Original Article

Upregulation of RACGAP1 is correlated with poor prognosis and immune infiltration in hepatocellular carcinoma

Yangyang Zhou[1], Shimeng Zheng[2], Qihang Guo[3], Ning Wei[1], Zhuanglong Xiao[1], Yuhu Song[1]

[1]Department of Gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [2]Immunology Research Center for Oral and Systemic Health, Beijing Friendship Hospital, Capital Medical University, Beijing, China; [3]Department of Gastroenterology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Contributions: (I) Conception and design: Y Zhou, Y Song; (II) Administrative support: S Zheng, Q Guo; (III) Provision of study materials or patients: N Wei, Z Xiao; (IV) Collection and assembly of data: Y Zhou, Y Song; (V) Data analysis and interpretation: Y Zhou, Y Song; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Yuhu Song, MD, PhD. Department of Gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277 Jiefang Avenue, Jianghan District, Wuhan 430022, China. Email: yuhusong@163.com.

Background: Hepatocellular carcinoma (HCC) is a primary liver cancer with high mortality worldwide. Even though the patients with HCC received standard therapies, patients with HCC continue to experience unsatisfactory therapy outcomes. Presently, immune therapy acts as a novel therapeutic management for HCC. The aim of this study was to determine overexpressed genes in HCC and evaluate the association between overexpressed genes with the prognosis and immune infiltration.

Methods: Gene expression profiles of HCC were analyzed using multiple online databases, and then confirmed by qualitative real time-polymerase chain reaction (RT-qPCR) and immunohistochemical analysis. The correlations of gene overexpression with the prognosis and immune infiltration were determined.

Results: Top 11 common differentially expressed genes were identified in HCC, and RACGAP1 was selected for further analysis. RACGAP1 expression was significantly higher in HCC tissues than that in normal tissues. Upregulation of RACGAP1 was correlated with clinical stage and poor prognosis. Additionally, RACGAP1 overexpression was positively related to the infiltration of suppressive immune cells. Moreover, we speculate RACGAP1 may promote tumorigenesis of HCC through immunosuppression mediated by YAP activation.

Conclusions: Overexpression of RACGAP1 was associated with unfavorable prognosis and immune infiltration in HCC, which indicated that RACGAP1 could be a molecular target for HCC.

Keywords: RACGAP1; hepatocellular carcinoma (HCC); prognosis; immune infiltration; YAP

Submitted Aug 15, 2023. Accepted for publication Dec 13, 2023. Published online Feb 27, 2024.
doi: 10.21037/tcr-23-1474
View this article at: https://dx.doi.org/10.21037/tcr-23-1474

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and a leading cause of death around the world (1). Even though the patients with HCC received standard therapies including open/laparoscopic resection, transcatheter arterial chemoembolization (TACE), and radiofrequency ablation (RFA), patients with HCC continue to experience unsatisfactory therapy outcomes (2). Presently, immune therapy acts as a novel therapeutic management for HCC.

RACGAP1, a component of the centralspindlin complex, regulates rho-mediated signal pathways. RACGAP1 plays essential roles in cytokinesis, cell growth and differentiation. Previous studies have reported that RACGAP1 plays a critical role in the pathogenesis of cancers and is identified as a prognosis biomarker (3-7). A study performed by Pliarchopoulou *et al.* showed that RACGAP1 overexpression was associated with poor overall

survival (OS) and recurrence-free survival (RFS) in breast cancer (8). Saigusa *et al.* reported that the overexpression of RACGAP1 was related to lymph node metastasis and unfavorable outcomes in gastric cancer (4). Moreover, some recent findings revealed that knockdown of RACGAP1 in HCC cells induced cytokinesis failure and cell apoptosis (9). All of these findings indicate the involvement of RACGAP1 in HCC. Thus, our study sought to examine the specific role of RACGAP1 in the pathogenesis of HCC. Firstly, differential expression gene (DEG) analysis was performed based on Gene Expression Omnibus (GEO) database, and RACGAP1 was chosen as the candidate gene for further research. Then, the expression of RACGAP1 was assessed, and the relationship between RACGAP and the outcomes of HCC was analyzed. Furthermore, the correlation between immune infiltration and RACGAP1 expression was evaluated using Tumor Immune Estimation Resource (TIMER) or Gene Expression Profiling Interactive Analysis (GEPIA) database. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1474/rc).

Methods

GEO datasets

The GEO database is a public database containing a large number of high-throughput gene expression data. GSE112791, GSE102079 and GSE62232 gene expression profile datasets were screened (10-12). GEO2R was applied for the DEG analysis.

Highlight box

Key findings

- This study found that RACGAP1 has predictive value for outcomes of hepatocellular carcinoma (HCC) patients.
- Interestingly, RACGAP1 may promote tumorigenesis of HCC through immunosuppression mediated by YAP activation.

What is known and what is new?

- HCC is a primary liver cancer with high mortality worldwide. Even though the patients with HCC received standard therapies, patients with HCC continue to experience unsatisfactory therapy outcomes. Presently, immune therapy acts as a novel therapeutic management for HCC.
- We found overexpression of RACGAP1 was correlated with clinical stage and poor prognosis.

What is the implication, and what should change now?

- The RACGAP1 is a potential prognostic signature or therapeutic strategy and is significantly associated with the malignant biology of HCC.

Enrichment analysis

The database for annotation, visualization and integrated discovery (DAVID) was used for analyzing the selected genes (13).

Construction of protein-protein interaction (PPI) network and identification of hub gene

A PPI network with complex regulatory relationships was constructed using the STRING database (14). Cytoscape software (version 3.9.1) was employed to visualize the PPI network (15).

TIMER database

TIMER or TIMER2 is a comprehensive resource for analyzing immune infiltration in different tumor types (16,17).

GEPIA database

GEPIA is a user-friendly online tool for analyzing gene expression based on the data of The Cancer Genome Atlas (TCGA) and genotype-tissue expression (GTEx) (18). Cell type-level proportion analysis was analyzed using GEPIA2021 (19).

The University of Alabama at Birmingham Cancer (UALCAN) database

UALCAN is a web for in-depth analysis of TCGA and clinical proteomic tumor analysis consortium (CPTAC) data (20).

Tumor, normal and metastatic (TNM) plot database

TNM plot provides gene expressions in different tissues (21).

The Human Protein Atlas (HPA) database

HPA is a dataset which provides gene expression profiles at the protein level in different tissues (22).

Kaplan-Meier (KM) survival analysis

The KM plotter contains clinical profiles of patients, and was used to assess the relationship between the survival and genes in diverse cancers (23).

GeneMANIA database

GeneMANIA was applied to predict the function of genes or gene sets (24).

Tumor-Immune System Interaction Database (TISIDB)

TISIDB was used for analyzing the interaction between tumor and immune system (25).

Sangerbox database

The Sangerbox database is a comprehensive Chinese bioinformatics analysis platform which provides various visualization plots (26).

Rat HCC model

The male rats (6–8 weeks, Beijing Vital River Laboratory, Beijing, China) weighing 200 g were injected intraperitoneally with N-nitrosodiethylamine (DEN) (N0756; Sigma-Aldrich, St. Louis, MO, USA) or 0.9% NaCl (control) at a dose of 50 mg/kg once a week for 16 weeks (27).

Immunohistochemistry (IHC)

The IHC was applied to detect the protein expression of RACGAP1 in human and rat liver tissue. IHC was performed using a 2-step method as previously reported (27-29). RACGAP1 was used as the primary antibody for incubation and then appropriate secondary antibody was selected. The other steps followed that marked in the manual. The IHC scores were referred as the value of the staining intensity [0–3] and the portion of stained cells [1–4].

Statistical analysis

The different expression of RACGAP1 between tumors and normal tissues was analyzed using the Wilcoxon rank sum test or *t*-test, respectively. KM curve was plotted to assess the survival differences between the high expression and low expression groups. $P<0.05$ was considered as statistical significance.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval was not required because GEO and TCGA are publicly available sources of data. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Results

Screen of hub genes

Three microarray datasets (GSE112791, GSE102079 and GSE62232) were screened for DEGs. In GSE112791, GSE102079 and GSE62232, 163 DEGs (60 upregulated, 103 downregulated), 93 DEGs (23 upregulated, 70 downregulated) and 130 DEGs (39 upregulated, 91 downregulated) were found, respectively (*Figure 1A*). Then, 65 common DEGs were identified through the intersection of the Venn diagram (*Figure 1B*). Next, the 65 overlapped genes were analyzed to establish PPI network (*Figure 1C*). The combined scores larger than 0.4 in PPIs were imported to Cytoscape for subsequent analysis. Using Cytohubba plug-in, 11 hub genes were identified (*Figure 1D*,*1E*). Some of the overlapped genes, such as *ANLN*, *CDK1*, and *CCNB1* have been reported in several studies (30-32). However, the role of RACGAP1 in the pathogenesis of HCC has been rarely reported. Thus, RACGAP1 was selected as a candidate target gene for further analyses. As shown in *Figure 1F*, increased RACGAP1 expression in RNA level was observed in HCC tissues compared with non-cancer tissues, which was also confirmed in the three microarray datasets.

RACGAP1 message RNA (mRNA) and protein expression increased in HCC patients

To further examine RACGAP1 mRNA expression in HCC, the expression of RACGAP1 in the GEPIA, UALCAN and TNM plot was displayed. And we found increased expression of RACGAP1 mRNA in HCC tissues (*Figure 2A*, Figure S1A,S1B).

To detect the RACGAP1 protein expression, the CPTAC was applied through UALCAN website. The results revealed that the expression of RACGAP1 protein was

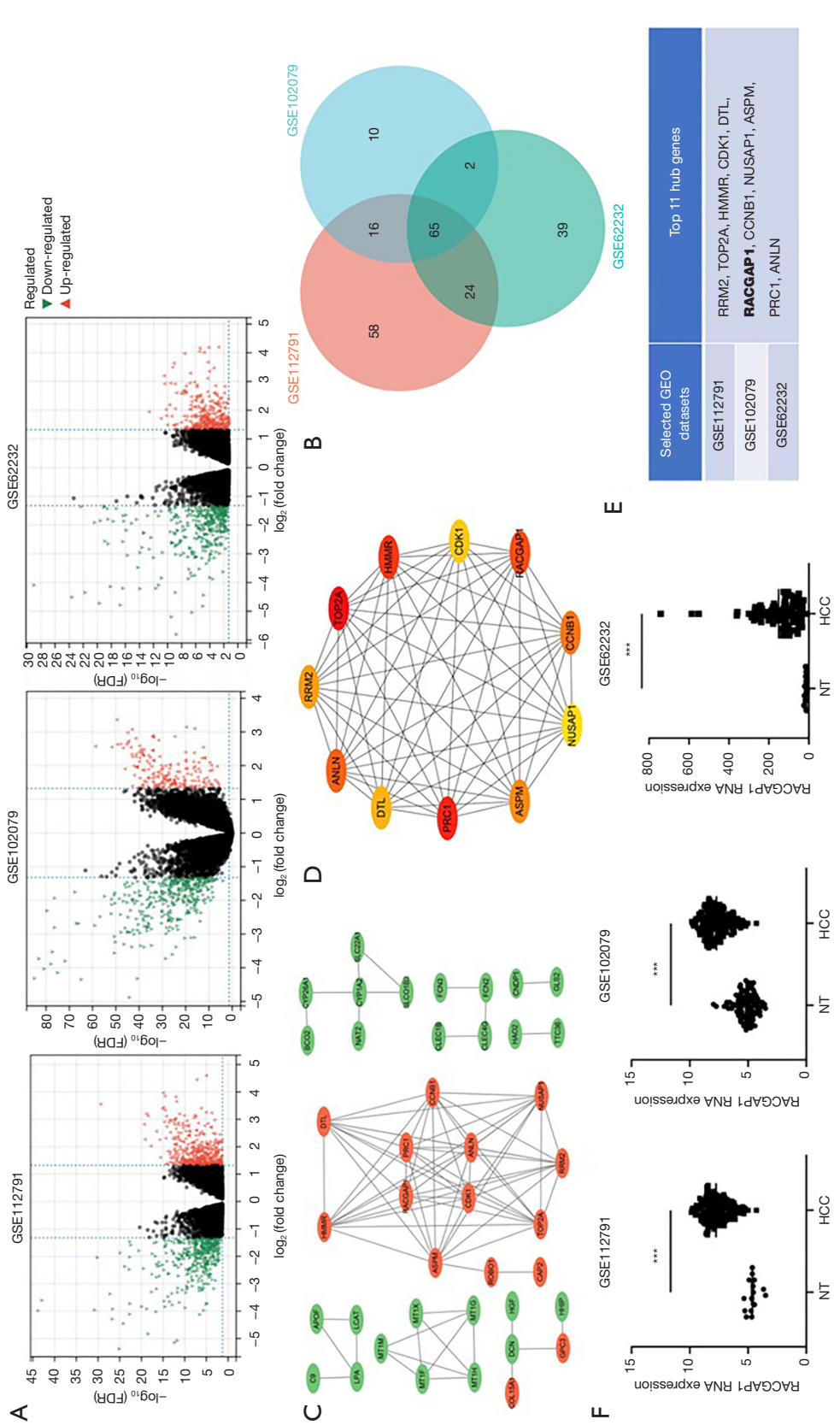

Figure 1 Identification of hub genes. (A) The volcano map of GSE112791, GSE102079 and GSE62232. (B) The Venn diagram showing overlapping differential expression genes. (C) Protein-protein interaction network diagram. (D) The significant genes clustering modules. (E) Top 11 hub genes of the overlapping genes. (F) RACGAP1 RNA expression in HCC and normal tissues. ***, P<0.001. FDR, false discovery rate; GEO, gene expression omnibus; NT, normal tissue; HCC, hepatocellular carcinoma.

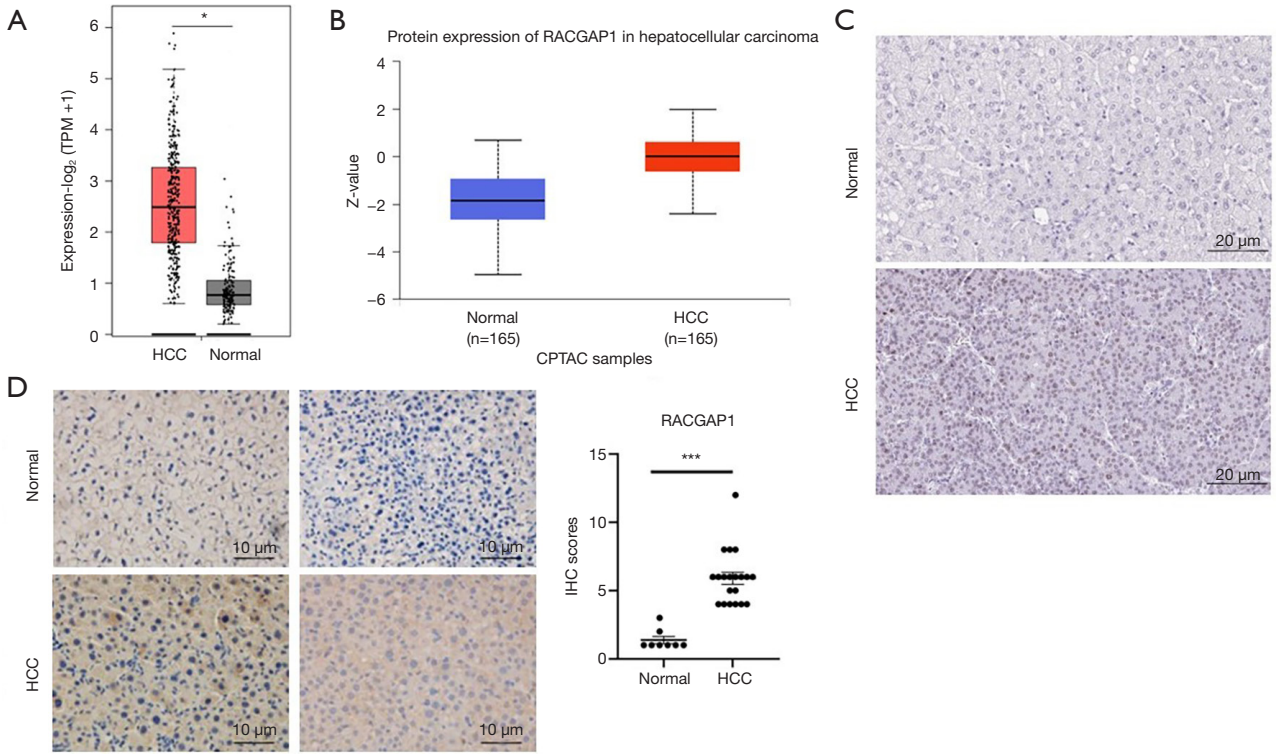

Figure 2 Expression of RACGAP1 in HCC. (A) RACGAP1 mRNA expression in HCC was examined by using the GEPIA. (B) RACGAP1 protein expression in HCC and normal tissues. (C) RACGAP1 protein expression in the HPA database by IHC with DAB staining. (D) RACGAP1 protein expression in human HCC tissues by IHC with DAB staining method. *, P<0.05; ***, P<0.001. HCC, hepatocellular carcinoma; mRNA, message RNA; GEPIA, Gene Expression Profiling Interactive Analysis; TPM, transcripts per million; CPTAC, clinical proteomic tumor analysis consortium; HPA, human protein atlas; IHC, immunohistochemistry; DAB, 3,3'-diaminobenzidine.

significantly higher in HCC tissues compared to that of the normal tissues (*Figure 2B*). IHC results from HPA database showed RACGAP1 protein expression was overexpressed in HCC tissues (*Figure 2C*). To confirm the result, the RACGAP1 expression in protein level was also determined. Our results revealed an up-regulation of RACGAP1 expression at the protein level in HCC tissues (*Figure 2D*).

Increased RACGAP1 expression was correlated with unfavorable prognosis in HCC patients

To investigate the relationship between RACGAP1 and clinicopathological characteristics in HCC patients, the analysis was performed using the online tool UALCAN. No difference was found in the RACGAP1 expression in different genders (*Figure 3A*). Then, the expression of RACGAP1 was determined in different stages of HCC. RACGAP1 expression increased gradually in cancerous tissues, which was accompanied by the development of HCC. However, lower RACGAP1 expression was observed in stage 4 compared with stage 3, which might be attributed to small size of enrolled patients in stage 4 (*Figure 3B*). Similar pattern was observed in the correlation between tumor grade and RACGAP1 expression (*Figure 3C*). Increased RACGAP1 expression was accompanied by lymph nodes metastasis (*Figure 3D*).

KM plotter survival analysis was performed to assess the prognosis (using "auto select best cutoff" as group cutoff). Firstly, the survival was analyzed using the KM plotter online tool. And KM analysis revealed HCC patients with increased RACGAP1 expression had poor OS, progression-free survival (PFS), RFS and disease-specific survival (DSS) compared with patients with lower level of RACGAP1 expression (*Figure 4A*). The data of the GEPIA database showed similar pattern in HCC patients with different levels of RACGAP1 expression (*Figure 4B*).

In order to further investigate the role of RACGAP1 expression in HCC patient's prognosis, the

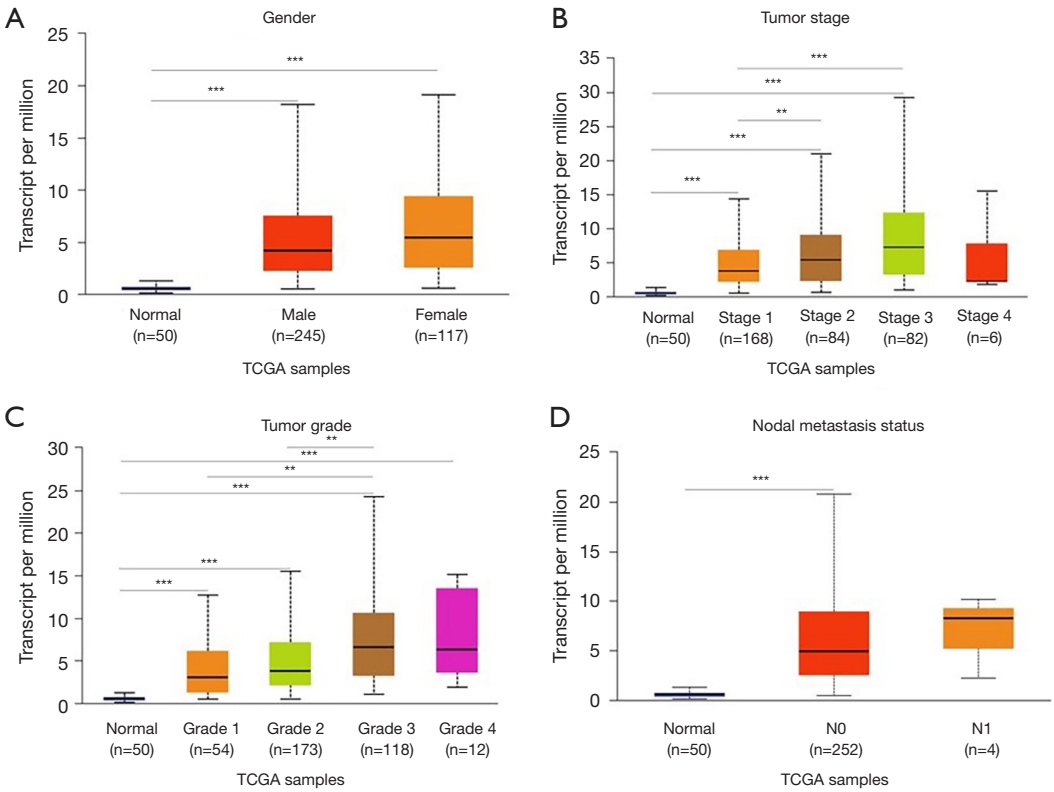

Figure 3 RACGAP1 is associated with clinicopathological data in hepatocellular carcinoma patients. (A) Gender. (B) Tumor stage. (C) Tumor grade. (D) Lymph node metastasis. **, P<0.01; ***, P<0.001. TCGA, the cancer genome atlas.

correlation between RACGAP1 mRNA expression and clinicopathological features was determined (*Table 1*). The overexpression of RACGAP1 was found to be significantly associated with worse OS and PFS. For HCC patients in Stages 1 to 3, high expression of RACGAP1 was significantly related to unfavorable OS and PFS. Additionally, increased expression of RACGAP1 was related to unfavorable OS and PFS in tumor grade and American Joint Committee on Cancer (AJCC) Tumor (T) stage but was not related to OS of grade 2 [hazard ratio (HR): 1.57, 95% CI: 0.93–2.62, P=0.086] and PFS of AJCC-T3 [HR: 1.94, 95% CI: 0.96–3.90, P=0.059]. These results revealed the expression of RACGAP1 was an important factor in the HCC patient's prognosis.

Identification and functional analysis of RACGAP1

Firstly, the gene interaction network for RACGAP1 was generated through the GeneMania database. The results revealed that the top 20 genes were strongly related to RACGAP1, including the *KIF23*, *ECT2*, *PRC1* and *AURKB* (*Figure 5A*). Then, the STRING tool was used to generate PPI network of RACGAP1. The network had 51 edges and 11 nodes, including *KIF23*, *ECT2*, *ANLN* and *CDK1* (*Figure 5B*).

To further uncover the function of RACGAP1, we searched the related genes of RACGAP1 using UALCAN website. The top 300 genes related to RACGAP1 were identified. And the results showed that the top 300 genes were positively associated with RACGAP1. Then, the top 50 related genes were selected through heat map analysis (*Figure 5C*). Subsequently, the top 300 genes received enrichment analysis to assess the potential signal pathways and function. The representative Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways are shown in Figure S2A-S2D. These genes were involved in cell division/cycle and DNA repair.

Expression of RACGAP1 was positively associated with immune infiltration

The tumor microenvironment (TME) plays a crucial role in tumor progression and recurrence (33-35). TME is

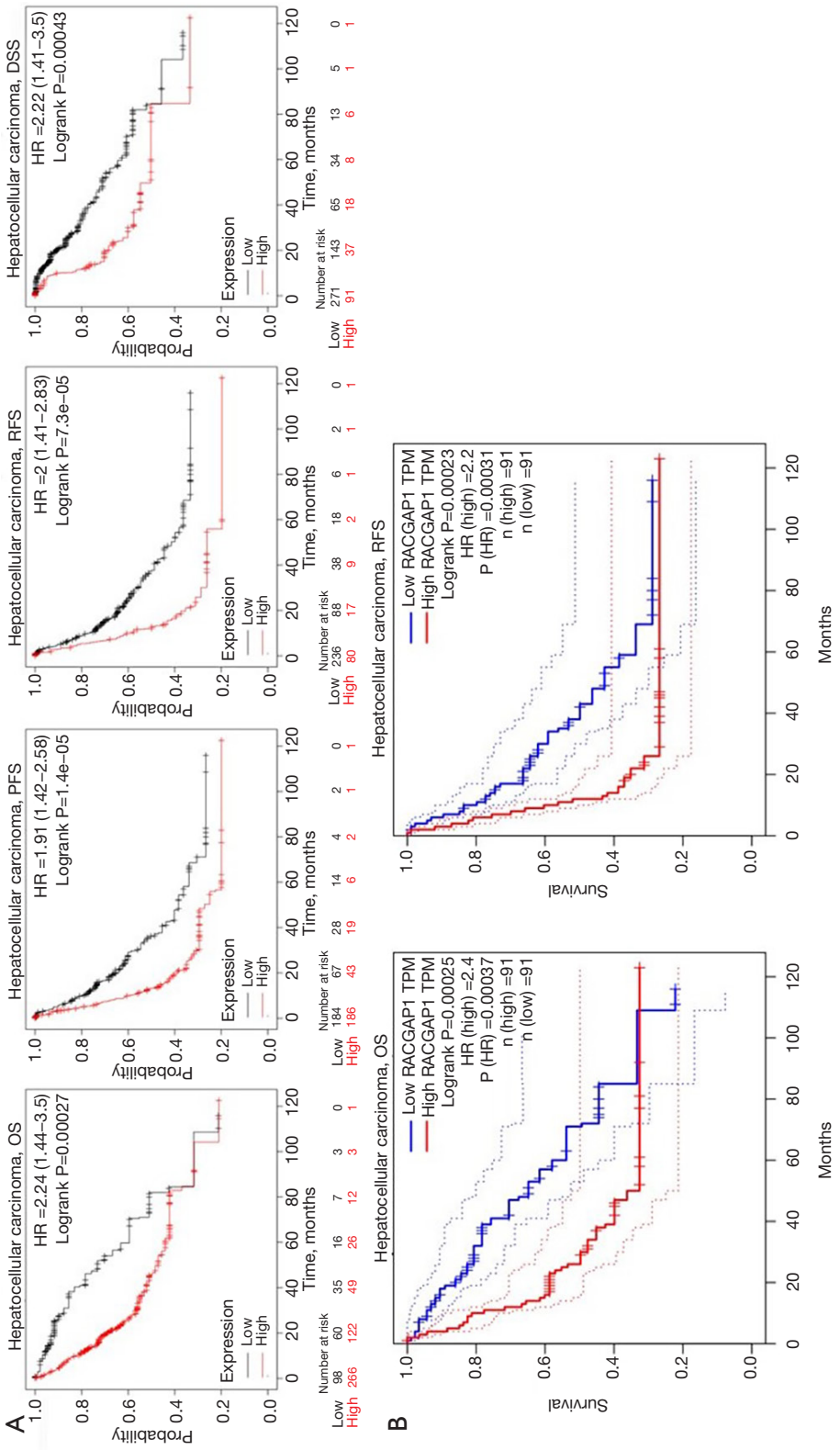

Figure 4 RACGAP1 is correlated with the prognosis of HCC. (A) Kaplan-Meier curves revealed HCC patients with high RACGAP1 mRNA expression had a shorter OS, PFS, RFS, and DSS. (B) GEPIA survival analysis of OS and RFS for HCC patients. HCC, hepatocellular carcinoma; mRNA, message RNA; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; DSS, disease-specific survival; GEPIA, Gene Expression Profiling Interactive Analysis; HR, hazard ratio; TPM, transcripts per million.

Table 1 Correlation of RACGAP1 mRNA expression and clinical prognosis in HCC with different clinicopathological factors by Kaplan-Meier plotter

Factors	Overall survival (n=364)			Progress-free survival (n=366)		
	N	HR (95% CI)	P	N	HR (95% CI)	P
Gender		1.81 (1.31–2.81)	0.00068*		1.81 (1.31–2.81)	0.00068*
Male	246	4.04 (1.94–8.40)	5.3e–05*	246	1.92 (1.33–2.76)	0.00038*
Female	118	1.98 (1.08–3.62)	0.024*	120	2.63 (1.49–4.66)	0.00055*
Alcohol						
Yes	115	3.26 (1.42–7.47)	0.0032*	115	2.90 (1.62–5.17)	0.00017*
None	202	2.05 (1.25–3.35)	0.0034*	204	2.06 (1.36–3.13)	0.00051*
Hepatitis virus						
Yes	150	2.70 (1.18–6.15)	0.014*	152	1.92 (1.14–3.25)	0.013*
None	167	2.19 (1.37–3.52)	0.00087*	167	3.07 (1.91–4.91)	1e–06*
Stage						
1	170	2.44 (1.23–4.88)	0.0088*	170	1.78 (1.06–3.00)	0.027*
2	83	2.75 (1.09–6.94)	0.025*	84	1.95 (1.00–3.80)	0.045*
3	83	2.60 (1.31–5.15)	0.0046*	83	2.27 (1.16–4.43)	0.014*
4	4	–	–	5	–	–
Grade						
1	55	3.9 (1.33–11.48)	0.0088*	55	2.69 (1.02–7.11)	0.039*
2	174	1.57 (0.93–2.62)	0.086	175	2.37 (1.53–3.68)	6.8e–05*
3	118	3.14 (1.71–5.76)	0.00011*	119	3.28 (1.93–5.57)	3.6e–06*
4	12	–	–	12	–	–
AJCC tumor						
1	180	2.31 (1.21–4.41)	0.0088*	180	1.80 (1.11–2.91)	0.015*
2	90	2.64 (1.12–6.21)	0.021*	92	1.98 (1.05–3.74)	0.031*
3	78	2.31 (1.22–4.4)	0.0085*	78	1.94 (0.96–3.90)	0.059
4	13	–	–	13	–	–
Vascular invasion						
None	203	1.99 (1.07–3.70)	0.027*	204	1.98 (1.25–3.15)	0.0033*
Micro	90	2.30 (0.93–5.73)	0.065	91	1.95 (1.08–3.54)	0.024*
Macro	16	–	–	16	–	–

"–" means sample number too low for meaningful analysis. *, P<0.05. Only patients for whom explicit information is available are listed in this table. mRNA, message RNA; HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; AJCC, American Joint Committee on Cancer.

consisted of immune cells, abundant immune molecules and other regulating cytokines, which is related to the occurrence, development and metastasis of tumor. Previous studies reported immune infiltration was closely associated with the prognosis of cancers (36,37). The TIMER database was used to explore the relationship between the expression of RACGAP1 and immune infiltration. RACGAP1 expression was closely related to B cell, $CD8^+$

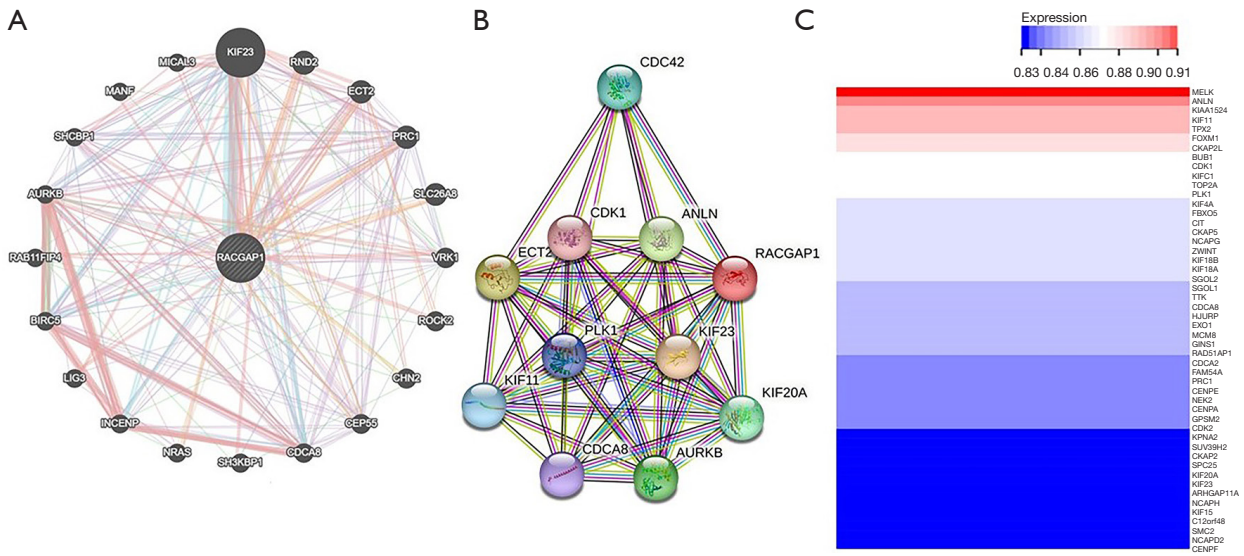

Figure 5 Identification of RACGAP1 interacted genes and proteins. (A) The gene-gene interaction network. (B) The protein-protein interaction network. (C) Co-expression heat map of the top 50 RACGAP1-related genes.

T cell and CD4$^+$ T cell (*Figure 6A*). Then, the results of TIMER2 database demonstrated RACGAP1 expression was positively related to the resting myeloid dendritic cell, M0 macrophage, myeloid derived suppressor cells (MDSCs), activated CD4$^+$ memory T cell, Tfh, and Treg cell, and negatively associated with CD4$^+$ memory resting T cell in TME.

Thereafter, the TISIDB database was applied to assess the relationship between RACGAP1 expression and tumor infiltrating lymphocytes (TILs). The result of heat map revealed the relationship between RACGAP1 and TILs across pan-cancers (*Figure 6B*). Meanwhile, the fraction of infiltrating immune cells was determined using the online tool of GEPIA2021. The result revealed that the proportion of Treg, macrophages M0, and mast cell resting was significantly higher in tumor tissues than that in normal tissues. However, the result for monocyte and mast cell activated showed the opposite trend (*Figure 6C*, Figure S3A-S3D). These findings indicated that RACGAP1 might regulate the infiltration of Treg, Tfh, Th2, M0 macrophage, MDSC in HCC TME.

RACGAP1 expression was positively correlated with immune suppressive microenvironment

It is well established that tumor immune escape involves Tregs which comprise an immune suppressive microenvironment. The TIMER and GEPIA datasets were utilized to explore the relationship between RACGAP1 expression and diverse immune signatures in HCC. Additionally, the association between RACGAP1 expression and the markers of diverse immune cells was explored.

The correlation between tumor purity and immune infiltration was studied. After adjusting for purity, there was a clear correlation between RACGAP1 expression and several immune cells, particularly Treg, T cell exhaustion, TAM, dendritic cell, M2 macrophage cell and MDSC (*Table 2*). In order to confirm the results, the association between RACGAP1 expression and the infiltration of immune cells in TME was analyzed using the GEPIA web. Similar results were observed in the TIMER web tool (*Table 3*).

The results of TIMER, TIMER2, GEPIA and GEPIA2021 revealed RACGAP1 was positively related to Treg cells in HCC. Moreover, further investigation was conducted to examine the relationship between RACGAP1 and the cytokine/receptor axis for Treg infiltration (CCR4-CCL22, CCR5-CCL5, CCR8-CCL1, and CCR10-CCL28). These results indicated RACGAP1 expression had a positive association with the expression levels of the aforementioned cytokine and receptors (Table S1).

Discussion

HCC is a common cancer with unfavorable prognosis (38). In China, the 5-year survival rate of HCC is still less than 5% (39). In this study, 65 common DEGs were screened

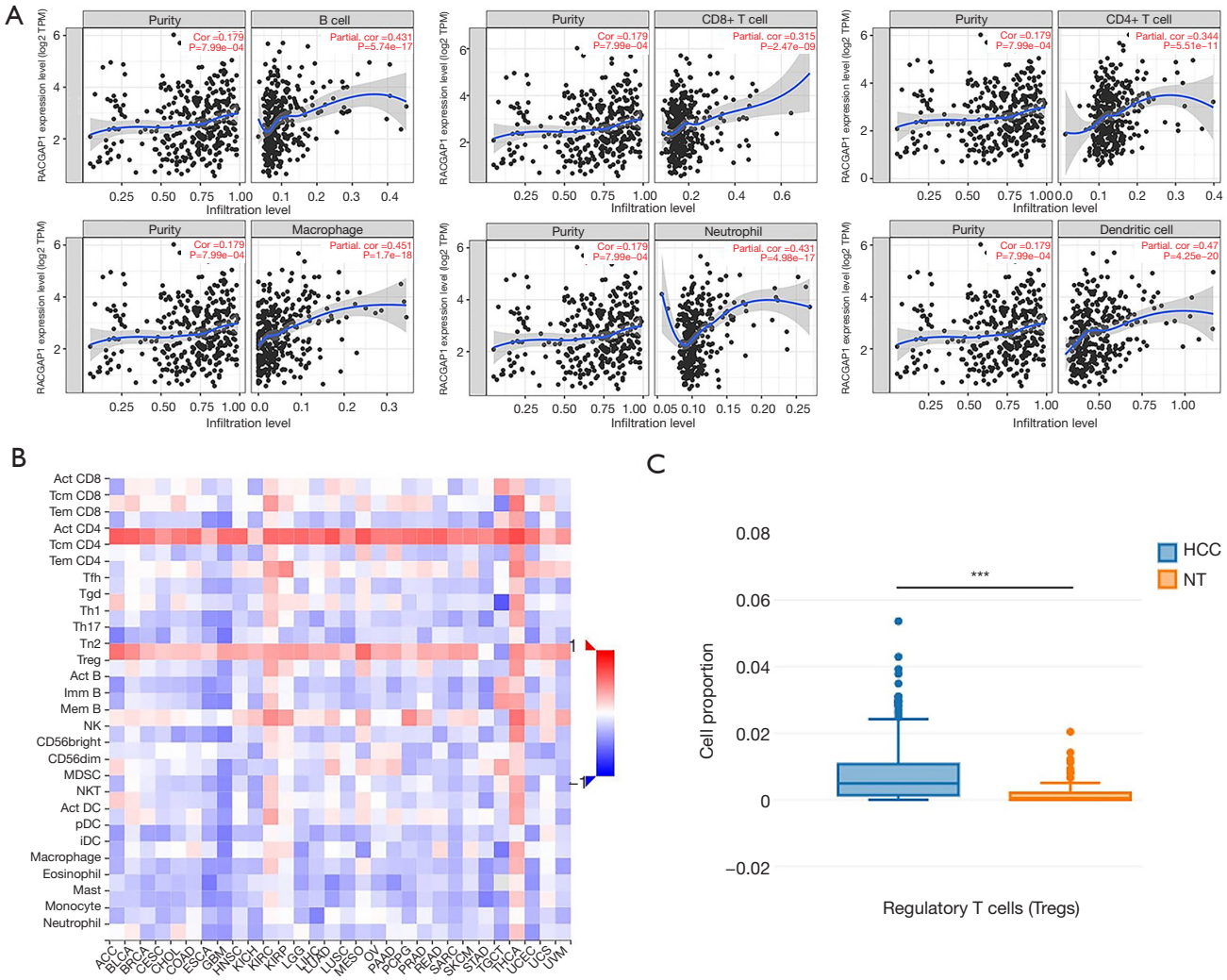

Figure 6 Correlation of RACGAP1 with immune infiltration. (A) RACGAP1 is correlated with tumor purity and different immune infiltration cells. (B) Pan-cancer analysis revealing the association between RACGAP1 and tumor infiltrating lymphocytes through TISIDB database. (C) The proportion of T regulatory cells in the HCC and NT. ***, P<0.001. TPM, transcripts per million; TISIDB, Tumor-Immune System Interaction Database; HCC, hepatocellular carcinoma; NT, normal tissues.

out from three GSE datasets, and then the top 11 hub genes were identified. The hub genes, including ANLN, CDK1 and CCNB1, have been studied previously. However, the function of RACGAP1 has rarely been explored in HCC. Thus, RACGAP1 was selected to explore its value in hepatocarcinogenesis.

RACGAP1 is a Rac GTPase-activating protein involved in process of cell division, cell transformation and differentiation. Recent studies found that it had an important function in oncogenesis. RACGAP1 knockdown could inhibit development of various tumors, especially HCC (9,40,41). However, the potential molecular mechanisms for this process have not been profoundly known.

In this study, the expression of RACGAP1 was upregulated in HCC. Higher expression of RACGAP1 was associated with tumor aggressive behavior, which is similar to findings from previous studies (7,42). All these results indicated that RACGAP1 could be applied as a candidate biomarker for predicting HCC prognosis. Enrichment analyses revealed that RACGAP1 regulated the cell division process. Furthermore, the results indicated that RACGAP1

Table 2 Correlation analysis between RACGAP1 and relate genes markers of immune cells using TIMER

Immune cells	Gene markers	Liver hepatocellular carcinoma			
		None		Purity	
		Cor	P	Cor	P
$CD8^+$ T cell	*CD8A*	0.152	3.34e–03*	0.267	4.60e–07*
	CD8B	0.115	2.68e–02*	0.227	2.12e–05*
T cell (general)	*CD2*	0.104	4.50e–02*	0.249	2.91e–06*
	CD3D	0.152	3.39e–03*	0.272	2.76e–07*
	CD3E	0.109	3.66e–02*	0.260	9.77e–07*
Th1	*IFN-γ* (*IFNG*)	0.231	6.89e–06*	0.324	6.76e–10*
	STAT1	0.412	0.00e+00*	0.470	2.18e–20*
	STAT4	0.142	6.20e–03*	0.210	8.28e–05*
	T-bet (*TBX21*)	0.052	3.13e–01	0.163	2.42e–03*
	TNF-α (*TNF*)	0.233	5.63e–06*	0.367	2.04e–12*
Th2	*GATA3*	0.125	1.63e–02*	0.269	3.76e–07*
	IL13	0.059	2.57e–01	0.070	1.96e–01
	STAT5A	0.366	3.15e–13*	0.431	4.65e–17*
	STAT6	0.297	5.40e–09*	0.281	1.05e–07*
Tfh	*BCL6*	0.356	2.07e–12*	0.358	7.68e–12*
	IL21	0.144	5.56e–03*	0.190	3.82e–04*
Th17	*IL17A*	0.119	2.16e–02*	0.125	2.02e–02*
	STAT3	0.240	3.12e–06*	0.288	5.13e–08*
Treg	*CCR8*	0.403	6.91e–16*	0.516	6.63e–25*
	FOXP3	0.190	2.41e–04*	0.255	1.56e–06*
	STAT5B	0.459	0.00e+00*	0.439	1.16e–17*
	TGFβ (*TGFB1*)	0.271	1.26e–07*	0.389	6.91e–14*
T cell exhaustion	*CTLA4*	0.249	1.22e–06*	0.369	1.36e–12*
	GZMB	0.107	4.04e–02*	0.179	8.60e–04*
	LAG3	0.214	3.48e–05*	0.271	3.11e–07*
	PD-1 (*PDCD1*)	0.251	9.76e–07*	0.358	6.81e–12*
	TIM-3 (*HAVCR2*)	0.282	3.86e–08*	0.457	3.31e–19*
B cell	*CD19*	0.221	1.68e–05*	0.301	1.18e–08*
	CD79A	0.061	2.38e–01	0.183	6.32e–04*
Monocyte	*CD86*	0.291	1.33e–08*	0.461	1.42e–19*
	CD115 (*CSF1R*)	0.177	6.20e–04*	0.333	2.13e–10*
TAM	*CCL2*	0.066	2.01e–01	0.175	1.09e–03*
	CD68	0.239	3.62e–06*	0.351	1.96e–11*
	IL10	0.225	1.17e–05*	0.351	1.90e–11*

Table 2 (*continued*)

Table 2 (*continued*)

Immune cells	Gene markers	Liver hepatocellular carcinoma			
		None		Purity	
		Cor	P	Cor	P
M1 macrophage	*COX2* (*PTGS2*)	0.147	4.65e–03*	0.283	8.62e–08*
	INOS (*NOS2*)	0.085	1.02e–01	0.099	6.62e–02
	IRF5	0.403	6.86e–16*	0.413	1.12e–15*
M2 macrophage	*CD163*	0.138	7.76e–03*	0.268	4.17e–07*
	MS4A4A	0.125	1.63e–02*	0.269	3.96e–07*
	VSIG4	0.120	2.06e–02*	0.250	2.47e–06*
Dendritic cell	*BDCA-1* (*CD1C*)	0.115	2.63e–02*	0.213	6.83e–05*
	BDCA-4 (*NRP1*)	0.442	0.00e+00*	0.478	4.19e–21*
	CD11C (*ITGAX*)	0.314	7.99e–10*	0.461	1.42e–19*
	HLA-DPA1	0.177	6.33e–04*	0.311	3.38e–09*
	HLA-DPB1	0.160	2.05e–03*	0.291	3.79e–08*
	HLA-DQB1	0.099	5.7e–02	0.215	5.88e–05*
	HLA-DRA	0.201	9.87e–05*	0.336	1.43e–10*
Natural killer cell	*KIR2DL1*	0.026	6.23e–01	0.009	8.69e–01
	KIR2DL3	0.191	2.19e–04*	0.237	8.73e–06*
	KIR2DL4	0.204	7.84e–05*	0.248	3.03e–06*
	KIR2DS4	0.076	1.42e–01	0.071	1.90e–01
	KIR3DL1	0.076	1.45e–01	0.106	4.86e–02*
	KIR3DL2	0.063	2.23e–01	0.117	2.93e–02*
	KIR3DL3	0.066	2.06e–01	0.059	2.73e–01
Neutrophils	*CCR7*	0.056	2.85e–01	0.187	4.74e–04*
	CD11b (*ITGAM*)	0.312	9.60e–10*	0.421	3.01e–16*
	CD66b (*CEACAM8*)	0.111	3.20e–02*	0.139	9.87e–03*
M-MDSC	*CD14*	–0.405	0.00e+00*	–0.376	5.13e–13*
PMN-MDSC	*ITGAM*	0.312	9.60e–10*	0.421	3.01e–16*
	FUT4	0.372	1.80e–13*	0.415	8.64e–16*

*, P<0.05. TIMER, Tumor Immune Estimation Resource; TAM, tumor-associated macrophage; MDSC, myeloid derived suppressor cell; M-MDSC, mononuclear MDSC; PMN-MDSC, polymorphonuclear MDSC.

expression was correlated with immune infiltration. Therefore, this study could provide a novel view to the potential value of RACGAP1 in tumor immunology.

Tregs are involved in the maintenance of immunological self-tolerance through immunosuppressive effects in various types of tumors, thereby helping tumor cells to escape from immune killing (43-46). In this study, several results indicated the recruitment of immunosuppressive Tregs as a key mechanism of immune escape in HCC. In HCC, the secretion of various chemokines recruits Treg to TME (47). We found that RACGAP1 upregulation was positively related to the chemokines and receptors expression for Treg recruitment. Increased secretion of chemokines and corresponding receptors following RACGAP1 may

Table 3 Correlation analysis between RACGAP1 and relate genes markers of immune cells using GEPIA

Immune cells	Gene markers	Liver hepatocellular carcinoma	
		R	P
$CD8^+$ T cell	*CD8A*	0.14	0.0066*
	CD8B	0.11	0.03*
T cell (general)	*CD2*	0.091	0.081
	CD3D	0.1	0.044*
	CD3E	0.092	0.077
Th1	*IFN-γ* (*IFNG*)	0.22	1.6e–05*
	STAT1	0.41	2.4e–16*
	STAT4	0.15	0.0035*
	T-bet (*TBX21*)	0.051	0.33
	TNF-α (*TNF*)	0.23	7.2e–06*
Th2	*GATA3*	0.15	0.0043*
	IL13	0.074	0.16
	STAT5A	0.39	1.6e–14*
	STAT6	0.33	8.5e–11*
Tfh	*BCL6*	0.38	3.3e–14*
	IL21	0.14	0.0056*
Th17	*IL17A*	0.09	0.084
	STAT3	0.27	1.1e–07*
Treg	*CCR8*	0.4	6.8e–16*
	FOXP3	0.12	0.02*
	STAT5B	0.49	2e–23*
	TGFβ (*TGFB1*)	0.22	2.2e–05*
T cell exhaustion	*CTLA4*	0.25	1.3e–06*
	GZMB	0.044	0.4
	LAG3	0.13	0.014*
	PD-1 (*PDCD1*)	0.25	1e–06*
	TIM-3 (*HAVCR2*)	0.28	5.8e–08*
B cell	*CD19*	0.24	4.3e–06*
	CD79A	0.064	0.22
Monocyte	*CD86*	0.29	1.2e–08*
	CD115 (*CSF1R*)	0.21	7e–05*
TAM	*CCL2*	0.056	0.29
	CD68	0.25	9.3e–07*
	IL10	0.19	2e–04*
M1 macrophage	*COX2* (*PTGS2*)	0.17	0.00085*
	INOS (*NOS2*)	0.12	0.025
	IRF5	0.39	8.3e–15*

Table 3 (*continued*)

Table 3 (*continued*)

Immune cells	Gene markers	Liver hepatocellular carcinoma	
		R	P
M2 macrophage	*CD163*	0.028	0.59
	MS4A4A	0.12	0.021*
	VSIG4	0.12	0.024*
Dendritic cell	*BDCA-1* (*CD1C*)	0.12	0.026*
	BDCA-4 (*NRP1*)	0.44	1.7e−18*
	CD11C (*ITGAX*)	0.32	6e−10*
	HLA-DPA1	0.18	5e−04*
	HLA-DPB1	0.17	0.00096*
	HLA-DQB1	0.031	0.55
	HLA-DRA	0.2	7.8e−05*
Natural killer cell	*KIR2DL1*	0.041	0.43
	KIR2DL3	0.2	0.00015*
	KIR2DL4	0.23	5.1e−06*
	KIR2DS4	0.073	0.16
	KIR3DL1	0.015	0.78
	KIR3DL2	0.15	0.0051*
	KIR3DL3	0.12	0.017*
Neutrophils	*CCR7*	0.058	0.26
	CD11b (*ITGAM*)	0.34	9.6e−12*
	CD66b (*CEACAM8*)	0.14	0.0067*
M-MDSC	*CD14*	-0.38	7.5e−14*
PMN-MDSC	*ITGAM*	0.34	9.6e−12*
	FUT4	0.36	1.7e−12*

*, P<0.05. GEPIA, gene expression profiling interactive analysis; TAM, tumor-associated macrophage; MDSC, myeloid derived suppressor cell; M-MDSC, mononuclear MDSC; PMN-MDSC, polymorphonuclear MDSC.

contribute to the Treg enrichment in HCC TME.

The enrichment analysis revealed RACGAP1 was involved in cell division processes, which suggested its contribution to tumorigenesis. Yang *et al.* demonstrated that overexpression of RACGAP1 contributed to tumorigenesis by promoting cytokinesis via Hippo/YAP signaling (9). Hippo-YAP pathway is regarded as a regulator of cell proliferation, contact inhibition, organ size and tumorigenesis. Interestingly, recent studies demonstrated YAP activation enhanced the initiation or maintenance of Treg differentiation in multiple tumors including gastric cancer, HCC and melanoma (48-50). Knockdown or knockout YAP decreased the expression of RACGAP1 and improved antitumor immunity. Therefore, we speculate RACGAP1 may promote tumorigenesis of HCC through immunosuppression mediated by YAP activation.

This study has several limitations. Firstly, the correlation between RACGAP1 and prognosis factors was analyzed using online public databases, which requires clinical studies to validate the conclusion. Secondly, the analyses were mainly performed according to the expression of RACGAP1 at the mRNA levels. Thirdly, the precise molecular mechanisms of RACGAP1 in immunosuppression need further studies to validate.

We demonstrated positive correlation of RACGAP1 overexpression with HCC prognosis and immune infiltration

in this study. Notably, we demonstrated that the immune suppressive effect of RACGAP1 was mediated by YAP activation. In summary, these results indicated that RACGAP1 could serve as a molecular target for HCC therapy.

Conclusions

In this study, we determined the prognostic value of RACGAP1 for HCC patients. Overexpression of RACGAP1 was associated with unfavorable prognosis and immune infiltration in HCC, which indicated that RACGAP1 could be a molecular target for HCC.

Acknowledgments

We would like to thank Robin Ahmed for his help in polishing our paper.
Funding: This work was supported by National Natural Science Foundation of China (No. 82070631).

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1474/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1474/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1474/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). Ethical approval was not required because GEO and TCGA are publicly available sources of data. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Kulik L, El-Serag HB. Epidemiology and Management of Hepatocellular Carcinoma. Gastroenterology 2019;156:477-491.e1.
3. Ge Q, Lu M, Ju L, et al. miR-4324-RACGAP1-STAT3-ESR1 feedback loop inhibits proliferation and metastasis of bladder cancer. Int J Cancer 2019;144:3043-55.
4. Saigusa S, Tanaka K, Mohri Y, et al. Clinical significance of RacGAP1 expression at the invasive front of gastric cancer. Gastric Cancer 2015;18:84-92.
5. Imaoka H, Toiyama Y, Saigusa S, et al. RacGAP1 expression, increasing tumor malignant potential, as a predictive biomarker for lymph node metastasis and poor prognosis in colorectal cancer. Carcinogenesis 2015;36:346-54.
6. Gu Y, Chen B, Guo D, et al. Up-Regulation of RACGAP1 Promotes Progressions of Hepatocellular Carcinoma Regulated by GABPA via PI3K/AKT Pathway. Oxid Med Cell Longev 2022;2022:3034150.
7. Wang SM, Ooi LL, Hui KM. Upregulation of Rac GTPase-activating protein 1 is significantly associated with the early recurrence of human hepatocellular carcinoma. Clin Cancer Res 2011;17:6040-51.
8. Pliarchopoulou K, Kalogeras KT, Kronenwett R, et al. Prognostic significance of RACGAP1 mRNA expression in high-risk early breast cancer: a study in primary tumors of breast cancer patients participating in a randomized Hellenic Cooperative Oncology Group trial. Cancer Chemother Pharmacol 2013;71:245-55.
9. Yang XM, Cao XY, He P, et al. Overexpression of Rac GTPase Activating Protein 1 Contributes to Proliferation of Cancer Cells by Reducing Hippo Signaling to Promote Cytokinesis. Gastroenterology 2018;155:1233-1249.e22.
10. Shimada S, Mogushi K, Akiyama Y, et al. Comprehensive molecular and immunological characterization of hepatocellular carcinoma. EBioMedicine 2019;40:457-70.

11. Chiyonobu N, Shimada S, Akiyama Y, et al. Fatty Acid Binding Protein 4 (FABP4) Overexpression in Intratumoral Hepatic Stellate Cells within Hepatocellular Carcinoma with Metabolic Risk Factors. Am J Pathol 2018;188:1213-24.
12. Schulze K, Imbeaud S, Letouzé E, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet 2015;47:505-11.
13. Sherman BT, Hao M, Qiu J, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res 2022;50:W216-21.
14. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res 2021;49:D605-12. Erratum in: Nucleic Acids Res 2021;49:10800.
15. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498-504.
16. Li T, Fan J, Wang B, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Res 2017;77:e108-10.
17. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020;48:W509-14.
18. Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45:W98-W102.
19. Li C, Tang Z, Zhang W, et al. GEPIA2021: integrating multiple deconvolution-based analysis into GEPIA. Nucleic Acids Res 2021;49:W242-6.
20. Chandrashekar DS, Karthikeyan SK, Korla PK, et al. UALCAN: An update to the integrated cancer data analysis platform. Neoplasia 2022;25:18-27.
21. Bartha Á, Győrffy B. TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. Int J Mol Sci 2021;22:2622.
22. Uhlen M, Zhang C, Lee S, et al. A pathology atlas of the human cancer transcriptome. Science 2017;357:eaan2507.
23. Lánczky A, Győrffy B. Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. J Med Internet Res 2021;23:e27633.
24. Franz M, Rodriguez H, Lopes C, et al. GeneMANIA update 2018. Nucleic Acids Res 2018;46:W60-4.
25. Ru B, Wong CN, Tong Y, et al. TISIDB: an integrated repository portal for tumor-immune system interactions. Bioinformatics 2019;35:4200-2.
26. Shen W, Song Z, Zhong X, et al. Sangerbox: A comprehensive, interaction-friendly clinical bioinformatics analysis platform. iMeta 2022;1:e36.
27. Chen Y, Meng L, Shang H, et al. β2 spectrin-mediated differentiation repressed the properties of liver cancer stem cells through β-catenin. Cell Death Dis 2018;9:424.
28. Wang Z, Liu F, Tu W, et al. Embryonic liver fodrin involved in hepatic stellate cell activation and formation of regenerative nodule in liver cirrhosis. J Cell Mol Med 2012;16:118-28.
29. Wang Z, Song Y, Tu W, et al. β-2 spectrin is involved in hepatocyte proliferation through the interaction of TGFβ/Smad and PI3K/AKT signalling. Liver Int 2012;32:1103-11.
30. Chen J, Li Z, Jia X, et al. Targeting anillin inhibits tumorigenesis and tumor growth in hepatocellular carcinoma via impairing cytokinesis fidelity. Oncogene 2022;41:3118-30.
31. Li L, Huang K, Zhao H, et al. CDK1-PLK1/SGOL2/ANLN pathway mediating abnormal cell division in cell cycle may be a critical process in hepatocellular carcinoma. Cell Cycle 2020;19:1236-52.
32. Xia P, Zhang H, Xu K, et al. MYC-targeted WDR4 promotes proliferation, metastasis, and sorafenib resistance by inducing CCNB1 translation in hepatocellular carcinoma. Cell Death Dis 2021;12:691.
33. Mi H, Ho WJ, Yarchoan M, et al. Multi-Scale Spatial Analysis of the Tumor Microenvironment Reveals Features of Cabozantinib and Nivolumab Efficacy in Hepatocellular Carcinoma. Front Immunol 2022;13:892250.
34. Ho WJ, Zhu Q, Durham J, et al. Neoadjuvant Cabozantinib and Nivolumab Converts Locally Advanced HCC into Resectable Disease with Enhanced Antitumor Immunity. Nat Cancer 2021;2:891-903.
35. Zhang S, Deshpande A, Verma BK, et al. Informing virtual clinical trials of hepatocellular carcinoma with spatial multi-omics analysis of a human neoadjuvant immunotherapy clinical trial. Preprint. bioRxiv 2023;2023.08.11.553000.
36. Gong X, Zheng C, Jia H, et al. A pan-cancer analysis revealing the role of LFNG, MFNG and RFNG in tumor prognosis and microenvironment. BMC Cancer 2023;23:1065.
37. Wang M, Chang M, Li C, et al. Tumor-Microenvironment-Activated Reactive Oxygen Species Amplifier for Enzymatic Cascade Cancer Starvation/

Chemodynamic /Immunotherapy. Adv Mater 2022;34:e2106010.
38. Ganesan P, Kulik LM. Hepatocellular Carcinoma: New Developments. Clin Liver Dis 2023;27:85-102.
39. Xia C, Dong X, Li H, et al. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J (Engl) 2022;135:584-90.
40. Pu J, Wang J, Wei H, et al. lncRNA MAGI2-AS3 Prevents the Development of HCC via Recruiting KDM1A and Promoting H3K4me2 Demethylation of the RACGAP1 Promoter. Mol Ther Nucleic Acids 2019;18:351-62.
41. Wu PH, Onodera Y, Recuenco FC, et al. Lambda-Carrageenan Enhances the Effects of Radiation Therapy in Cancer Treatment by Suppressing Cancer Cell Invasion and Metastasis through Racgap1 Inhibition. Cancers (Basel) 2019;11:1192.
42. Wang MY, Chen DP, Qi B, et al. Pseudogene RACGAP1P activates RACGAP1/Rho/ERK signalling axis as a competing endogenous RNA to promote hepatocellular carcinoma early recurrence. Cell Death Dis 2019;10:426.
43. Gu J, Zhou J, Chen Q, et al. Tumor metabolite lactate promotes tumorigenesis by modulating MOESIN lactylation and enhancing TGF-β signaling in regulatory T cells. Cell Rep 2022;39:110986.
44. Zhang Y, Guo J, Jia R. Treg: A Promising Immunotherapeutic Target in Oral Diseases. Front Immunol 2021;12:667862.
45. Villarreal DO, L'Huillier A, Armington S, et al. Targeting CCR8 Induces Protective Antitumor Immunity and Enhances Vaccine-Induced Responses in Colon Cancer. Cancer Res 2018;78:5340-8.
46. Bai F, Zhang P, Fu Y, et al. Targeting ANXA1 abrogates Treg-mediated immune suppression in triple-negative breast cancer. J Immunother Cancer 2020;8:e000169.
47. Liu L, Wang Q, Qiu Z, et al. Noncoding RNAs: the shot callers in tumor immune escape. Signal Transduct Target Ther 2020;5:102.
48. Wang J, Huang F, Shi Y, et al. RP11-323N12.5 promotes the malignancy and immunosuppression of human gastric cancer by increasing YAP1 transcription. Gastric Cancer 2021;24:85-102.
49. Fan Y, Gao Y, Rao J, et al. YAP-1 Promotes Tregs Differentiation in Hepatocellular Carcinoma by Enhancing TGFBR2 Transcription. Cell Physiol Biochem 2017;41:1189-98.
50. Ni X, Tao J, Barbi J, et al. YAP Is Essential for Treg-Mediated Suppression of Antitumor Immunity. Cancer Discov 2018;8:1026-43.

Cite this article as: Zhou Y, Zheng S, Guo Q, Wei N, Xiao Z, Song Y. Upregulation of RACGAP1 is correlated with poor prognosis and immune infiltration in hepatocellular carcinoma. Transl Cancer Res 2024;13(2):847-863. doi: 10.21037/tcr-23-1474

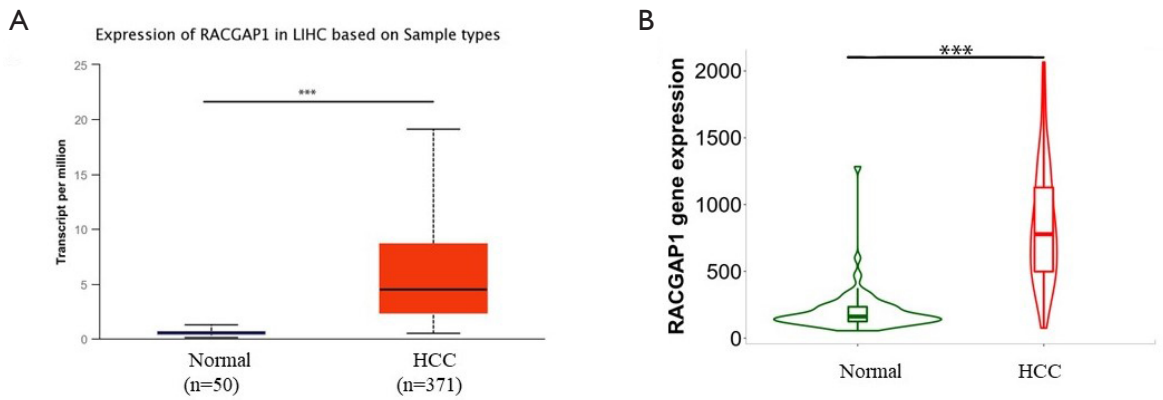

Figure S1 RACGAP1 expression in HCC. (A,B) RACGAP1 mRNA expression in HCC was examined through the UALCAN and TNM plot datasets. ***, P<0.001.

https://dx.doi.org/10.21037/tcr-23-1474

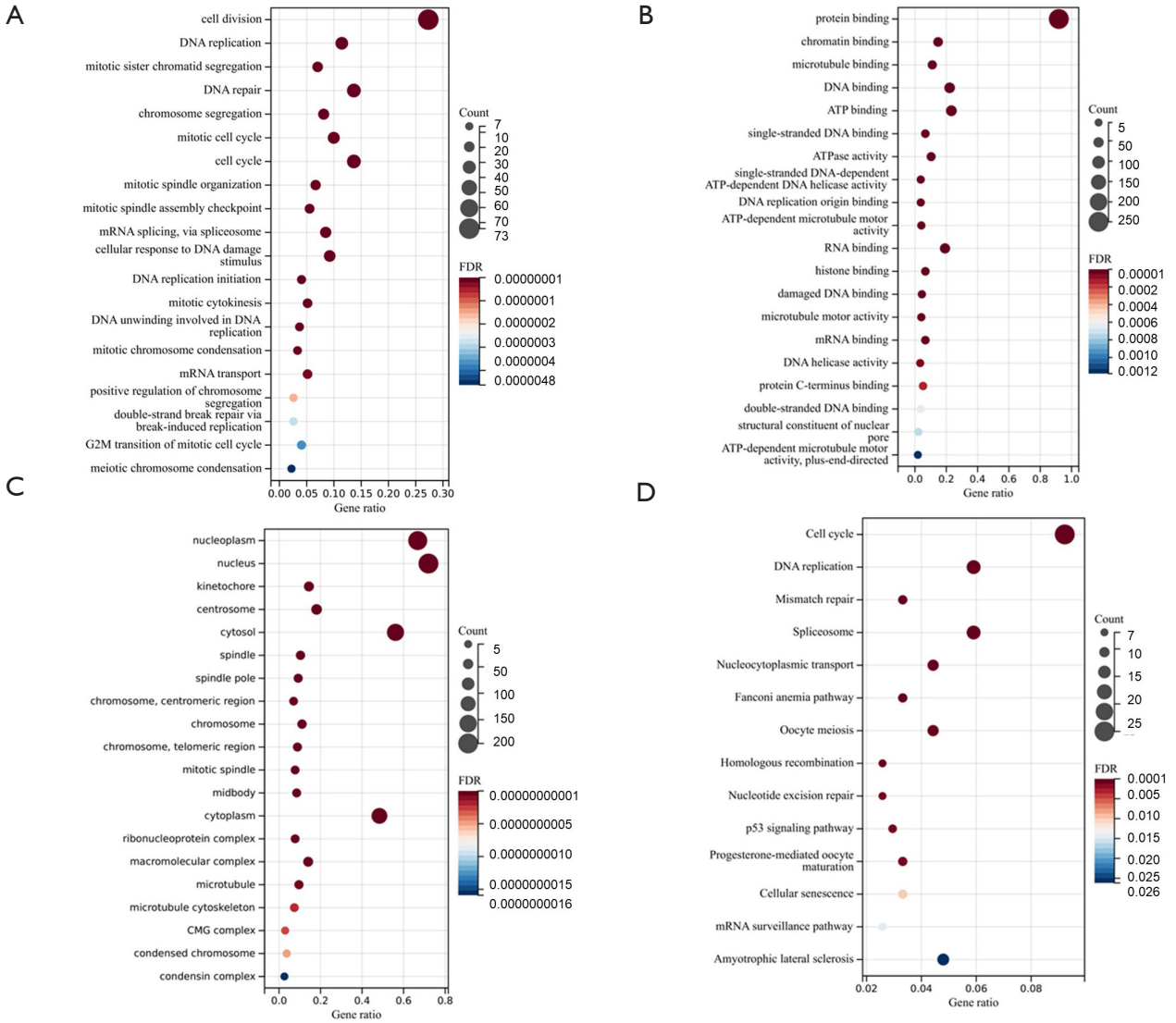

Figure S2 Functional enrichment analysis of RACGAP1-interacting genes and proteins. (A) Biological process (BP), (B) molecular function (MF), (C) cellular component (CC) and (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of the genes related to RACGAP1.

https://dx.doi.org/10.21037/tcr-23-1474

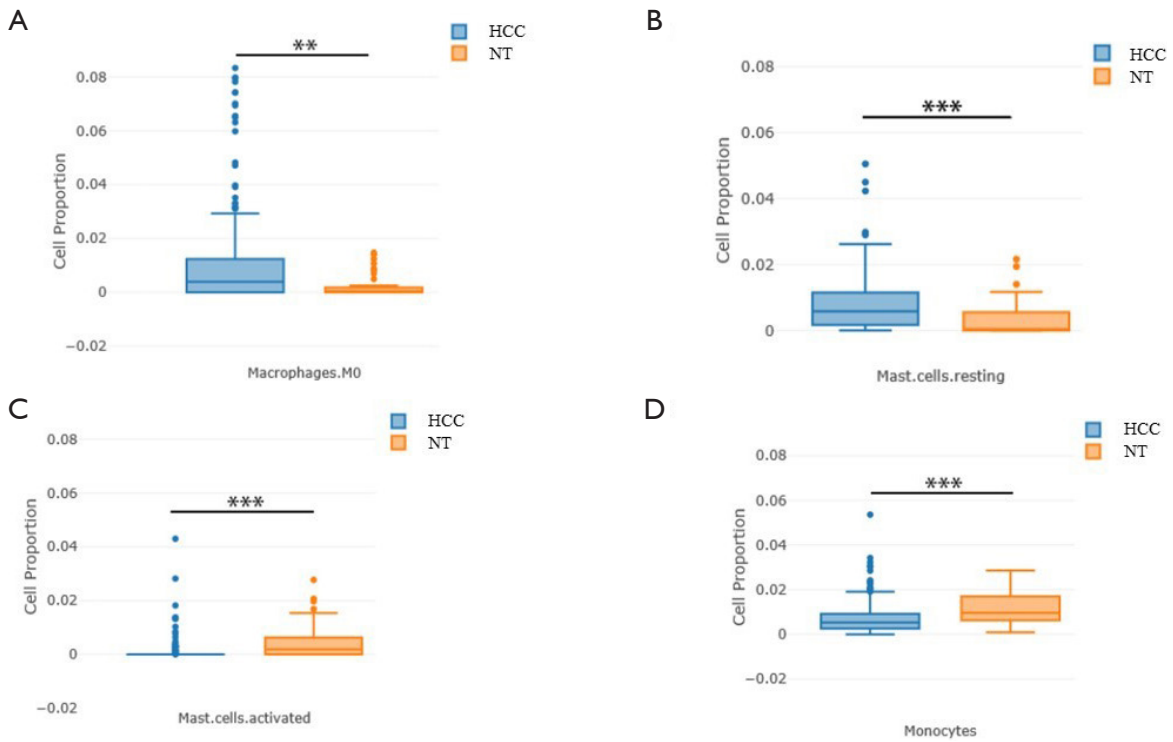

Figure S3 The proportion of tumor-infiltrating lymphocytes in hepatocellular carcinoma and normal tissues. (A-D) the proportion of macrophages M0, resting mast cell, activated mast cell and monocyte in HCC and NT. **, P<0.01; ***, P<0.001.

https://dx.doi.org/10.21037/tcr-23-1474

Table S1 Correlation analysis between RACGAP1 and cytokines using TIMER

Cytokines	Liver hepatocellular carcinoma			
	None		Purity	
	Cor	P	Cor	P
CCR4	0.219	2.10–05*	0.329	3.75e–10*
CCR5	0.199	1.14e–04*	0.371	1.03e–12*
CCR8	0.403	6.91e–16*	0.516	6.63e–25*
CCR10	0.438	7.56e–19*	0.474	9.43e–21*
CCL22	0.052	3.20e–01	0.169	1.67e–03*
CCL5	0.065	2.14e–01	0.185	5.42e–04*
CCL1	0.147	4.61e–03*	0.173	1.26e–03*
CCL28	0.334	3.98e–11*	0.311	3.46e–09*

*, P<0.05.

https://dx.doi.org/10.21037/tcr-23-1474