expression and CD8 positive cells in RAP1GAP high-low group with bladder cancer. **I** Association between RAP1GAP expression and overall survival (OS) in bladder cancer

Table 1 Clinical features of patients with bladder cancer in validation cohort

Characteristics	Low expression of RAP1GAP (n = 20)	High expression of RAP1GAP (n = 16)	P value
Gender, n (%)			0.492
Female	2 (10.0%)	0	
Male	18 (90.0%)	16 (100%)	
Age, n (%)			0.749
≤ 70	10 (50.0%)	7 (43.8%)	
> 70	10 (50.0%)	9 (56.2%)	
Pathological classification, n (%)			1.00
Urothelial carcinoma	18 (90.0%)	15 (93.8%)	
Other types[a]	2 (10.0%)	1 (6.2%)	
Histologic grade, n (%)			0.259
High grade	16 (80.0%)	15 (93.8%)	
Low grade	2 (10.0%)	0	
Unknown	2 (10.0%)	1 (6.2%)	
Lymph node positive, n (%)			1.00
Yes	4 (20.0%)	3 (18.7%)	
No	16 (80.0%)	13 (81.3%)	
Pathologic T stage, n (%)			0.503
T1 and T2	8 (40.0%)	9 (56.2%)	
T3 and T4	12 (60.0%)	7 (43.8%)	
Pathologic N stage, n (%)			
N0	16 (80.0%)	15 (93.8%)	0.355
N1, N2 and N3	4 (20.0%)	1 (6.2%)	
Pathologic M stage, n (%)			
M0	20 (100%)	16 (100%)	1.00
M1	0	0	
AJCC clinical staging (7th edition), n (%)			1.00
I–II	8 (40.0%)	7 (43.8%)	
III–IV	12 (80.0%)	9 (56.2%)	

[a]Other types: adenocarcinoma, squamous cell carcinoma, and sarcoma

analysis indicated that higher RAP1GAP expression correlated with improved OS in bladder cancer patients based on the TCGA-BLCA cohort (Fig. 4I).

3.5 Functional enrichment analysis of RAP1GAP expression in bladder cancer

The 10 most strongly co-expressed genes were identified, including TRAK1, KLHDC7A, SCNN1G, SCNN1B, SNX31, RBBP8NL, PDCD1LG2, WARS1, CARD16, MT2A, and IFITM3 (Fig. 5A). Subsequently, we analyzed differentially expressed genes (DEGs) between the high- and low-RAP1GAP expression groups in the TCGA-BLCA cohort. A heatmap illustrates the top 50 DEGs between the two groups (Fig. 5B). Functional enrichment analysis revealed that these DEGs were primarily associated with biological processes such as leukocyte-mediated immunity, lymphocyte-mediated immunity, and positive regulation of cytochrome production (Fig. 5C). In terms of cellular components, the DEGs were enriched in the T cell receptor complex, external side of the plasma membrane, and plasma membrane signaling receptor complex (Fig. 5C). For molecular functions, they were primarily involved in antigen binding, immune receptor activity, and cytokine activity (Fig. 5C). Pathway analysis showed significant enrichment in cytokine–cytokine receptor interaction, Th17 cell differentiation, and the JAK-STAT signaling pathway (Fig. 5D). GSEA indicated that GO terms were predominantly enriched in cornification, keratinization, intermediate filament, T cell receptor complex, and antigen binding (Fig. 5E). KEGG pathway analysis highlighted enrichment in the chemokine signaling pathway, cytokine–cytokine receptor interaction, focal adhesion, hematopoietic cell lineage, and natural killer cell-mediated cytotoxicity (Fig. 5F).

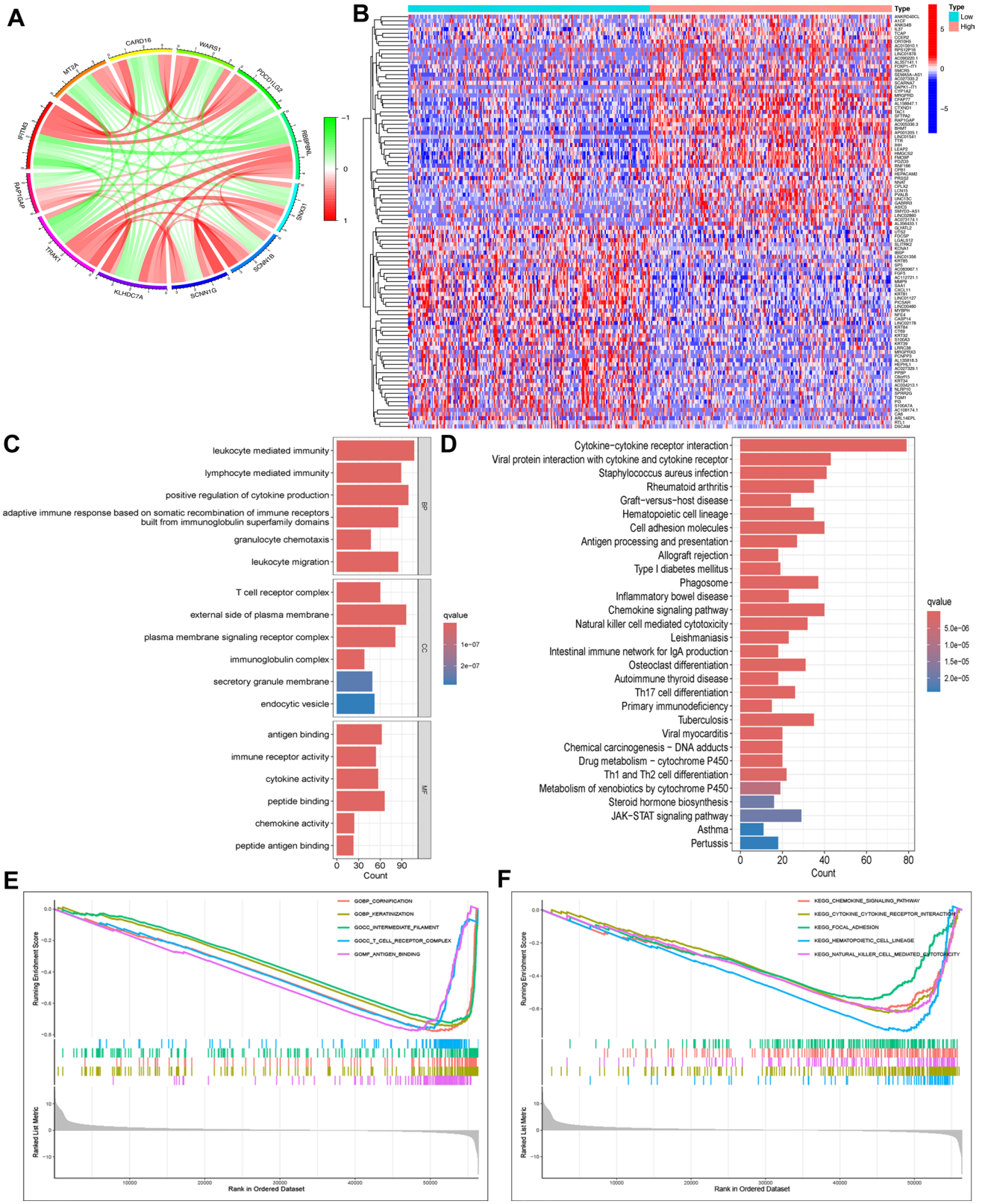


Fig. 5 Functional enrichment analysis of RAP1GAP in bladder cancer. **A** Co-expressed genes significantly associated with RAP1GAP were shown as correlation circle plots. Red, positive correlation; green, negative correlation. **B** Heatmap showing the top 50 up-regulated DEGs and top 50 down-regulated DEGs; red, up-regulated genes; blue, down-regulated genes. **C** Gene ontology analysis results of RAP1GAP expression in bladder cancer. **D** Kyoto encyclopedia of genes and genomes analysis results of RAP1GAP expression in bladder cancer. **E** Gene ontology analysis results of RAP1GAP expression in bladder cancer based on gene set enrichment analysis. **F** Kyoto encyclopedia of genes and genomes analysis results of RAP1GAP expression in bladder cancer based on gene set enrichment analysis

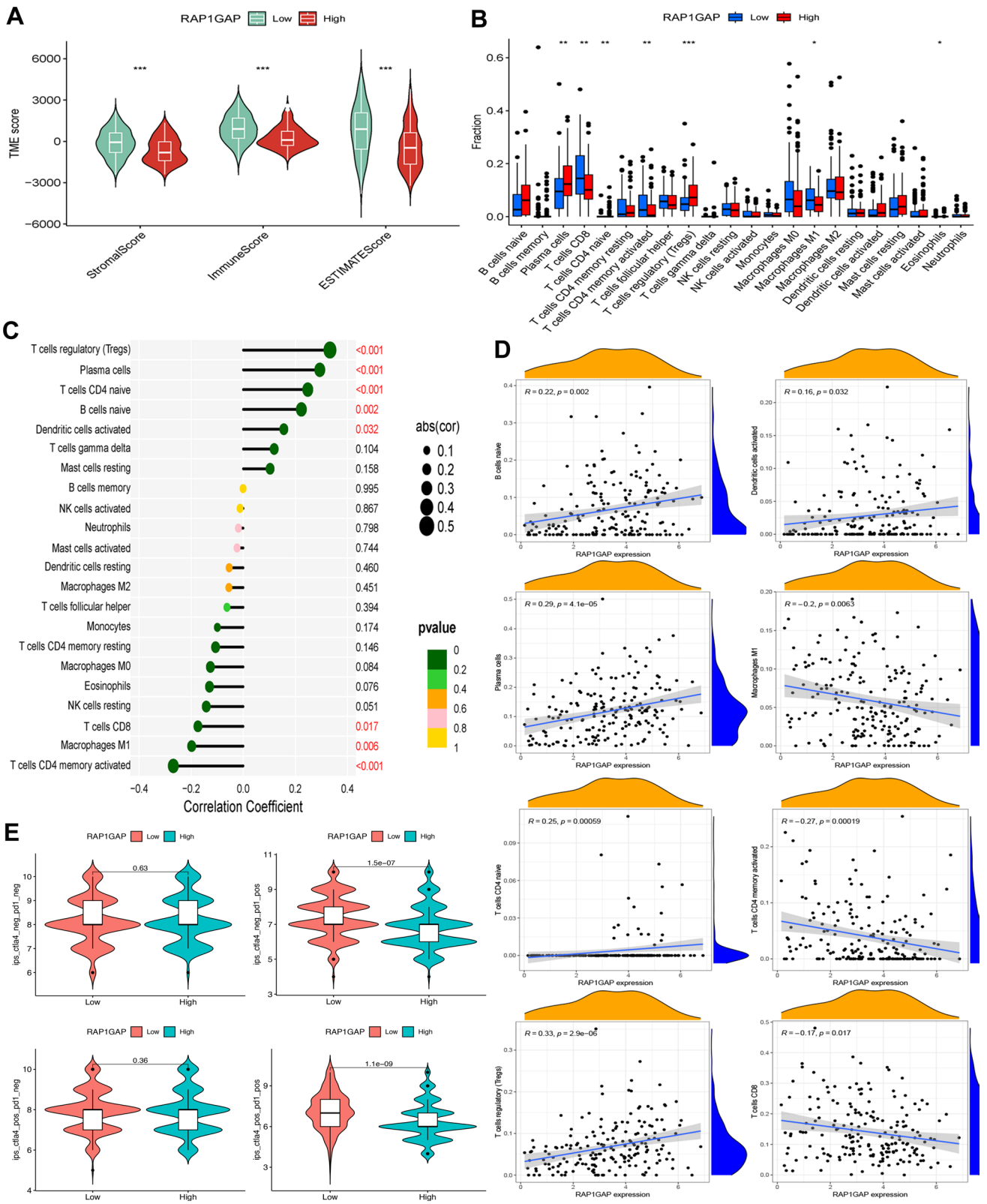


Fig. 6 Immunological effects and immune response prediction of RAP1GAP in bladder cancer. **A** ESTIMATE algorithm was used to investigate the correlation between the two groups in tumor immune microenvironment score. **B** The proportion of 22 immune cells infiltrating in low and high RAP1GAP expression groups. **C** Relationships between the expression of RAP1GAP and 22 types of immune infiltration cells. **D** The relationships between the expression of RAP1GAP and 8 kinds of immune infiltrating cells. **E** The correlation between immunophenoscore and different RAP1GAP expression groups

3.6 Analysis of immunological roles of RAP1GAP expression in bladder cancer

Low RAP1GAP expression was associated with higher stromal, immune, and ESTIMATE scores in bladder cancer patients (Fig. 6A). Using the CIBERSORT algorithm, we found that RAP1GAP expression significantly correlated with seven types of immune-infiltrating cells. High RAP1GAP expression was linked to increased infiltration of plasma cells, naïve $CD4^+$T cells, regulatory T cells (Tregs), and eosinophils, whereas low RAP1GAP expression was associated with elevated infiltration of CD8 + T cells, activated memory CD4 + T cells, and M1 macrophages (Fig. 6B). Further analysis showed that RAP1GAP expression was positively correlated with five immune cell types, including Tregs, plasma cells, naïve CD4 + T cells, naïve B cells, and activated dendritic cells, while negatively correlated with $CD8^+$T cells, M1 macrophages, and activated memory $CD4^+$T cells (Fig. 6C, D). Finally, we evaluated the potential of RAP1GAP expression as a predictor of immunotherapy response in bladder cancer patients. In PD-1-positive patients, lower RAP1GAP expression was associated with better immunotherapy outcomes (Fig. 6E).

4 Discussion

RAP1GAP has been shown to suppress cancer progression in various tumors [30, 31]. Its expression is closely linked to patient prognosis across multiple cancer types [32–34].In this study, RAP1GAP was differentially expressed in tumors compared to normal tissues and strongly correlated with patient outcomes. In most solid tumors, low RAP1GAP expression is associated with greater aggressiveness, poor prognosis, and increased metastasis rates [32, 35]. Notably, in gastric and breast cancers, reduced RAP1GAP levels correlate with high-grade differentiation, local invasion, and distant metastasis [36, 37].

Dynamic changes in the tumor microenvironment influence immune responses, ultimately affecting the prognosis of cancer patients undergoing immunotherapy [38]. Beyond its role in tumor cell proliferation and migration, RAP1GAP may also modulate tumor progression by shaping the immune microenvironment [14, 39]. Studies suggest that RAP1GAP expression is closely associated with various immunomodulatory genes [40, 41]. Our findings indicate that RAP1GAP regulates immune cell recruitment and function by modulating chemokines, cytokines, and their receptors across different cancers. Additionally, it may influence immune checkpoint gene expression, including PD-1, PD-L1, and CTLA-4. Given the immunosuppressive role of checkpoint pathways in tumors, RAP1GAP could contribute to immune evasion by altering their activity [14, 41]. Although research on this topic remains limited, RAP1GAP emerges as a potential regulator of the TME, warranting further investigation into its role in immune modulation.

In recent years, genomic markers such as TMB and MSI have gained significant attention for predicting immunotherapy responses [42, 43]. TMB reflects the total number of tumor mutations, with higher mutation loads often correlating with better immunotherapy outcomes [44]. MSI, a form of genomic instability resulting from DNA mismatch repair defects, enhances tumor immunogenicity and increases sensitivity to immune checkpoint inhibitors (ICIs) [45]. Our study suggests a potential link between RAP1GAP expression and these genomic features. Low RAP1GAP levels may correspond to higher TMB or MSI, influencing tumor responsiveness to immunotherapy. Additionally, RAP1GAP's association with DNAss and RNAss may contribute to genomic stability, further impacting immunotherapy sensitivity [23]. Future studies should explore RAP1GAP's regulatory role in these genomic characteristics and its potential as an immunotherapy biomarker.

Bladder cancer, a common urinary malignancy, exhibits high recurrence, aggressiveness, and complex pathogenesis [46, 47]. Public data suggest that low RAP1GAP expression correlates with higher tumor grade, increased lymph node metastasis, and poorer prognosis, implying its role in suppressing tumor progression by regulating cell adhesion and migration. Studies further highlight RAP1GAP's anticancer function, linking its expression to both patient outcomes and the TME [14, 39, 41]. Our findings indicate that RAP1GAP may modulate immune responses in bladder cancer by influencing immune cell infiltration. We hypothesize that RAP1GAP enhances antitumor immunity by inhibiting macrophage polarization and reducing immunosuppression. It may regulate immune checkpoint molecules such as PD-L1, potentially affecting sensitivity to immune checkpoint inhibitors. In our validation cohort, RAP1GAP was upregulated in tumors, but its association with clinical features, including PD-L1 and CD8 + cells, lacked statistical significance, likely due to the small sample size. Further research is needed to clarify RAP1GAP's interaction with immune cells and its role in bladder cancer immunotherapy. Integrating RAP1GAP expression with microenvironment profiling may offer new strategies for optimizing immunotherapy.

However, this study has certain limitations, requiring further validation through additional experiments and clinical samples. In bladder cancer, its low expression is associated with poor outcomes and immune evasion. Further research on RAP1GAP's role in immune regulation, genomic instability, and immunotherapy response could provide valuable insights and identify novel targets for precision bladder cancer treatment.

5 Conclusion

In conclusion, RAP1GAP not only influences tumor cell behavior but also plays a crucial role in shaping the tumor immune microenvironment. Its association with tumor genomic features underscores its potential relevance in immunotherapy.

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Author contributions ZS, XL and XYL carried out the interpretation of data and drafted the manuscript. XL completed the experimental verification. BL and YZ conceived and designed the study. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate We obtained a commercial bladder cancer tissue microarray (Cat. No. HBlaU050CS01) along with corresponding clinical information from Shanghai Outdo Biotechnology Company (China). All tissue samples and clinical data were collected with informed patient consent and approved by the institutional ethics committee of Shanghai Outdo Biotech Company (No. YB M-05-02). Since all the data used in this study were from publicly available databases and no human subjects were recruited from our institution, ethical approval was not required.

Consent to participate Written informed consent was obtained from all participants.

Competing interests The authors declare no competing interests.

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