Original Article

# *RAC1* as a potential pan-cancer diagnostic, prognostic, and immunological biomarker

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**Background:** Ras-related C3 botulinum toxin substrate 1 (*RAC1*) is an important member of the Rho GTPase family involved in tumorigenesis. However, its role and potential clinical utility across cancer entities in solid tumors is unknown.

**Methods:** We analyzed data from various databases, including The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression Project, cBioPortal, Tumor Immune Estimation Resource 2 (TIMER2), and published articles. A prognostic nomogram for liver hepatocellular carcinoma (LIHC) patients was developed based on RAC1-guanosine triphosphate (GTP) gene expression levels, which were validated using immunohistochemistry (IHC).

**Results:** In this study, *RAC1* was highly expressed in most cancers and correlated with prognosis and pathological stages. Furthermore, significant associations were observed between *RAC1* and DNA methylation, immune cell infiltration, immune-related genes, tumor mutational burden, and microsatellite instability in most tumors. As a use case, we employed gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) to analyze the biologic importance of *RAC1* expression and established a prognostic nomogram based on tumor stage and *RAC1* expression, which can better predict the overall survival rate of patients with LIHC better than tumor stage alone. The gene expression results were validated with IHC, which confirmed a higher expression of the RAC1-GTP protein in LIHC compared to paracancerous tissues.

**Conclusions:** This extensive solid tumor analysis provides sound evidence that *RAC1* can serve as both as an immunotherapy target and as a diagnostic and prognostic biomarker.

**Keywords:** Ras-related C3 botulinum toxin substrate 1 (*RAC1*); solid tumors; liver hepatocellular carcinoma (LIHC); prognostic analysis; immunotherapy

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## Introduction

Cancer has become a global health crisis, impacting millions of people worldwide, with increasing morbidity and mortality (1). Cancer not only causes suffering to individuals but also imposes a considerable economic burden on society (2). Over the past decade, even with improvements in the diagnosis and treatment of the disease, many cancer patients still have poor prognosis (3). Thus, it is necessary to find new ways to detect and treat cancer early and effectively. The Cancer Genome Atlas (TCGA) and other public databases are continually improving and developing. Whole genome sequencing provides insight into the similarities and differences among different types of cancer and will hopefully lead to the identification of new potential diagnostic, prognostic and therapeutic targets sometimes with agnostic characteristics.

There is evidence suggesting that genetic and epigenetic alterations are involved in the occurrence and development of tumors. Genetic alterations confer selective growth advantages to cancer cells (4). As an epigenetic alteration, DNA methylation can control gene expression without altering the sequence of the DNA (5). Tumor-suppressor genes that are abnormally methylated have been associated with prognoses in esophageal cancer (6). Additionally, immune infiltrating cells in the tumor microenvironment can contribute both to immune escape of tumors and to long-term disease control (7). In recent years, tumor immunotherapy has shown significant efficacy, especially immune checkpoint inhibitors (ICIs) (8-10). However, most patients remain insensitive to immunotherapy. Thus, the identification of new targets that may better stratified cancer about prognosis and or are predictive for immunotherapy/targeted therapies efficacy constitutes an important challenge in this setting.

Ras-related C3 botulinum toxin substrate 1 (*RAC1*) is a vital member of the Rho GTPase family. Proteins in this group can be transformed between the guanosine diphosphate (GDP)-bound state (inactive) and guanosine triphosphate (GTP)-bound state (active) (11). Many cellular processes are regulated by *RAC1*, including cell proliferation, apoptosis, invasion and metastasis, and angiogenesis, which promote tumorigenesis and development. *RAC1* has also been implicated in cancer therapy resistance (12). Over the past few years, several studies have demonstrated the abnormal expression or activation of *RAC1* in some cancers that contributes to poor prognosis (13-15). A relationship between *RAC1* and immunity has also been found. *RAC1* P29S mutant melanoma cells have a higher level of programmed cell death ligand-1 expression than do *RAC1* wild-type cells and can evade immune surveillance (16). In B-cell chronic lymphocytic leukemia, lenalidomide, as an immunomodulatory agent, restores normal levels of *RAC1* activity in T cells, thereby maintaining T-cell adhesion and motility (17). Based on these findings, it may be possible to analyse *RAC1* expression/alteration for cancer diagnosis, prognosis, and predictive factor for immunotherapy. Thus far, most studies on the *RAC1* gene have been limited to specific tumors, and systematic research on its role is lacking. Thus, our study aimed to explore the role of *RAC1* in a wider range of solid tumors in order to reveal innovative ideas and methods for clinical studies and treatments in cancer.

In this study, database information was used to analyze *RAC1* expression and investigate its prognostic value (*Table 1*). The level of *RAC1* expression was notably associated with pathological stages, *RAC1* promoter methylation, immune cell infiltration, immune-related genes, tumor mutational burden, and microsatellite instability. As a use case, we employed gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) to analyze the biological function and pathway of *RAC1* in liver hepatocellular carcinoma (LIHC). Based on tumor stage and *RAC1* expression, the existing prognostic nomogram

### Highlight box

**Key findings**

- Ras-related C3 botulinum toxin substrate (*RAC1*) can serve as a solid tumor biomarker.

**What is known and what is new?**

- *RAC1* is involved in tumor development and immunotherapy in specific tumors.
- Our results provided a new perspective on the role of *RAC1* in pan-cancer diagnosis, prognosis, and potential predictive factor regarding immune-check point inhibitors. Moreover, immunohistochemistry (IHC) results showed that liver hepatocellular carcinoma (LIHC) tissues expressed more RAC1-guanosine triphosphate (GTP) than did normal tissues.

**What is the implication, and what should change now?**

- *RAC1* may be both an immunotherapy target and diagnostic and prognostic biomarker.
- Our study also provides a theoretical basis for studying *RAC1 in vitro* and *in vivo*, which can enhance our understanding of how *RAC1* can be targeted therapeutically, thereby providing a superior immune-based anticancer strategy.

**Table 1** *RAC1* gene expression, relationship between *RAC1* gene expression and OS, TAM infiltration in 33 types of tumors

| Tumor | *RAC1* Expression | Prognosis (OS) | TAMs |
|---|---|---|---|
| ACC | Higher | Poor | N |
| BLCA | Higher | Poor | N |
| BRCA | Higher | Poor | Positive |
| CESC | Higher | Poor | Positive |
| CHOL | Higher | Better | N |
| COAD | Higher | Poor | N |
| DLBC | Higher | Better | N |
| ESCA | Higher | N | N |
| GBM | Higher | Poor | N |
| HNSC | Higher | Poor | N |
| KICH | N | Poor | N |
| KIRC | Higher | Poor | Positive |
| KIRP | Higher | N | Negative |
| LAML | Lower | N | N |
| LGG | Higher | Poor | Positive |
| LIHC | Higher | Poor | Positive |
| LUAD | Higher | Poor | Positive |
| LUSC | Higher | N | Positive |
| MESO | N | Poor | N |
| OV | Higher | Poor | N |
| PAAD | Higher | Poor | Positive |
| PCPG | N | Better | Positive |
| PRAD | Higher | N | N |
| READ | Higher | N | N |
| SARC | Higher | Poor | Positive |
| STAD | Higher | N | Positive |
| SKCM | Higher | Poor | Positive |
| TGCT | Higher | N | Negative |
| THCA | Higher | N | Positive |
| THYM | Higher | N | Positive |
| UCEC | Higher | N | N |
| UCS | Higher | N | N |
| UVM | N | Poor | N |

*RAC1*, Ras-related C3 botulinum toxin substrate 1; OS, overall survival; TAM, tumor-associated macrophage; ACC, adrenocortical carcinoma; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; STAD, stomach adenocarcinoma; SKCM, skin cutaneous melanoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; N, non-significant.

 

was better able to predict patients' overall survival (OS) rates. Finally, through immunohistochemistry (IHC), we further confirmed the expression of RAC1-GTP in LIHC. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2016/rc).

## Methods

### *RAC1 gene expression pattern*

Case information regarding messenger RNA (mRNA) expression and clinical features (18,086 samples from 33 cancers) were obtained from TCGA and the Genotype-Tissue Expression (GTEx) project and downloaded from the website(https://xenabrowser.net/datapages/). The TCGA database contained information on paracancerous tissues, however, due to the absence of corresponding paracancerous tissue data for certain tumors in TCGA, data from both TCGA and GTEx were utilized to analyze and compare the expression levels of *RAC1* in cancerous and normal tissues. We transformed the RNA sequencing (RNA-seq) data to transcripts per million (TPM), and a log2 (1 + TPM) transformation was performed. Analysis of tumor and normal tissue expression was performed using *t*-tests. A clinical dataset from TCGA was analyzed to determine whether the expression of *RAC1* affected pathological stages. The "ggplot2" R package (version 4.1.1, The R Foundation of Statistical Computing) was used to analyze and visualize the results.

### *Gene alteration, copy number alteration (CNA), and RAC1 DNA methylation analysis*

cBioPortal database (https://www.cbioportal.org/) is a web platform for analyzing tumor genomic characteristics in the *RAC1* gene. We used this database to analyze the types and frequency of *RAC1*. Pearson rank correlation coefficient was calculated to analyze the relationship between *RAC1* expression and CNA and DNA methylation. TCGA samples from the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN; http://ualcan.path.uab.edu/) were used to compare the promoter methylation level of *RAC1* between tumors and normal tissues.

### *Prognostic value of RAC1 gene*

TCGA provided us with survival data of tumor patients (n=9,784). Using routing algorithms, we divided samples into low and high groups based on the optimal cutoff point, OS, disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) were evaluated. The "surv-cutpoint" function within the survminer R package was utilized to identify the optimal split point through a comprehensive evaluation of all potential cut points. Forest plot analysis was conducted using the R package "forestplot", and R packages "survival" and "survminer" were used to draw survival curves. Log-rank P values, hazard ratios (HRs), and 95% confidence intervals were examined.

### *Establishment and assessment of the nomogram*

To determine the prognostic value of the *RAC1* expression level in patients with LIHC, we used an online platform (https://www.home-for-researchers.com/static/index.html#/) to conduct univariate and multivariate Cox regression analysis and develop a nomogram of tumor stage and *RAC1* expression. Finally, a correction curve was used to determine whether the nomogram was predictive.

### *GSEA and GSVA of RAC1 gene in LIHC*

Based on GSEA and GSVA, we examined the biological function and significance of *RAC1* in LIHC. GSEA was implemented using the R package "clusterProfiler". A set of gene signatures for the Hallmark pathway was obtained from the Molecular Signatures Database and by using the R package "GSVA", and we obtained the hallmark pathway scores for LIHC.

### *Relationship between RAC1 gene expression, immune cell infiltration, and immune-related genes*

The Tumor Immune Estimation Resource 2 (TIMER2; http://timer.comp-genomics.org/) offers three distinct modules, namely Immune Association, Cancer Exploration, and Immune Estimation, which facilitate the examination of relationships between immune infiltration and various genetic or clinical attributes.

Analysis of the data revealed there to be a relationship between *RAC1* levels and immune cells. Next, our study utilized data on pan-cancer immune cell infiltration obtained from a previous study (18). Furthermore, we examined the relationship between *RAC1* and genes associated with immunity. To visualize the data, a heatmap

 

was produced using the "pheatmap" package in R.

### *Correlational research on RAC1 gene expression and tumor mutational burden, as well as microsatellite instability*

Using data derived from TCGA, we examined tumor mutational burden in 33 cancer types. The microsatellite instability data were obtained from a published article (19), and the Pearson rank correlation coefficient was calculated to analyze the relationship between *RAC1* expression and tumor mutational burden and microsatellite instability. In order to visualize data, a radar map was generated using the "ggradar" package in R.

### *Immunohistochemical analysis*

The Pathological Center of Ningbo City provided paraffin sections of five pairs of LIHC tissue specimens and adjacent tissue specimens from postoperative patients with LIHC from Department of Radiation Oncology, The Affiliated Lihuili Hospital, Ningbo University between January 2021 to December 2021. RAC1-GTP antibody was purchased from NewEast Biosciences (cat no. 26903; RRID: AB_1961793; NewEast Biosciences, Wuhan, Zhejiang). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The Ethics Committee of The Affiliated Lihuili Hospital, Ningbo University approved this study (No. KY2022SL322-01). Individual consent for this retrospective analysis was waived. Determine the proportion of positive cells based on sample color and staining intensity, and evaluate the expression intensity of cells. If 0–10% is negative, the score is 0; 11–30% are weakly positive, with a score of 1; 31–60% is positive, with a score of 2; 61–100% are strongly positive, with a score of 3.

### *Statistical analysis*

All statistical analyses were performed in R software (version 4.1.1). Changes in *RAC1* expression levels between cancer and normal tissues were assessed through *t*-tests. Univariate Cox regression analysis was employed to calculate the HR and P value for survival analysis. Kaplan-Meier analysis was utilized to examine the survival duration of patients categorized based on high or low *RAC1* expression levels. The Pearson rank correlation coefficient was computed in order to examine the association between the variables. A value of $P<0.05$ (2-sided) was considered significant for the abovementioned statistical analyses.

## Results

### *Tumor and normal tissue expression of RAC1 gene*

We combined TCGA data with GTEx data to examine *RAC1* expression between 33 common cancers and their corresponding normal tissues. A comparison of *RAC1* expression between corresponding normal tissues and tumor tissues revealed no significant difference in kidney chromophobe (KICH) or pheochromocytoma and paraganglioma (PCPG), and significant upregulation in other tumor tissues. However, the expression of RAC1 is elevated in normal tissues compared to tumor tissues in acute myeloid leukemia (LAML) (*Figure 1A*). In 33 tumor tissues from TCGA, esophageal carcinoma (ESCA) exhibited the highest levels of *RAC1* and KICH exhibited the lowest levels of *RAC1*. According to the GTEx database, *RAC1* was expressed at the highest level in the skin and the lowest in the pancreas (*Figure 1B*,*1C*). TCGA database was used to retrieve paired samples and evaluate *RAC1* expression. In tumor tissues from patients with bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), ESCA, head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), LIHC, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ), and stomach adenocarcinoma (STAD), *RAC1* expression was higher than in adjacent normal tissues. By contrast, its expression was downregulated in KICH (*Figure 2*).

### *Relationship between RAC1 gene expression and pathological stages*

The expression of *RAC1* was analyzed in patients' various tumors according to the cancer stage to determine if a relationship exists between *RAC1* expression and pathological features. The results showed that *RAC1* was differentially expressed in different stages of adrenocortical carcinoma (ACC), colon adenocarcinoma (COAD), HNSC, KICH, KIRC, LIHC, LUAD, mesothelioma (MESO), pancreatic adenocarcinoma (PAAD), uterine corpus endometrial carcinoma (UCEC), and ovarian serous cystadenocarcinoma (OV), with the expression level generally being even higher in the more advanced stages (Figure S1).

 

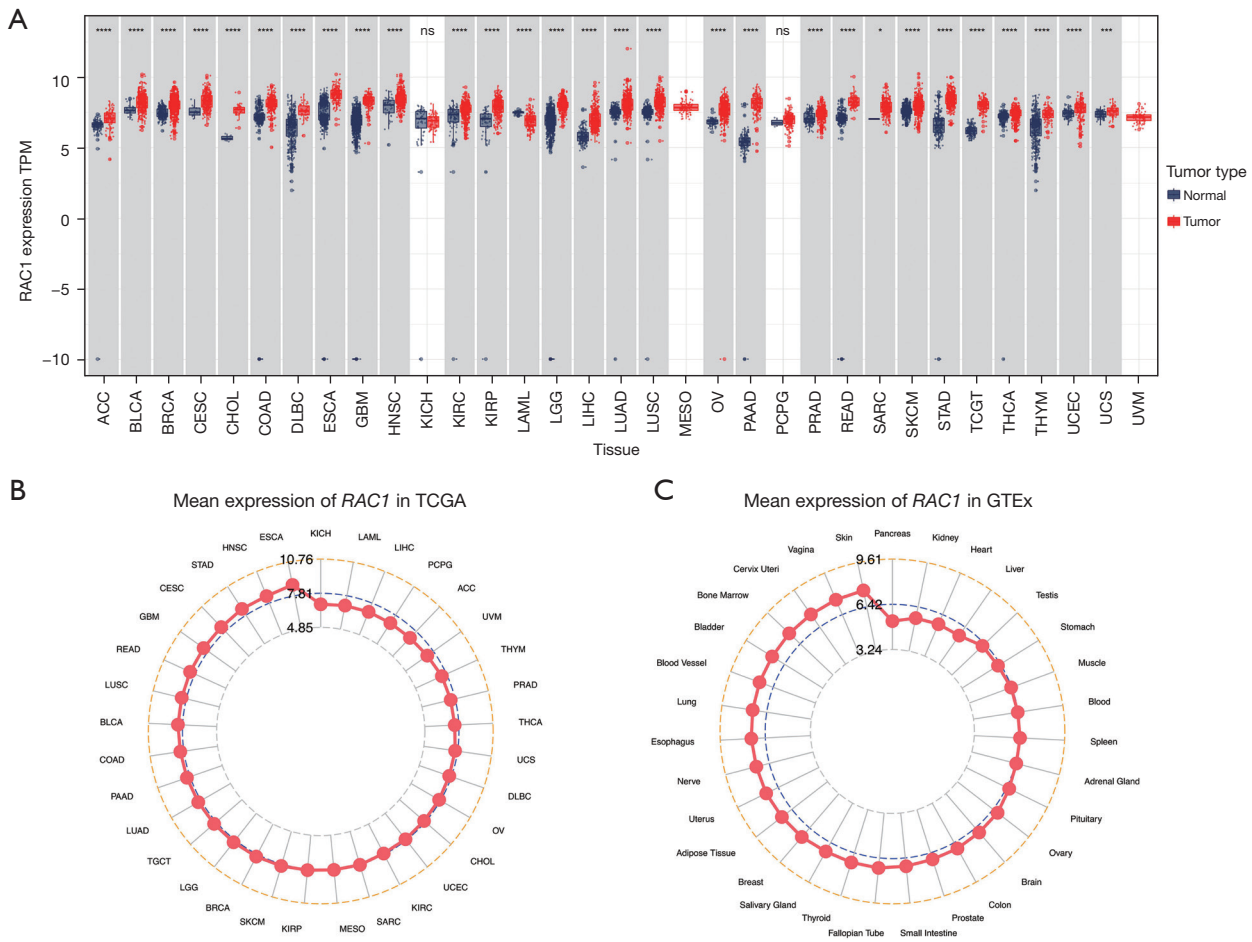


**Figure 1** *RAC1* expression pattern. (A) The pan-cancer expression patterns for *RAC1* from the TCGA and GTEx databases. (B) The expression of *RAC1* in various tumor tissues was derived from TCGA. (C) The expression of the *RAC1* gene in various normal tissues was derived from GTEx. *, P<0.05; ***, P<0.001; ****, P<0.0001. The dashed blue line represents the median. ns, no significance; *RAC1*, Ras-related C3 botulinum toxin substrate 1; TPM, transcripts per million; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, 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; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

### *Gene alteration, CNA, and methylation levels of RAC1 gene promoter*

We used the cBioPortal database to study *RAC1* gene alteration and CNA. The results showed that *RAC1* gene alteration was most common in patients with skin cutaneous melanoma (SKCM), at about an 8.7% incidence. The alteration of the *RAC1* gene included mutation, amplification, deep deletion, and multiple alterations. The most common form of gene alteration was amplification. Amplification was also a major alteration

 

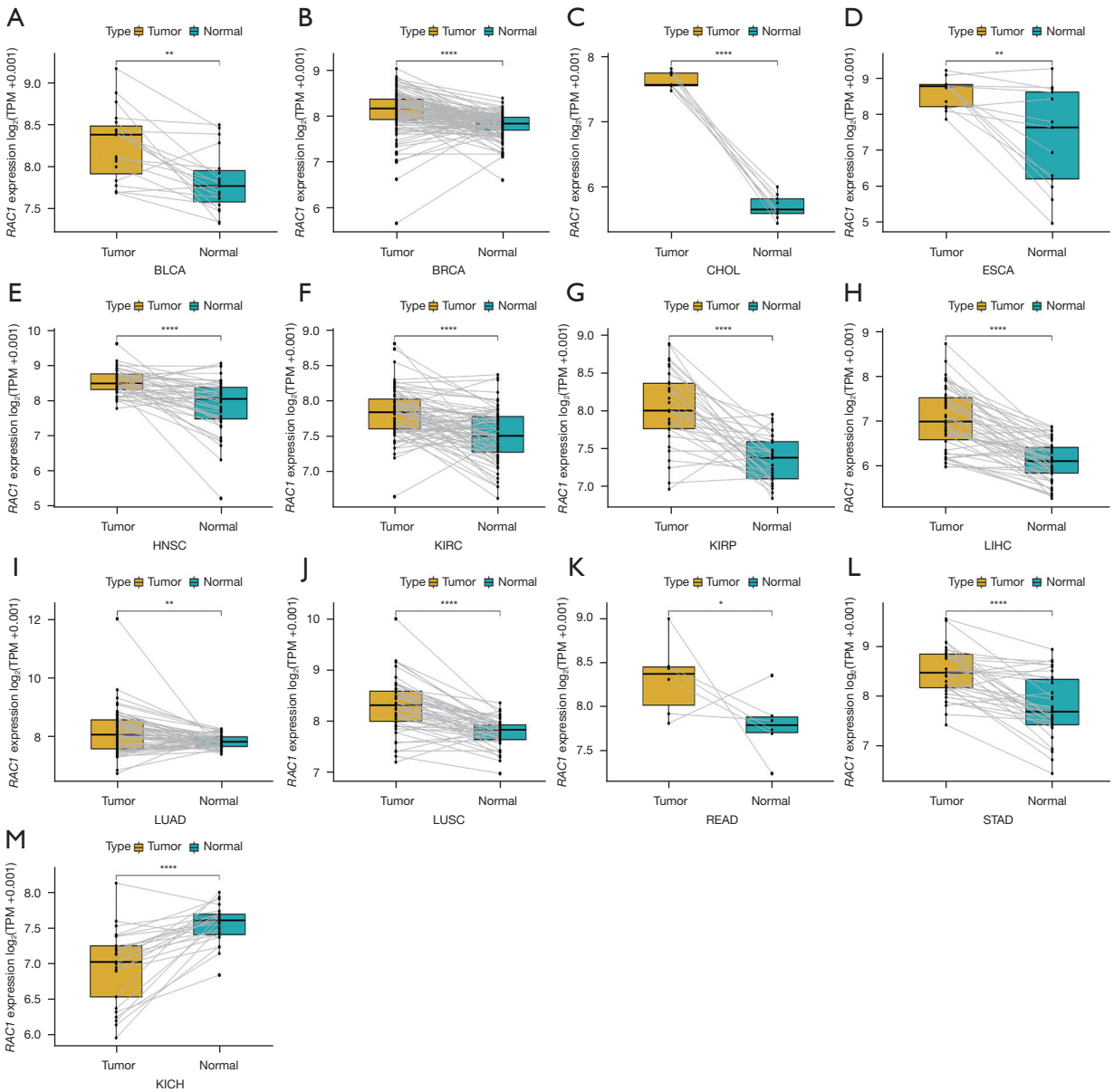


**Figure 2** Expression of *RAC1* in tumor and paired normal tissues based on TCGA. (A-L) *RAC1* expression was high in 12 cancer types. (M) The expression of *RAC1* was low in KICH. *, P<0.05; **, P<0.01; ****, P<0.0001. *RAC1*, Ras-related C3 botulinum toxin substrate 1; TPM, transcripts per million; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CHOL, cholangiocarcinoma; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; KICH, kidney chromophobe; TCGA, The Cancer Genome Atlas.

in uterine carcinosarcoma (UCS), BLCA, ESCA, sarcoma (SARC), LUAD, ACC, STAD, OV, testicular germ cell tumors (TGCT), brain lower grade glioma (LGG), KIRP, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), PCPG, BRCA, glioblastoma multiforme (GBM), LIHC, and thyroid carcinoma (THCA) (*Figure 3A*). Additionally, rather than KICH, pancreatic cancer (PANC), thymoma (THYM), and THCA, *RAC1* expression correlated positively with CNA (*Figure 3B*). Furthermore, *RAC1* promoter hypermethylation was accompanied by

 

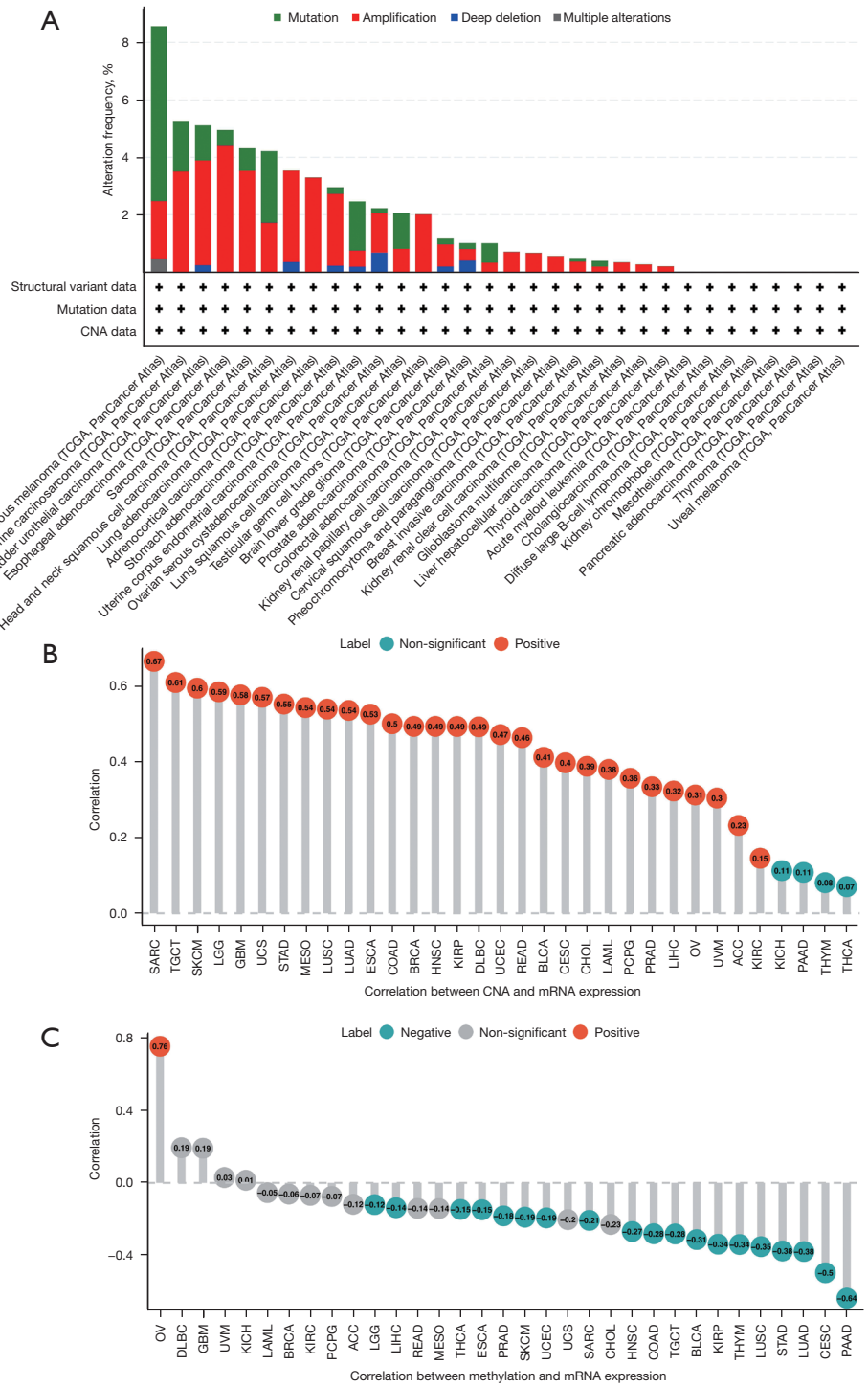


**Figure 3** Gene mutation, CNA, and methylation of *RAC1* promoter. (A) The *RAC1* mutation frequency in cancers from cBioPortal. (B) The relationship between *RAC1* expression levels and CNA. (C) The association between DNA methylation and *RAC1* expression. CNA, copy number alteration; TCGA, The Cancer Genome Atlas; SARC, sarcoma; TGCT, testicular germ cell tumors; SKCM, skin cutaneous melanoma; LGG, lower grade glioma; UCS, uterine carcinosarcoma; STAD, stomach adenocarcinoma; MESO, mesothelioma; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; ESCA, esophageal carcinoma; COAD, colon adenocarcinoma; BRCA, breast invasive carcinoma; HNSC, head and neck squamous cell carcinoma; KIRP, kidney renal papillary cell carcinoma; DLBC, lymphoid

 

neoplasm diffuse large B-cell lymphoma; UCEC, uterine corpus endometrial carcinoma; READ, rectum adenocarcinoma; BLCA, bladder urothelial carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; LAML, acute myeloid leukemia; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; LIHC, liver hepatocellular carcinoma; OV, ovarian serous cystadenocarcinoma; UVM, uveal melanoma; ACC, adrenocortical carcinoma; KIRC, kidney renal clear cell carcinoma; KICH, kidney chromophobe; PAAD, pancreatic adenocarcinoma; THYM, thymoma; THCA, thyroid carcinoma; GBM, glioblastoma multiforme.

down-regulation of *RAC1* expression in LGG, LIHC, THCA, ESCA, prostate adenocarcinoma (PRAD), SKCM, UCEC, SARC, HNSC, COAD, TGCT, BLCA, KIRP, THYM, LUSC, STAD, LUAD, CESC, and PANC tumor tissues; conversely, *RAC1* expression increased with hypermethylation of its promoter in OV tumor tissues (*Figure 3C*).

Next, we investigated the association between *RAC1* promoter methylation level and cancers well as its prognostic value. Compared with those of normal tissue, *RAC1* promoter methylation levels were significantly decreased in BLCA, BRCA, CESC, CHOL, ESCA, HNSC, UCEC, LIHC, LUAD, LUSC, PRAD, and TGCT tumor tissues. However, the methylation level exhibited a modest increase in KIRP tumor tissues (Figure S2). In order to determine whether *RAC1* promoter methylation has prognostic significance, Kaplan-Meier analyses were conducted. The hypermethylation of *RAC1* promoter in LIHC, LUAD, PANC, and THYM suggested increased OS but was associated with reduced OS in KIRP. The hypermethylation of *RAC1* promoter was a good prognostic factor in patients with LUAD for DSS and in PANC for DFI. Regarding PFI, high *RAC1* promoter methylation level in CHOL was significantly associated with better PFI, whereas *RAC1* promoter hypermethylation level in KIRP was significantly associated with poor PFI (Figure S3).

### *Survival analysis of patients with altered RAC1 gene expression*

We sought to determine whether *RAC1* expression is associated with survival outcomes. Indicators of survival prognosis included OS, DSS, DFI, and PFI. Univariate Cox regression analysis of 33 tumor outcomes indicated that *RAC1* expression had a significant correlation with OS in 11 tumors. *RAC1* was a risk factor in some tumor types, particularly in KICH. However, it was a protective factor in lymphoid neoplasm diffuse large B-cell lymphoma (DLBC) (*Figure 4A*). The DSS analysis revealed that *RAC1* was a risk factor for 11 tumor types, including LGG, MESO, ACC, LIHC, GBM, uveal melanoma (UVM), KICH, PANC, LUAD, CESC, and SKCM (*Figure 4B*). In the DFI analysis, *RAC1* was found to be a risk prognostic factor for ACC and PANC (*Figure 4C*). In terms of PFI, high *RAC1* expression was found to be associated with clinical outcomes of LGG, ACC, MESO, UVM, PANC, and KIRC (*Figure 4D*).

Kaplan-Meier curves were then generated to determine the prognostic value of *RAC1* expression. Results indicated that high *RAC1* expression was associated with poor OS in 18 cancer types, including ACC, BLCA, and BRCA, among others. In contrast, the high expression of *RAC1* in CHOL, DLBC, and PCPG was associated with better survival (*Figure 5*). With regards to DSS, the analysis revealed that an increase in *RAC1* expression was associated with shorter survival in most cancers, but increased expression of *RAC1* in THCA and KIRP was associated with longer DSS (Figure S4).

The upregulation of the *RAC1* gene was found to be linked to unfavorable DFI in eight cancer types, including ACC, LGG, LIHC, and others, but linked to better DFI in HNSC (Figure S5). Furthermore, *RAC1* expression was upregulated in the majority of cancers, which was related to a worse PFI. However, in the case of LUSC, TGCT, THCA, and KIRP, *RAC1* upregulated was associated with a better PFI (Figure S6).

### *Nomogram of LIHC*

Due to the former evidences seems to emerge that patients with LIHC who exhibit high expression of *RAC1* are at risk of a poor prognosis. Univariate Cox regression analysis was conducted, and tumor size, metastasis sites, and *RAC1* were associated with survival and prognosis in LIHC. According to multivariate Cox regression analysis, tumor size and *RAC1* were independent risk factors in patients with LIHC (*Figure 6A,6B*). We then developed a nomogram based on tumor stage and *RAC1* expression level to the predict 1-, 3-, and 5-year survival (*Figure 6C*). In order to determine the accuracy of the prediction model, calibration plots were drawn. The results demonstrated that the nomogram had

 

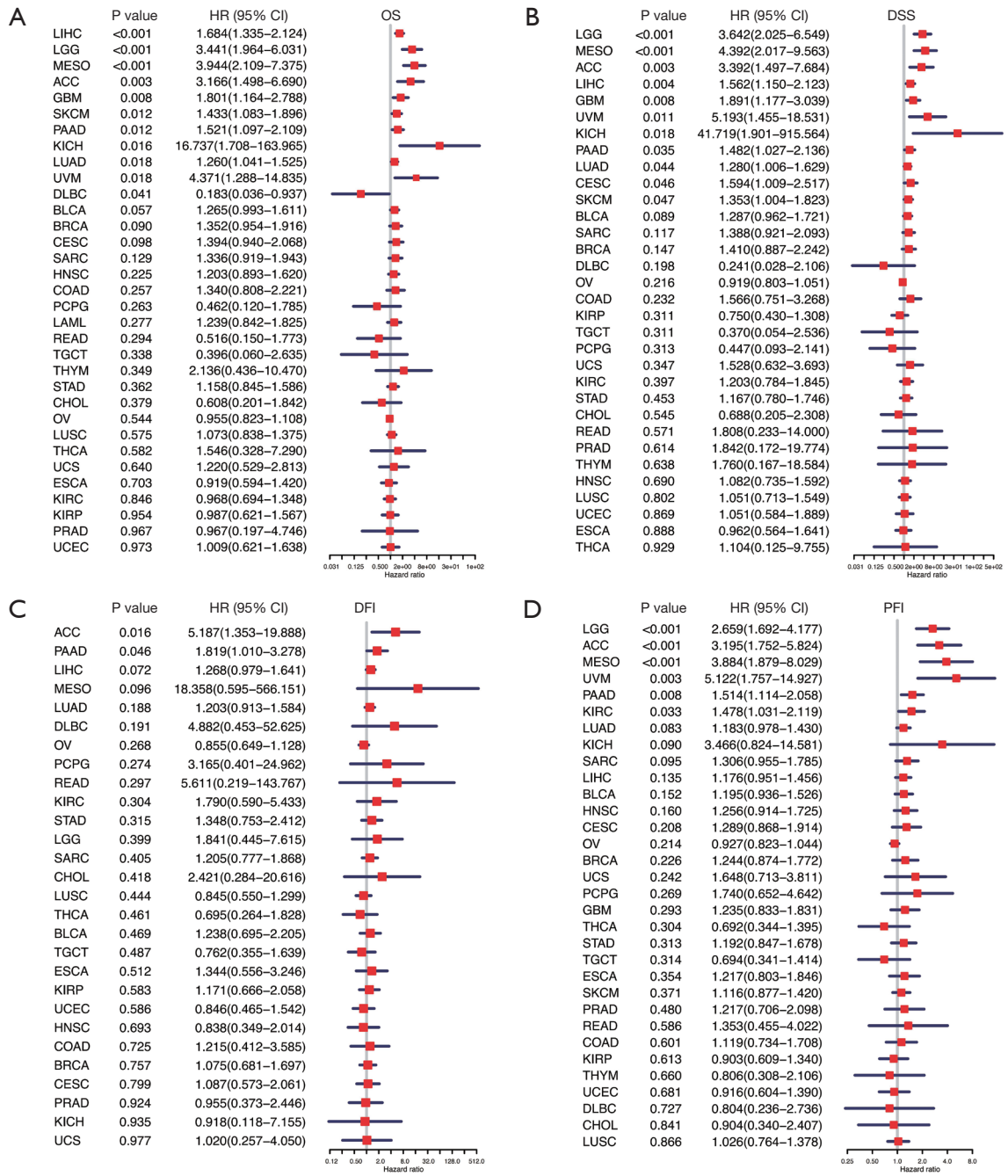


**Figure 4** Association between *RAC1* expression and prognosis. A forest plot of hazard ratios of *RAC1* in cancers for OS (A), DSS (B), DFI (C), and PFI (D). LIHC, liver hepatocellular carcinoma; LGG, lower grade glioma; MESO, mesothelioma; ACC, adrenocortical carcinoma; GBM, glioblastoma multiforme; SKCM, skin cutaneous melanoma; PAAD, pancreatic adenocarcinoma; KICH, kidney chromophobe; LUAD, lung adenocarcinoma; UVM, uveal melanoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; SARC, sarcoma; HNSC, head and neck squamous cell carcinoma; COAD, colon adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; LAML, acute myeloid leukemia; READ, rectum adenocarcinoma; TGCT, testicular germ cell tumors; THYM, thymoma; STAD, stomach adenocarcinoma; CHOL, cholangiocarcinoma; OV, ovarian serous cystadenocarcinoma; LUSC, lung squamous cell carcinoma; THCA, thyroid carcinoma; UCS, uterine carcinosarcoma; ESCA, esophageal carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; PRAD, prostate adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; HR, hazard ratio; CI, confidence interval; OS, overall survival; DSS, disease-specific survival; DFI, disease-free interval; PFI, progression-free interval; *RAC1*, Ras-related C3 botulinum toxin substrate 1.

 

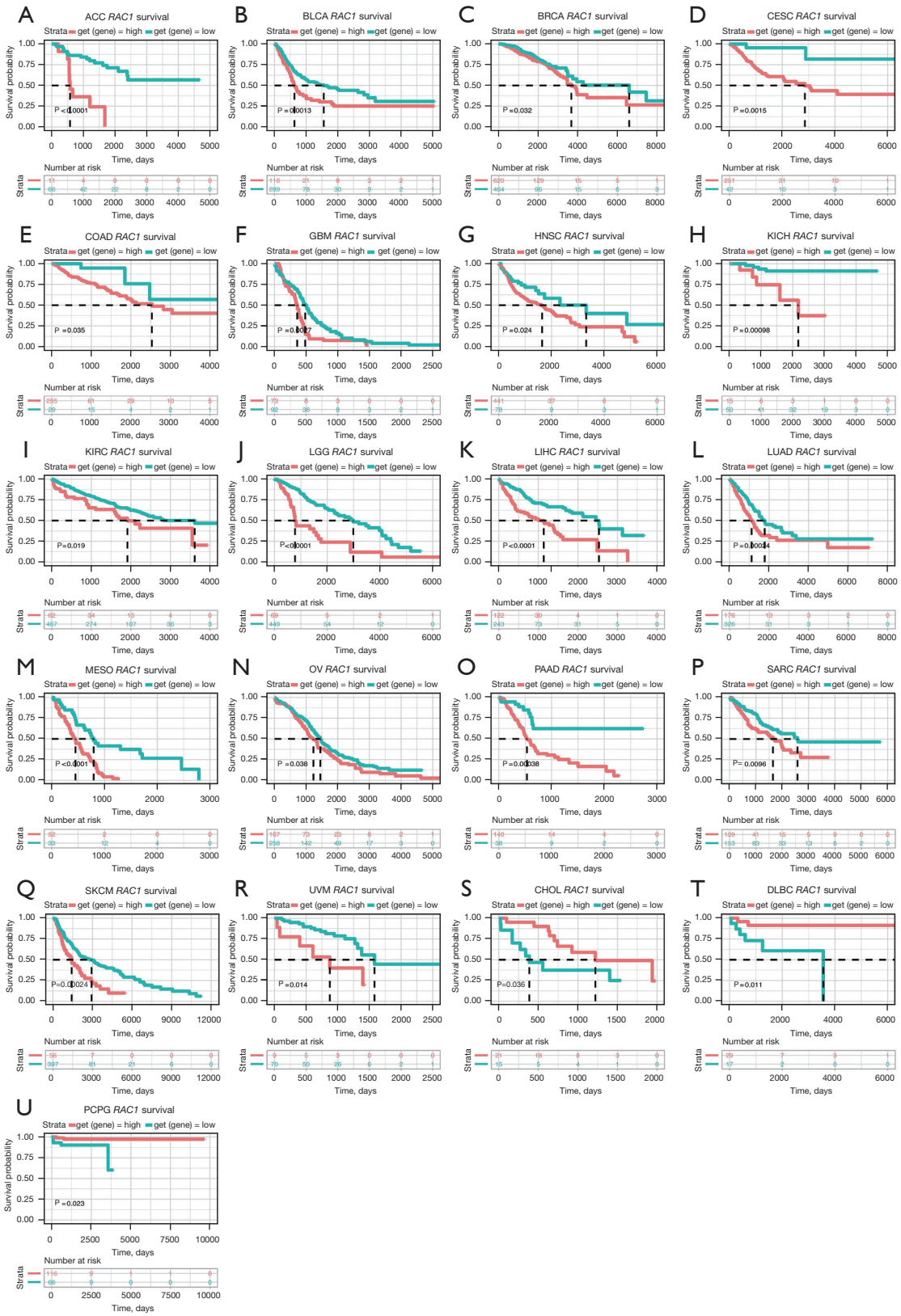


**Figure 5** The association between the *RAC1* expression and survival outcomes. (A-R) Kaplan-Meier analysis indicated that high *RAC1*

 

expression was related to poor OS in 18 tumor types. (S-U) Kaplan-Meier analysis indicated that high *RAC1* expression was associated with better OS in some tumor types. ACC, adrenocortical carcinoma; *RAC1*, Ras-related C3 botulinum toxin substrate 1; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; COAD, colon adenocarcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; UVM, uveal melanoma; CHOL, cholangiocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; PCPG, pheochromocytoma and paraganglioma; OS, overall survival.

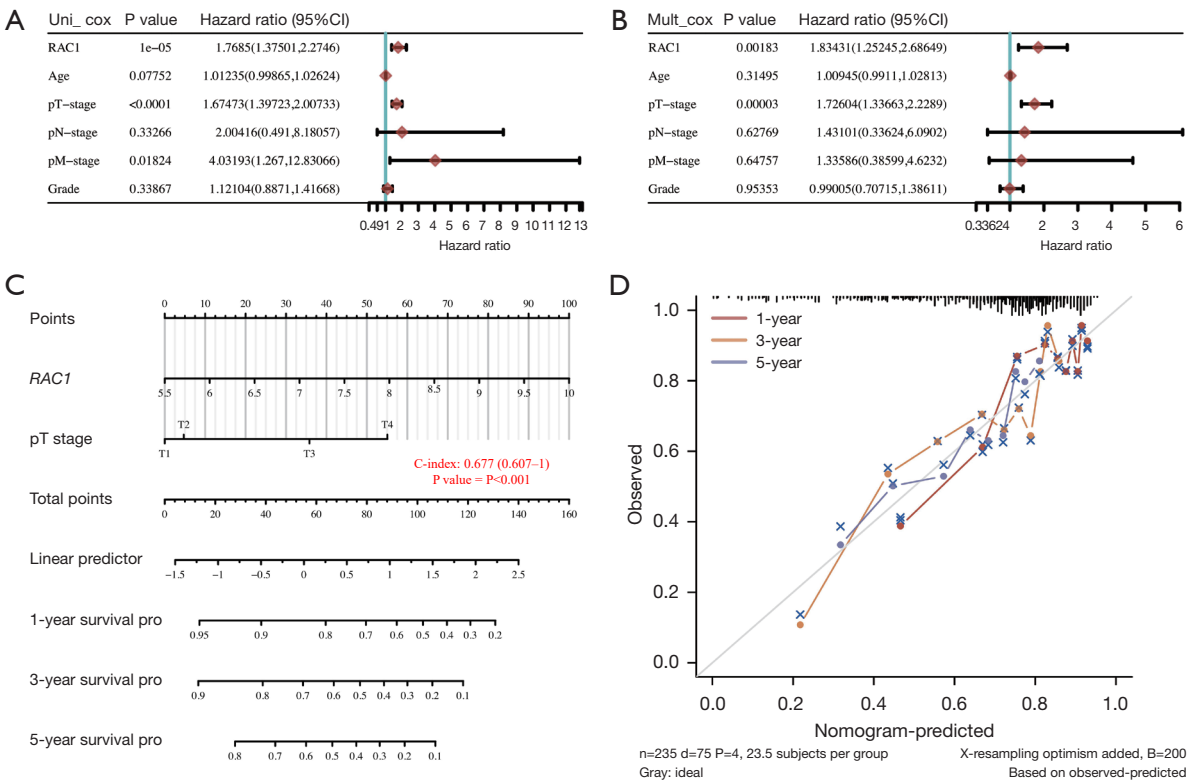


**Figure 6** Cox regression and correction curve analysis. Univariate analysis (A) and multivariate analysis (B) of overall survival for LIHC. (C) The nomogram could predict the overall survival of patients with LIHC. (D) The correction curve used to evaluate the nomogram. CI, confidence interval; *RAC1*, Ras-related C3 botulinum toxin substrate 1; pT, pathology tumor; pN, pathology lymph node; pM, pathology metastasis; LIHC, liver hepatocellular carcinoma.

good predictive ability (*Figure 6D*).

### *Analysis of GSEA and GSVA in LIHC for RAC1 gene*

To investigate the biological function of *RAC1*, we conducted GSEA on individuals with LIHC using the "clusterProfiler" package in R. The results showed major enrichment in immune regulation and cell cycle pathways, including the neutrophil, myeloid cell, and leukocyte activation involved in the immune response; neutrophil- and myeloid leukocyte-mediated immunity; adaptive and innate immune systems; neutrophil degranulation; cytokine signaling in the immune system, G2/M and G1/S transition of mitotic cell cycle; and cell cycle checkpoint (*Figure 7A-7C*). Meanwhile, the GSVA results indicated that *RAC1* is related to pathways that promote tumor development and immunity, including the mitotic spindle, G2/M checkpoint, DNA repair, PI3K-AKT-mTOR

 

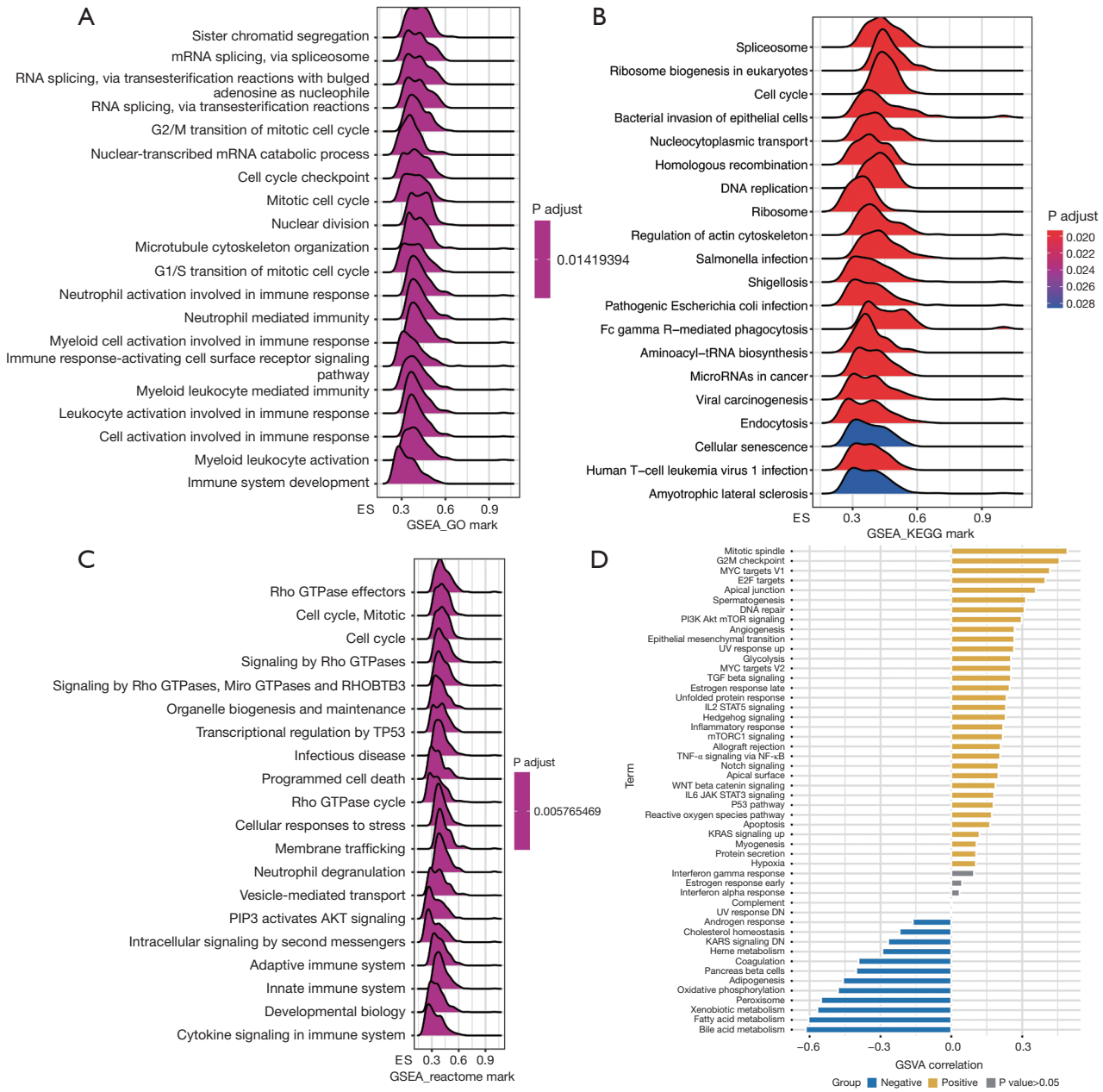


**Figure 7** Results of GSEA and GSVA for *RAC1* in LIHC. (A-C) The GSEA results of *RAC1* in LIHC, including GO, KEGG, and Reactome. (D) Analysis of the 50 hallmark pathways in LIHC according to GSVA. ES, enrichment score; GSEA, gene set enrichment analysis; GSVA, gene set variation analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; tRNA, transfer RNA; *RAC1*, Ras-related C3 botulinum toxin substrate 1; LIHC, liver hepatocellular carcinoma; MTOR, mammalian target of rapamycin; UV, ultraviolet; TGF, transforming growth factor; TNFA, tumor necrosis factor α; NFKB, nuclear factor kappa-B; JAK STAT3, Janus kinase signal transducer and activator of transcription 3; KRAS, Kirsten rat sarcoma; DN, dysplastic nodule; RHO, Ras homology; MYC, myelocytomatosis oncogene.

 

signaling, interleukin 2 (IL-2)-STAT5 signaling, the inflammatory response, and IL-6-JAK-STAT3 signaling (*Figure 7D*). According to the results of *RAC1*, this biomarker may serve as an indicator of Immunotherapy sensitivity in LIHC.

### *Correlation between RAC1 gene and tumor-associated macrophages (TAMs) infiltration level in tumor microenvironment*

Tumor microenvironment immune cells are usually dysfunctional, resulting in immune escape. TAMs are critically involved in tumorigenesis and tumor progression (20). A study has suggested a role for *RAC1* in some cancers, but it remains unclear whether abnormal expression of *RAC1* has an effect on the infiltration of TAMs (12). To elucidate the role of *RAC1* in tumors, we used the TIMER2 database and found a significant positive association between TAM infiltration and *RAC1* for most tumors (*Figure 8*). A published study (18) on immune cell infiltration, also found that the TAM infiltration level was consistent with the *RAC1* gene expression (*Figure 9*).

### *Correlation between RAC1 gene and immune-related genes, tumor mutational burden, and microsatellite instability*

We additionally investigated the association of immune-related genes with *RAC1*. Notably, most genes related to immune stimulation and immunosuppression were positively correlated with *RAC1* expression in OV, KICH, UVM, and PCPG. In contrast, in ESCA and LUSC, *RAC1* expression showed a negative correlation with many immune stimulation (*Figure 10A*) and immunosuppression genes (*Figure 10B*). Among the immune-suppressing genes, *IL10RB*, *TGFBR1*, and *TGFB1* expression correlated significantly with that of *RAC1* in most tumors. Several studies have demonstrated that tumor mutational burden and microsatellite instability are useful biomarkers, which can be helpful in predicting the effectiveness of immunotherapy in cancers (21,22). We examined if *RAC1* expression correlated with tumor mutational burden or microsatellite instability. We found that *RAC1* expression in MESO, LUAD, and LGG was positively correlated with tumor mutational burden, whereas *RAC1* expression in THCA, OV, and COAD was significantly negatively correlated with tumor mutational burden (*Figure 11A*). Microsatellite instability and *RAC1* expression were positively correlated in UVM, MESO, LIHC, and SARC, but negatively correlated in HNSC, LGG, PRAD, and COAD (*Figure 11B*).

### *Expression of RAC1-GTP in LIHC and adjacent normal tissues*

Further evidence for the role of *RAC1* in LIHC was obtained using immunohistochemical analyses performed on postoperative pathological sections from patients with LIHC. The analyses revealed that the expression of RAC1-GTP was higher in LIHC tissues than adjacent normal tissues (*Figure 11C*).

## Discussion

*RAC1* participates in a wide range of cellular events, which promote tumorigenesis and development. Abnormal expression or activation of *RAC1* is related to inferior prognosis in patients with tumor (13-15), and *RAC1* is also involved in tumor microenvironment-mediated immune escape (16,17). The aim of this study was to examine the role of *RAC1* in immunotherapy and tumor diagnosis and prognosis. In our literature search, we were unable to find any studies analyzing a full relationship between *RAC1* expression in a wide range of cancers. By analyzing whole raw data, similarities and differences between different types of cancer can be identified, thus facilitating the development of personalized cancer prevention and suggest treatment options.

In the study, the expression of *RAC1* was examined comprehensively across pan-cancer datasets. In the comparison of tumors with normal tissues, most tumors expressed higher levels of *RAC1*. Next, we investigated the association between *RAC1* expression and pathological stages. For most tumors, *RAC1* expression was higher in the advanced/metastatic stages. Furthermore, Cox proportional hazards model and Kaplan-Meier analysis indicated that upregulation of *RAC1* expression was correlated with poorer OS, DSS, DFI, and PFI in several cancers, while in other cancers, this was associated with better prognosis. These results suggest that *RAC1* could play a role in promoting tumors and could be a biomarker for diagnosis and prognosis.

The chemical modification of DNA, DNA methylation, alters the epigenetic state of a genome without changing its sequence, thus controlling gene expression. Aberrant methylation is a hallmark of cancer progression (5).

 

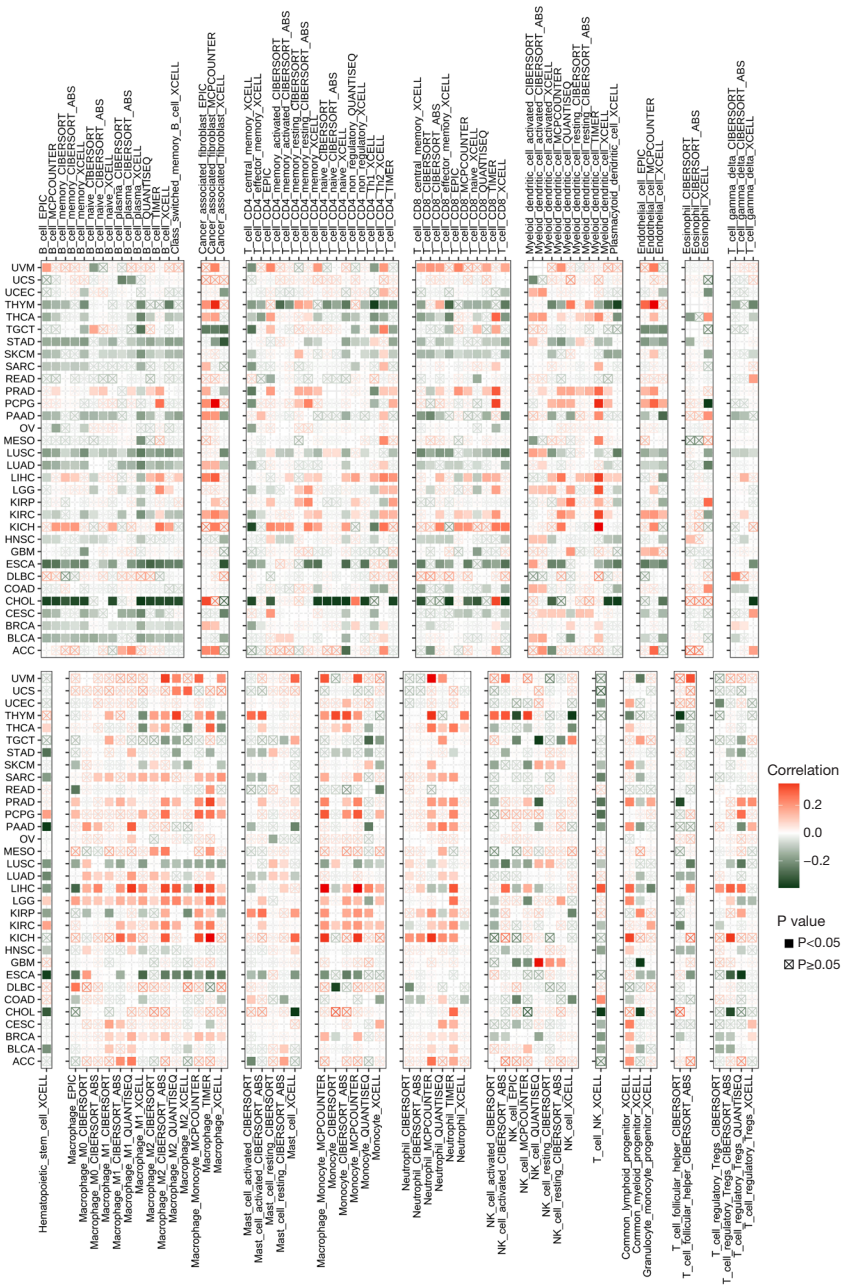


**Figure 8** Relationship of *RAC1* expression with immune infiltration level in tumors. Correlation analysis between *RAC1* gene expression and immune infiltration cells from TIMER2. UVM, uveal melanoma; UCS, uterine carcinosarcoma; UCEC, uterine corpus endometrial carcinoma; THYM, thymoma; THCA, thyroid carcinoma; TGCT, testicular germ cell tumors; STAD, stomach adenocarcinoma; SKCM, skin cutaneous melanoma; SARC, sarcoma; READ, rectum adenocarcinoma; PRAD, prostate adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PAAD, pancreatic adenocarcinoma; OV, ovarian serous cystadenocarcinoma; MESO, mesothelioma; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; LIHC, liver hepatocellular carcinoma; LGG, lower grade glioma; KIRP, kidney renal papillary cell carcinoma; KIRC, kidney renal clear cell carcinoma; KICH, kidney chromophobe; HNSC, head and neck squamous cell carcinoma; GBM, glioblastoma multiforme; ESCA, esophageal carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; BRCA, breast invasive carcinoma; BLCA, bladder urothelial carcinoma; ACC, adrenocortical carcinoma; *RAC1*, Ras-related C3 botulinum toxin substrate 1; TIMER2, Tumor Immune Estimation Resource 2.

 

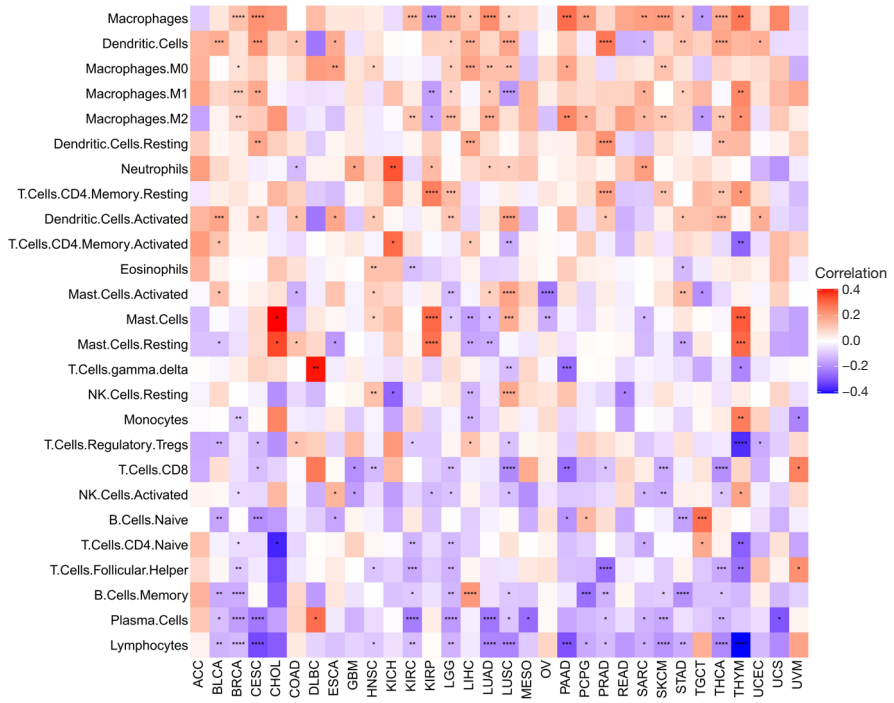


**Figure 9** Relationship of *RAC1* expression with immune infiltration level in tumors. Correlation analysis between *RAC1* gene expression and immune infiltration cells from a previous study. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. *RAC1*, Ras-related C3 botulinum toxin substrate 1; NK, natural killer; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, 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; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

Currently, promoter methylation is thought to be more easily detectable than that in other locations in genes, which makes it useful for the early detection of cancer (23,24). Our study found that downregulation of *RAC1* DNA promoter methylation was accompanied by the upregulation of *RAC1* expression in most common cancers. In addition, hypermethylation in some cancers was associated with a good prognosis, but not in KIRP. Therefore, abnormal methylation of *RAC1* promoter may affect the development of tumors and the prognosis of patients. These findings suggest that the status of *RAC1* DNA promoter methylation could be an early diagnostic marker.

There is evidence supporting the close association of tumor microenvironment with tumorigenesis and the development of tumor. The tumor microenvironment is primarily composed of tumor cells, immune cells, and supporting cells (25). TAMs are immune cells that participate in the immune escape of tumors (7). M2-like TAMs, in particular, as anti-inflammatory cells, play a significant role in promoting various pro-tumorigenic effects in cancer, including regulation of angiogenesis and lymphangiogenesis, immune suppression, induction of hypoxia, promotion of tumor cell proliferation, and facilitation of metastasis (26,27). *RAC1* expression levels and prognosis have been found to be closely related in several types of cancer, but the effect of *RAC1* on immune cells is unknown. In our study, the association between *RAC1* and TAM infiltration was found in a number of

 

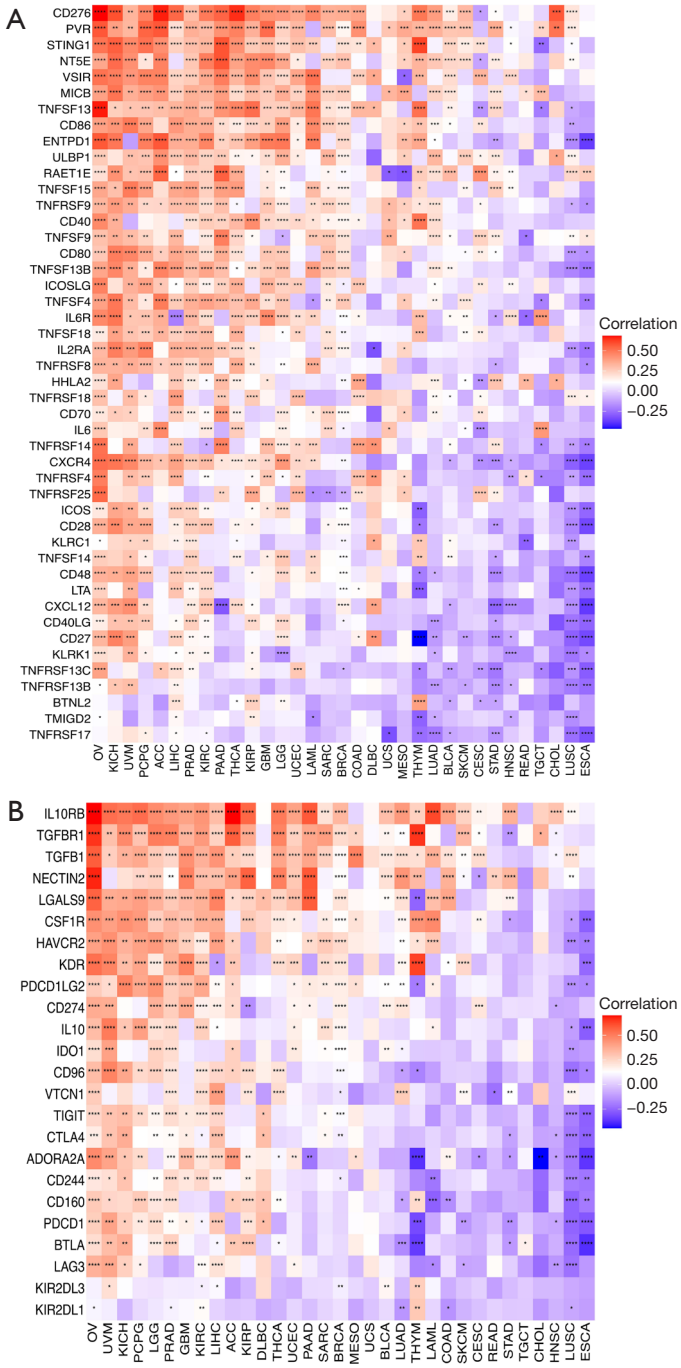


**Figure 10** Correlation analyses of *RAC1* and immune-related genes. (A) Immune stimulation-associated genes. (B) Immunosuppressive-associated genes. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. OV, ovarian serous cystadenocarcinoma; KICH, kidney chromophobe; UVM, uveal melanoma; PCPG, pheochromocytoma and paraganglioma; ACC, adrenocortical carcinoma; LIHC, liver hepatocellular carcinoma; PRAD, prostate adenocarcinoma; KIRC, kidney renal clear cell carcinoma; PAAD, pancreatic adenocarcinoma; THCA, thyroid carcinoma; KIRP, kidney renal papillary cell carcinoma; GBM, glioblastoma multiforme; LGG, lower grade glioma; UCEC, uterine corpus endometrial carcinoma; LAML, acute myeloid leukemia; SARC, sarcoma; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; UCS, uterine carcinosarcoma; MESO, mesothelioma; THYM, thymoma; LUAD, lung adenocarcinoma; BLCA, bladder urothelial carcinoma; SKCM, skin cutaneous melanoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; STAD, stomach adenocarcinoma; HNSC, head and neck squamous cell carcinoma; READ, rectum adenocarcinoma; TGCT, testicular germ cell tumors; CHOL, cholangiocarcinoma; LUSC, lung squamous cell carcinoma; ESCA, esophageal carcinoma; *RAC1*, Ras-related C3 botulinum toxin substrate 1.

 

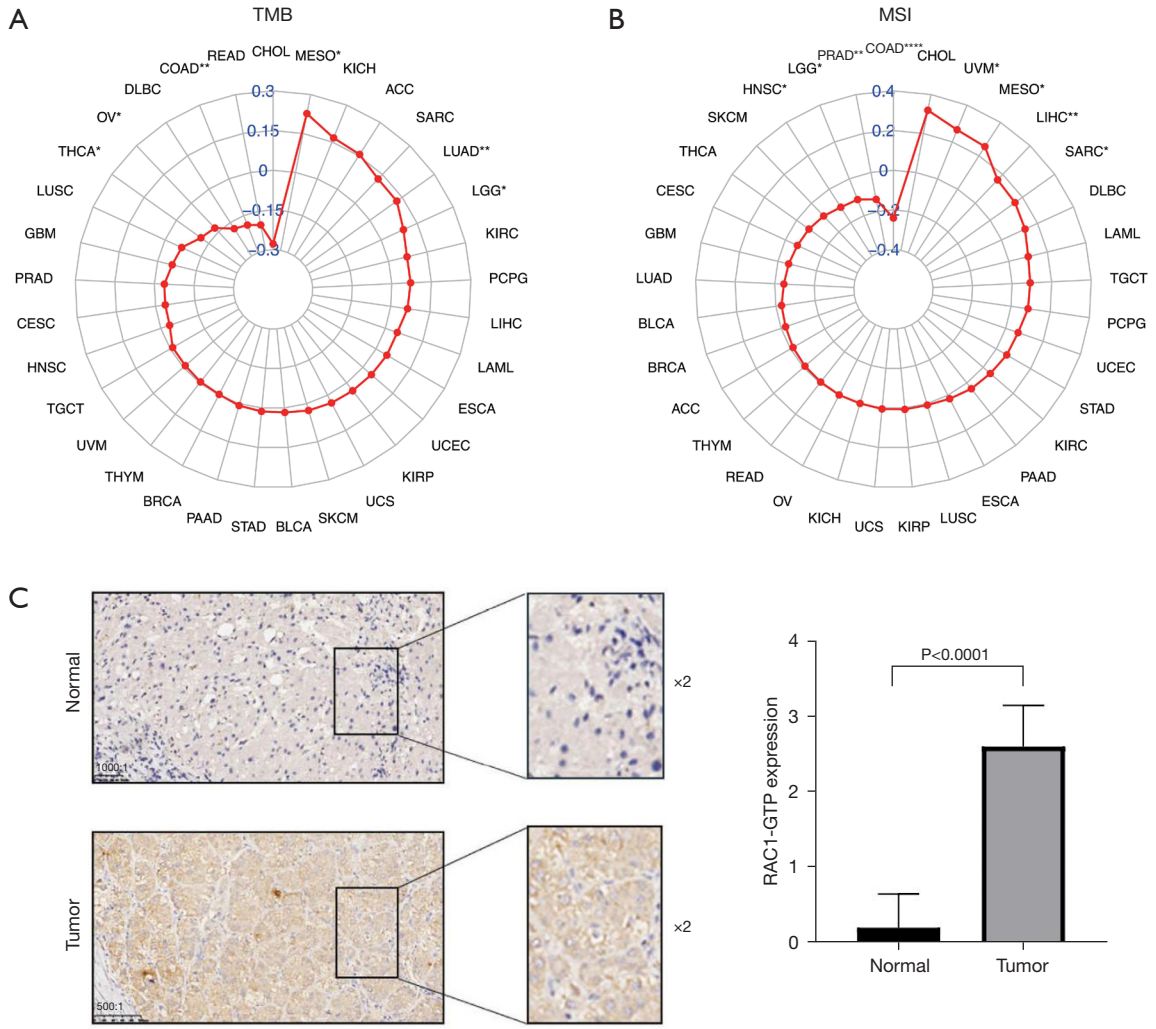


**Figure 11** The relationship between *RAC1* expression and tumor mutational burden and microsatellite instability, and the expression of RAC1-GTP in LIHC and adjacent normal tissues. Expression of *RAC1* correlated with tumor mutational burden (A) and microsatellite instability (B) in 33 cancers by Pearson's correlation analysis. (C) Immunohistochemical analysis of RAC1-GTP expression in patients with LIHC (scale bar, 1,000:1; 500:1). The red color refers to Pearson correlation coefficient "r". *, P<0.05; **, P<0.01; ****, P<0.0001. TMB, tumor mutational burden; MSI, microsatellite instability; CHOL, cholangiocarcinoma; MESO, mesothelioma; KICH, kidney chromophobe; ACC, adrenocortical carcinoma; SARC, sarcoma; LUAD, lung adenocarcinoma; LGG, lower grade glioma; KIRC, kidney renal clear cell carcinoma; PCPG, pheochromocytoma and paraganglioma; LIHC, liver hepatocellular carcinoma; LAML, acute myeloid leukemia; ESCA, esophageal carcinoma; UCEC, uterine corpus endometrial carcinoma; KIRP, kidney renal papillary cell carcinoma; UCS, uterine carcinosarcoma; SKCM, skin cutaneous melanoma; BLCA, bladder urothelial carcinoma; STAD, stomach adenocarcinoma; PAAD, pancreatic adenocarcinoma; BRCA, breast invasive carcinoma; THYM, thymoma; UVM, uveal melanoma; TGCT, testicular germ cell tumors; HNSC, head and neck squamous cell carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; PRAD, prostate adenocarcinoma; GBM, glioblastoma multiforme; LUSC, lung squamous cell carcinoma; THCA, thyroid carcinoma; OV, ovarian serous cystadenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; COAD, colon adenocarcinoma; READ, rectum adenocarcinoma; *RAC1*, Ras-related C3 botulinum toxin substrate 1; GTP, guanosine triphosphate; LIHC, liver hepatocellular carcinoma.

 

tumors. Additionally, the expression of *RAC1* was positively correlated with that of immunosuppressive genes including *TGFB1* and *IL-10*. *IL-10* promotes the conversion of TAM1 to TAM2, and TAM2 is able to produce *TGFB1*, which suppresses the antitumor T response (20). The existence of a link between *TGFB1*, *IL-10*, and TAM2 further supports the significance of *RAC1* in regulating tumor microenvironment. The results suggest a role of *RAC1* in the tumor microenvironment, which indicates that it may be an ideal tumor immunotherapy target.

Recently, a breakthrough has been made in immunotherapy in treating some malignant tumors (8-10). However, as immunotherapy has poor efficacy and low response rates for some tumors, only a small portion of patients benefit, limiting its widespread use. Hence, new biomarkers are needed to identify sensitive patients and predict treatment response. Currently, a study has demonstrated that tumor mutational burden and microsatellite instability are useful biomarkers that are helpful in predicting the effectiveness of immunotherapy in cancers (21). High tumor mutational burden and microsatellite instability in patients may indicate a better therapeutic response to ICIs (22,28). In this study, by exploring the relationship between *RAC1* expression, tumor mutational burden, and microsatellite instability, we found that *RAC1* expression in MESO, LUAD, and LGG was positively correlated with tumor mutational burden. Moreover, an association was observed between microsatellite instability and *RAC1* expression in UVM, MESO, LIHC, and SARC. These findings thus point to *RAC1* as a potential candidate for predicting the efficacy of immunotherapy for tumors.

Our findings indicate that patients with LIHC who exhibit abnormal expression of *RAC1* are at risk of a poor prognosis. To determine the prognostic value of *RAC1* among patients with LIHC, a prognostic nomogram was constructed to predict survival. This model had good predictive ability, and will assist clinicians in predicting the prognosis of LIHC so that suitable treatment measures can be administered. The high expression of a protein does not always indicate high activity, as activity level is also related to modification states such as phosphorylation, acetylation, and methylation, as well as the binding of the protein to GTP or GDP. Therefore, studying the activity of a protein by simply detecting the expression level of the total protein provides a limited assessment. At present, no studies exist on RAC1-GTP in LIHC tissues. As shown by IHC, LIHC tissues expressed more RAC1-GTP than did the adjacent normal tissues. The possibility to a relationship between *RAC1* expression and immunotherapy sensitivity pave the way to prospective clinical trials in order to find a new predictive factor to atezolizumab activity in this hard-to-treat cancer. But there are also some flaws of this study: (I) in order to understand the true prognostic role of *RAC1* we should have had data from an untreated population versus a sample of patients treated with uniform first-line therapy. (II) The final results don't permit to conclude that *RAC1* is an agnostic cancer biomarker due to the controversial results regarding the good or worse prognosis on different tumors. (III) The analysis conducted in this study are derived from raw data and some confounding variables may lead to different biases which may reduce the value of the prognostic and predictive aspect of *RAC1*. (IV) For these reasons our findings must be confirmed in prospective clinical trials and final conclusion could be considered as a proof of concept for future research.

## Conclusions

Our pan-cancer analysis provides insights into the similarities and differences of cancers in relation to *RAC1*. The findings indicate that *RAC1* is a promising biomarker in cancer diagnosis and prognosis and may prove valuable in personalized immunotherapy. However, we used public databases and bioinformatics data, which have limitations. It is necessary to further explore the specific mechanism of *RAC1*. Future *in vivo* and *in vitro* studies of *RAC1* may enhance our understanding of how *RAC1* can be targeted therapeutically, thereby providing a superior immune-based anticancer strategy.

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## Footnote

*Reporting Checklist:* The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2016/rc

*Data Sharing Statement:* Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2016/dss

 

*Peer Review File:* Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2016/prf

*Conflicts of Interest*: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2016/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethics Committee of The Affiliated Lihuili Hospital, Ningbo University (No. KY2022SL322-01). Individual consent for this retrospective analysis was waived.



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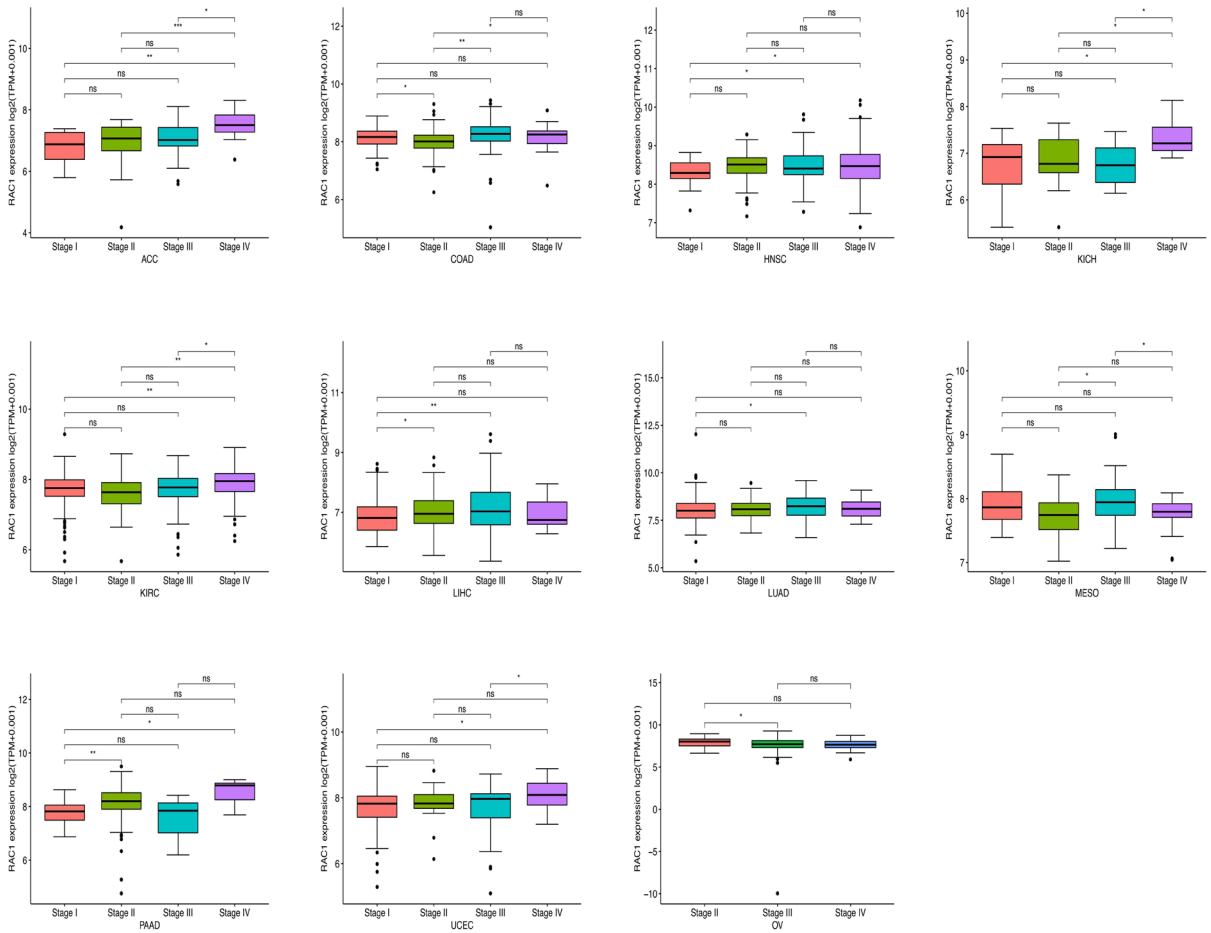


**Figure S1** Expression levels of *RAC1* in different pathological stages for pan-cancer tumors. *, P<0.05; **, P<0.01. *RAC1*, Ras-related C3 botulinum toxin substrate 1; TPM, transcripts per million; ns, no significance; ACC, adrenocortical carcinoma; COAD, colon adenocarcinoma; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; MESO, mesothelioma; PAAD, pancreatic adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; OV, ovarian serous cystadenocarcinoma.


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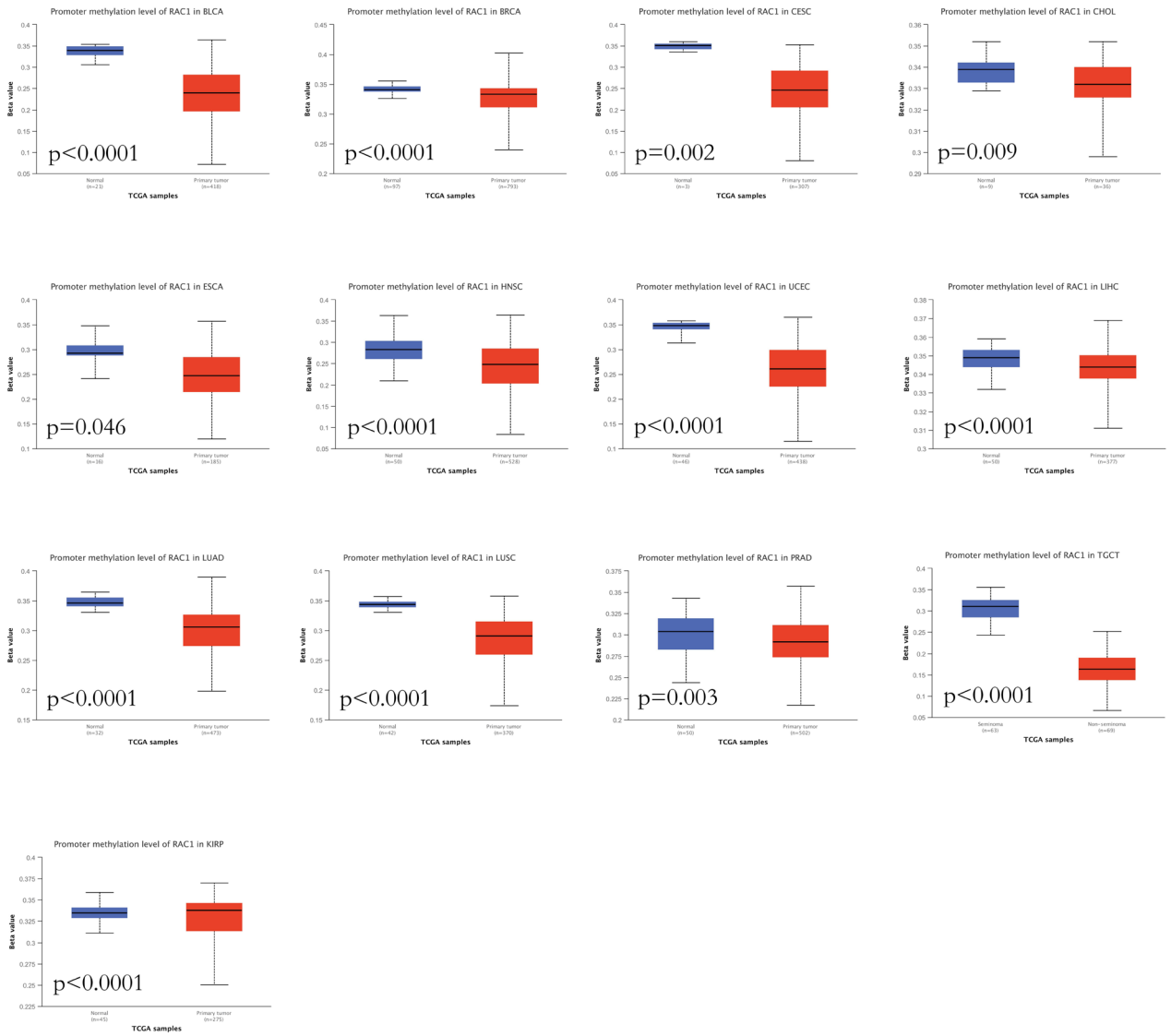


**Figure S2** Promoter methylation of *RAC1* in tumor and normal tissues. *RAC1*, Ras-related C3 botulinum toxin substrate 1; TCGA, The Cancer Genome Atlas; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; UCEC, uterine corpus endometrial carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PRAD, prostate adenocarcinoma; TGCT, testicular germ cell tumors; KIRP, kidney renal papillary cell carcinoma.


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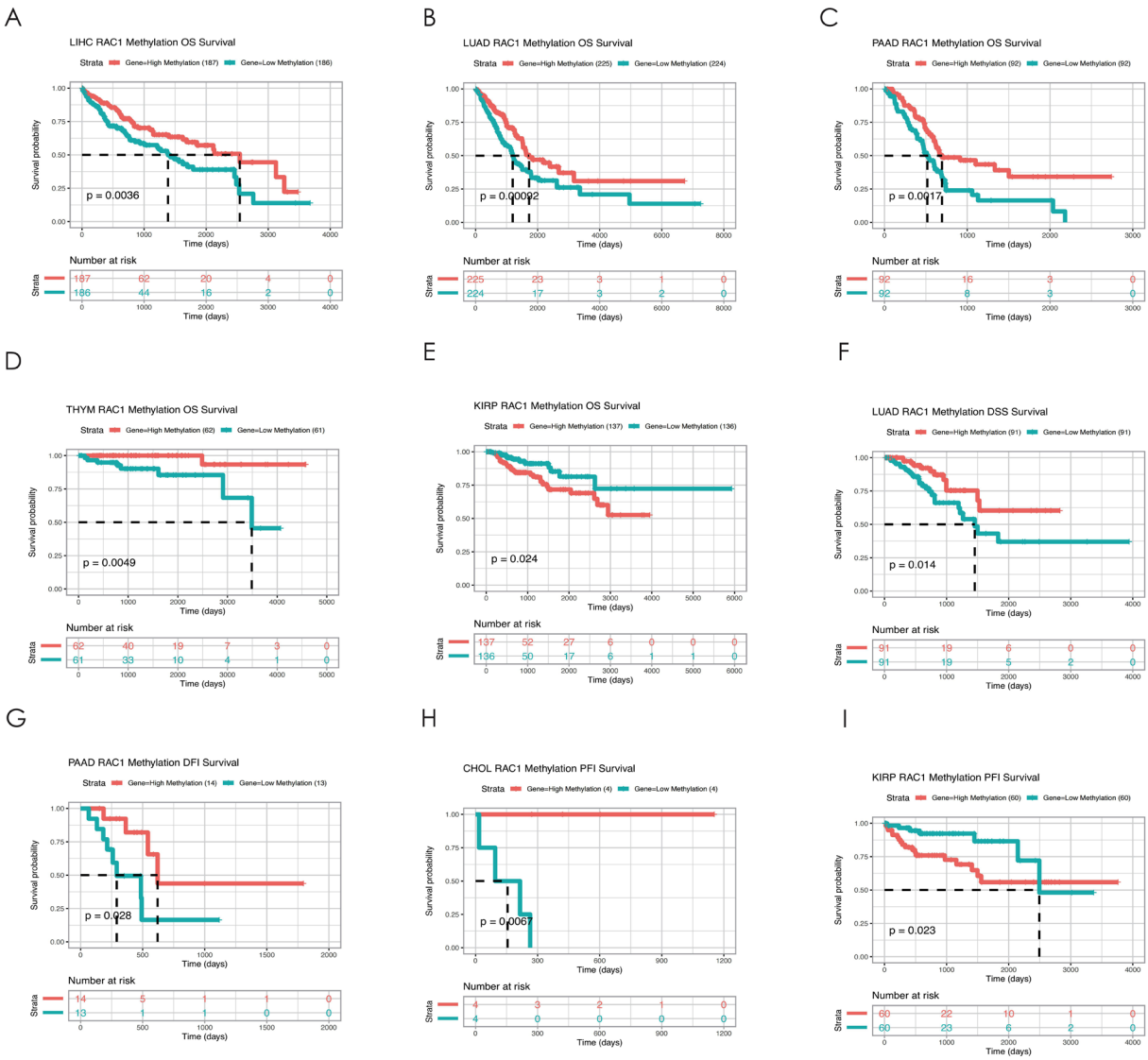


**Figure S3** Correlation between *RAC1* promoter methylation and the survival of patients with tumor. Correlation between RAC1 promoter methylation and OS (A-E), DSS (F), DFI (G), and PFI (H,I). LIHC, liver hepatocellular carcinoma; *RAC1*, Ras-related C3 botulinum toxin substrate 1; OS, overall survival; LUAD, lung adenocarcinoma; PAAD, pancreatic adenocarcinoma; THYM, thymoma; KIRP, kidney renal papillary cell carcinoma; DSS, disease-specific survival; DFI, disease-free interval; CHOL, cholangiocarcinoma; PFI, progression-free interval.


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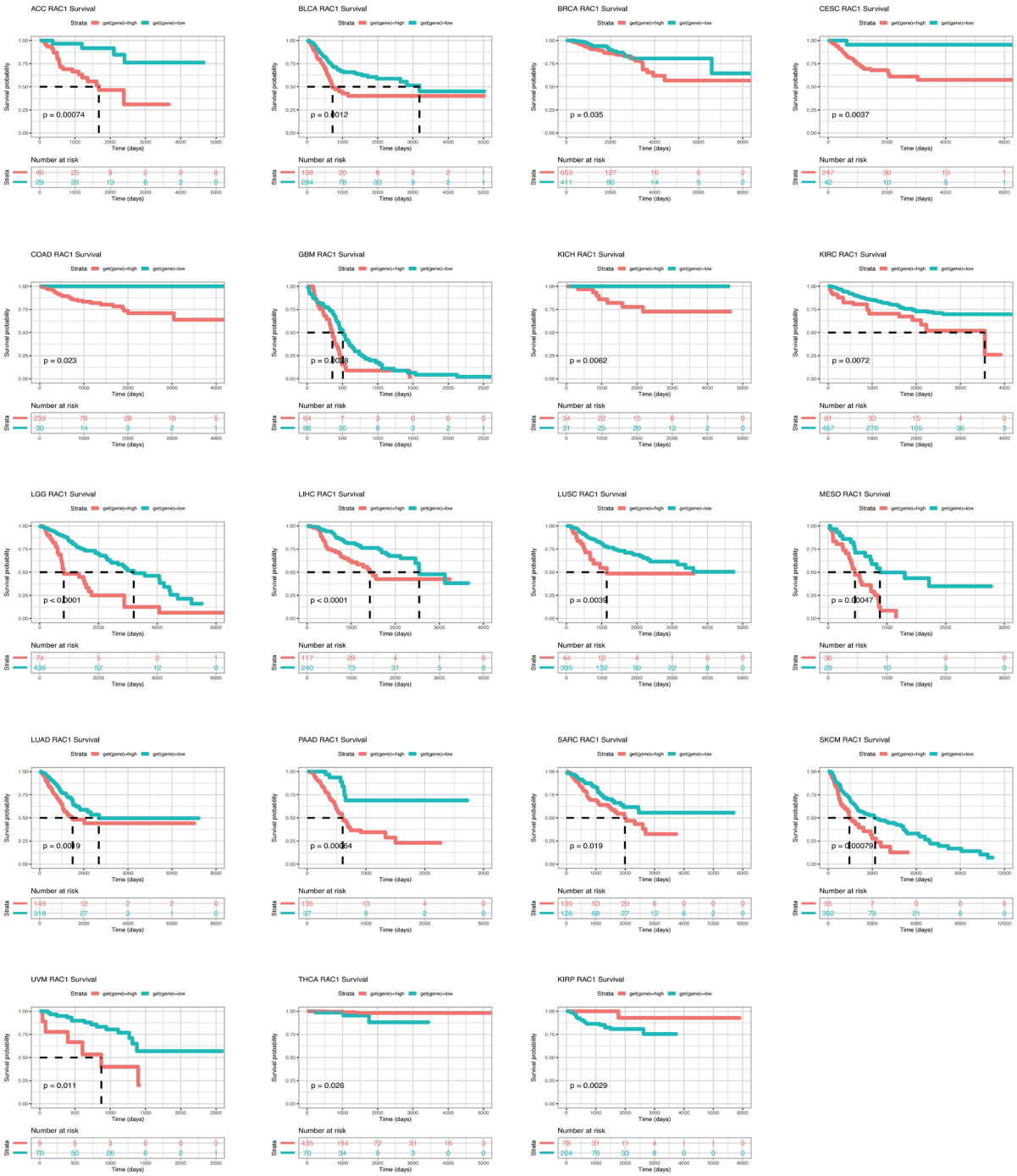


**Figure S4** Correlation between the *RAC1* expression and DSS. *RAC1*, Ras-related C3 botulinum toxin substrate 1; DSS, disease-specific survival; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; COAD, colon adenocarcinoma; GBM, glioblastoma multiforme; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; LUAD, lung adenocarcinoma; PAAD, pancreatic adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; UVM, uveal melanoma; THCA, thyroid carcinoma; KIRP, kidney renal papillary cell carcinoma.


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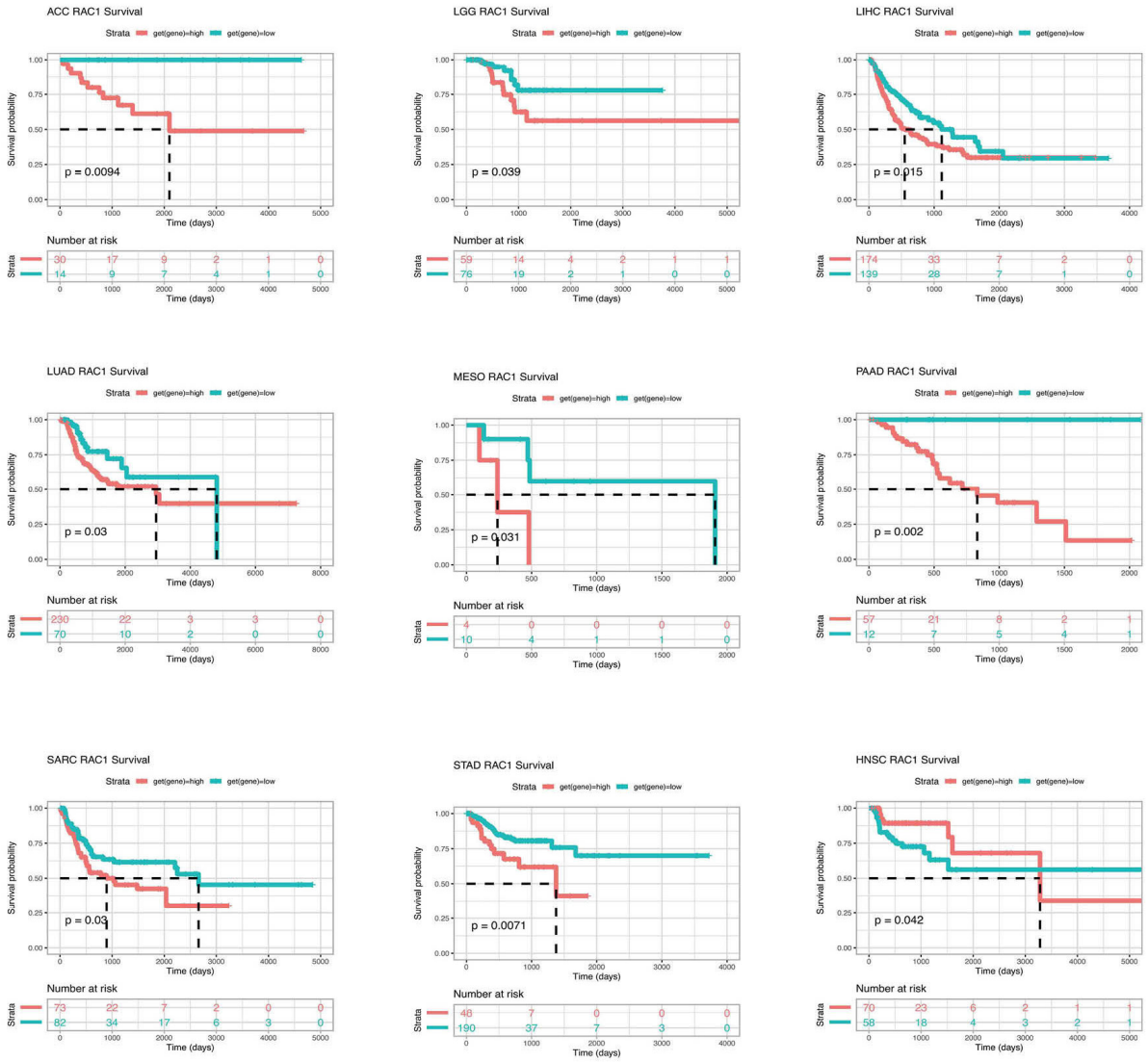


**Figure S5** Correlation between *RAC1* expression and DFI. ACC, adrenocortical carcinoma; *RAC1*, Ras-related C3 botulinum toxin substrate 1; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; MESO, mesothelioma; PAAD, pancreatic adenocarcinoma; SARC, sarcoma; STAD, stomach adenocarcinoma; HNSC, head and neck squamous cell carcinoma; DFI, disease-free interval.


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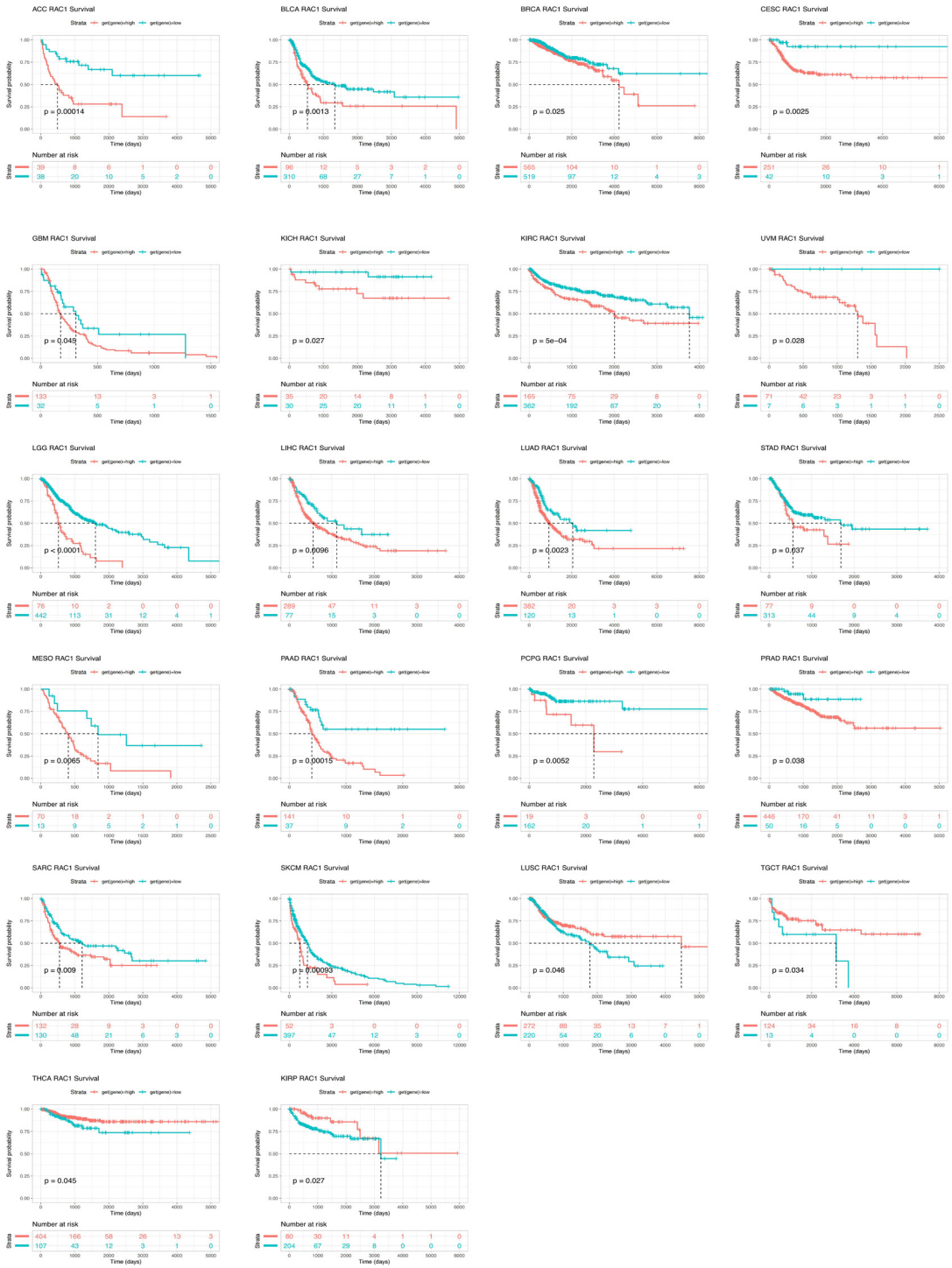


**Figure S6** Correlation between *RAC1* expression and PFI. *RAC1*, Ras-related C3 botulinum toxin substrate 1; PFI, progression-free interval; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; GBM, glioblastoma multiforme; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; UVM, uveal melanoma; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; STAD, stomach adenocarcinoma; MESO, mesothelioma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; LUSC, lung squamous cell carcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; KIRP, kidney renal papillary cell carcinoma.


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