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# A pan-cancer analysis of the oncogenic and immunological roles of RGS5 in clear cell renal cell carcinomas based on in vitro experiment validation

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## Abstract

**Background** RGS5, the first gene identified in tumor-resident pericytes, plays a crucial role in angiogenesis. However, its effects on immunology and prognosis in human cancer are still mostly unknown. This study investigates the carcinogenic and immunological roles of RGS5 through a comprehensive pan-cancer analysis.

**Methods** A standardized pan-cancer dataset for RGS5 was obtained from the public database. R software and relevant packages were utilized to analyze the oncogenic and immunological roles. Clinical samples and cellular experiments were conducted to validate RGS5 expression and its biological function in renal cancer.

**Results** Bioinformatics analysis revealed that RGS5 is dysregulated in a variety of human malignancies and is significantly associated with patient prognosis. Additionally, RGS5 expression is closely linked to tumor heterogeneity and stemness indicators across different cancer types. Co-expression of RGS5 with genes involved in MHC, immune activation, immunosuppressive proteins, chemokines, and chemokine receptors was observed in various tumors. High expression of RGS5 predicts a good prognosis in patients with renal cancer. In the renal cancer cohort, RGS5 expression strongly correlated with the distribution of tumor-associated fibroblasts. Silencing RGS5 expression can affect the proliferation, migration, and invasion of renal carcinoma cells.

**Conclusions** RGS5 expression in tumors is intricately associated with various clinical features, particularly concerning tumor progression and patient prognosis.

**Keywords** RGS5, Pan-cancer analysis, Angiogenesis, Renal cancer

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## Introduction

Mutations in the G protein-coupled receptor (GPCR) signaling pathway are more prevalent in cancer than previously recognized, according to Kankanamge et al. as referenced [1]. GPCR signaling involves the activation of receptors by external ligands, which subsequently initiate intracellular signaling cascades [2]. These pathways play pivotal roles in cancer progression by contributing to hallmark traits such as sustained proliferative signaling and enhanced angiogenesis [3, 4]. GPCRs influence the immune microenvironment by modulating the migration and activation of immune cells, which can either promote or inhibit tumor growth depending on the context [5, 6]. Overall, GPCR signaling has emerged as a desirable target for cancer treatment due to its ability to stimulate tumor cell proliferation and alter the tumor microenvironment (TME).

GTPase-accelerating proteins (GAPs) for Gα subunits are among the regulators of G protein signaling (RGS) that are essential for stopping GPCR signaling [3]. The discovery of new immunotherapy targets within the RGS family and their correlations with patient outcomes have been made possible by developments in open databases such as The Cancer Genome Atlas (TCGA) [7, 8]. A crucial regulator of GPCR signaling, which is closely related to both physiological and pathological processes, is the RGS family [9]. RGS5, a member of the RGS family, plays a crucial role in regulating G-protein-mediated signaling pathways [10]. It is involved in various physiological functions, including blood pressure regulation and vascular development [11]. RGS5 was discovered in early research to be a smooth muscle cell marker that stimulates arterial growth during arteriogenesis [12]. RGS5 is abundantly expressed in the blood arteries around kidney carcinoma nests and facilitates angiogenesis, a mechanism necessary for tumor growth [13]. RGS5 affects the capacity of cancer cells to infiltrate adjacent tissues and spread to other locations by stabilizing or disrupting blood vessels [14]. RGS5 is highly expressed in cancer-associated fibroblasts (CAFs), and recent developments in single-cell RNA sequencing (scRNA-seq) have shown that it may play a role in the spread of cancer [15]. RGS5 is being researched as a possible therapeutic target because of its functions in angiogenesis and the TME [16, 17]. However, there is no comprehensive pan-cancer evidence examining the relationship between RGS5 and various cancers.

In this study, we comprehensively explored RGS5's oncogenic and immunological roles by integrating pan-cancer analyses with experimental validation in clear cell renal cell carcinoma (ccRCC). By identifying RGS5 as a key factor in tumor progression and immune interactions, our findings establish a foundation for developing targeted therapeutic strategies, particularly in renal cancer.

## Materials and methods

### Data acquisition, processing, and differential and prognostic analysis

Consistent with a previous study [18], we retrieved a comprehensive pan-cancer dataset from the UCSC database, encompassing RNA sequencing and clinical data [19]. Using the GTEx database (https://www.gtexportal.org), we included RGS5 differential expression data in tumor and normal tissues. The GENT2 database included additional pan-cancer datasets based on the Gene Expression Omnibus (GEO) cohort [20]. Additionally, prognostic information was acquired for 34 tumor types, including progression-free interval (PFI), disease-specific survival (DSS), overall survival (OS), and disease-free interval (DFI) [21]. We performed prognostic analysis using the Cox proportional hazards regression model, which was implemented using the "survival" package. The log-rank test was used to assess statistical significance [22]. The Cancer Cell Line Encyclopedia (CCLE) provided the RGS5 expression data for kidney cancer cell lines [23]. RGS5 expression levels in various cell subpopulations in renal carcinoma were also assessed using single-cell sequencing data from the TISCH website [24, 25].

### Pan-cancer analysis of tumor heterogeneity, stemness, and gene mutation

With an emphasis on tumor mutation burden (TMB), microsatellite instability (MSI), neoantigen (NEO), and tumor purity, we used Pearson correlation analysis to investigate the connection between tumor genomic heterogeneity and RGS5 expression. The GDC website (https://portal.gdc.cancer.gov/) provided the data for these analyses, which were then processed using the R package "maftools" and the Mutect2 software [26, 27]. MSI scores from prior studies were utilized to calculate Pearson correlations with RGS5 expression [28]. By combining tumor methylation and mRNA expression profiles, we were able to derive a tumor stemness score that included both RNA expression-based stemness (RNAss) and DNA methylation-based stemness (DNAss) [29]. Furthermore, by combining mutation data and domain information, the gene mutation landscape was created, enabling us to evaluate mutation frequency across different tumor types [30].

### Pan-cancer analysis of tumor microenvironment, RNA modification, and drug sensitivity

We examined the relationship between RGS5 expression and immunological checkpoint genes and computed the

Pearson correlation between RGS5 expression and immunomodulatory genes across different tumor types [31]. Furthermore, we looked into the connection between RGS5 expression and genes linked to RNA modification in various tumor types. The R package "IOBR" was used to examine immune cell infiltration in connection to RGS5 expression [32, 33]. Additionally, information from the Genomics of Drug Sensitivity in Cancer (GDSC) and the Cancer Therapeutics Response Portal (CTRP) was used to assess the relationship between RGS5 expression and drug sensitivity through the GSCALite website [34].

#### Functional enrichment analysis

Using the GeneMANIA website, we were able to identify RGS5 co-expressed genes [35]. Two groups were created from the TCGA-KIRC dataset according to the median RGS5 expression level. The "limma" program was used to identify genes that were differentially expressed, with the criteria of $|\log2(\text{Fold Change})| > 1$ and P-value $< 0.05$. The R packages "clusterProfiler," "GSEABase," and "GSVA" were then used to carry out functional enrichment analysis [36, 37].

#### Collecting clinical samples

From surgical specimens, kidney cancer tissues—including 14 cases of renal cell carcinoma—as well as nearby normal tissues were extracted. Before surgery, all patients signed an informed permission form for tissue collection. Criteria for inclusion: (1) Patients who received surgical treatment after being diagnosed with kidney cancer at our hospital. (2) Patients who willingly completed informed permission forms and agreed to the collection of tumor tissues. (1) Postoperative pathology results that show a diagnosis other than ccRCC are an exclusion criterion. (2) Patients who refused to have a tumor sample taken. Table S1 displays the clinical features of these patients.

#### Immunohistochemistry (IHC) assay

The detailed steps for IHC have been described in previous studies [38, 39]. After dewaxing, hydrating, and retrieving the antigen, tissue samples were incubated with an RGS5 antibody (rabbit, 1:150; OriGene) throughout the entire night. Two senior pathologists independently completed the IHC scoring.

#### Immunofluorescence (IF) assay

As described in previous studies [40], three cases of renal cell carcinoma and adjacent normal tissues were selected for IF analysis (Table S2 contains comprehensive details about the three patients). Following the proper preparation, the tissues were co-incubated with primary antibodies, such as α-SMA (rabbit, 1:100, Abcam) and RGS5 (mouse, 1:100, OriGene). Following the manufacturer's directions, a fluorescent secondary antibody was then added. HALO software (Indica Labs, USA) was used to assess the fluorescence intensity of proteins, and the average fluorescence intensity of five images per specimen was used to compute the histochemistry score (H-Score) for group comparisons [41].

#### Cell culture and siRNA transfection

Human cell lines were purchased from Procell (Wuhan, China) and cultured in 1640 or DMEM/F12 medium containing 10% FBS. OriGene Technologies GmbH (EU) provided the RGS5-specific siRNA (Locus ID: 8490). The 786-O and Caki-1 cells were plated in 6-well plates and transfected with siRNA using Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific) following the manufacturer's protocol.

#### Western blotting (WB) assay

The detailed procedure for WB has been reported previously [42]. Protein samples were processed through electrophoresis, membrane transfer, and blocking before being incubated overnight with primary antibodies, including RGS5 (1:1000, OriGene), and GAPDH (1:2000, Affinity Bio). Following incubation with a secondary antibody (1:10,000, Affinity Bio), the results were examined using an enhanced chemiluminescence kit (Thermo Fisher Scientific).

#### Transwell migration and invasion assay

As previously mentioned, Transwell Chambers (Corning Inc.) were used for the migration and invasion experiments [42]. The bottom chambers were filled with medium containing 10% FBS, while the top chambers were filled with $2\times10^5$ cells that had been resuspended in serum-free medium. For the invasion assay, 50 μL Matrigel (Corning Inc.) was added to the upper chamber prior to cell seeding. After 24 h of incubation, cells were fixed, stained with crystal violet, and analyzed.

#### EdU cell proliferation assay

Cell proliferation was assessed using the BeyoClick™ EdU-488 Cell Proliferation Assay Kit (Beyotime Bio.) following the manufacturer's instructions. A total of $3\times10^5$ cells were seeded on glass slides in a 6-well plate. After processing, the results were visualized using a fluorescence microscope (Leica).

#### Statistic analysis

R software (version 4.2.1) was used for all statistical analyses. A two-sided P-value of less than 0.05 was considered statistically significant. Pearson correlation analysis was utilized to investigate associations between variables,

and Wilcoxon rank-sum and signed-rank tests were performed to evaluate pairwise differences.

## Results

### RGS5 expression and prognosis analysis in human cancer

In the TCGA cohort, we found that RGS5 transcription was higher in 12 tumor types than in normal tissues (Fig. 1A). Conversely, significant downregulation was observed in 17 tumor types (Fig. 1A). Eleven tumor types in the GEO cohort had increased RGS5 transcription levels (Fig. 1B). Eleven tumor types showed reduced expression (Fig. 1B). According to OS analysis, a bad prognosis was linked to high RGS5 expression in six tumor types, whereas a better prognosis was related to it in two tumor types (Fig. 1C). In the DSS analysis, high RGS5 expression in three tumor types correlated with poor prognosis, whereas low expression in two tumor types was associated with poor prognosis (Fig. 1D). According to DFI analysis, a higher expression of RGS5 in PCPG predicted a better prognosis, but a higher expression in KIRP indicated a bad prognosis (Fig. 1E). In the PFI analysis, a poor prognosis was associated with high RGS5 expression in four tumor types, while a better prognosis was predicted by raised expression in five tumor types (Fig. 1F).

### Association of RGS5 expression with genomic heterogeneity and tumor stemness in human cancers

We calculated the correlation between RGS5 expression levels and four genomic heterogeneity indicators as well as two tumor stemness scores. RGS5 expression was negatively correlated with TMB in six tumor types (Fig. 2A). A significant positive correlation between RGS5 expression and MSI was observed in TGCT. Conversely, negative correlations were identified in 13 tumor types (Fig. 2B). RGS5 expression showed a positive correlation with NEO in TGCT, but negative correlations in five tumor types (Fig. 2C). RGS5 expression was significantly positively correlated with tumor purity in 2 tumor types, such as THYM and TGCT, significantly negatively correlated in 18 tumor types (Fig. 2D). RGS5 expression was significantly positively correlated with DNAss in 7 tumor types and negatively correlated with (DNA methylation-based stemness) DNAss in 10 tumor types (Fig. 2E). RGS5 expression was significantly positively correlated with RNA expression-based stemness (RNAss) in LGG and negatively correlated with RNAss in 27 tumor types (Fig. 2F).

### Association of RGS5 expression with clinical features in human cancers

We analyzed the relationship between RGS5 expression and clinical features across various tumor types. We observed that RGS5 expression was significantly positively correlated with age in 6 tumor types, and negatively correlated with age in 6 tumor types (Fig. 3A). In gender analysis, significant differences in RGS5 expression were observed in KIRP and KIPAN (Fig. 3B). For the M stage, RGS5 expression differed significantly in LUAD, KIPAN, KIRC, and LUSC (Fig. 3C). As regards grade, Significant differences were found in ESCA, STES, KIPAN, HNSC, and KIRC (Fig. 3D). In terms of the N stage, RGS5 expression varied significantly in STES, KIRP, THCA, BLCA, and ACC (Fig. 3E). For the T stage, RGS5 expression showed significant differences in STEC, KIRP, KIPAN, PRAD, KIRC, and THCA (Fig. 3F).

### Association between RGS5 expression and gene variation in human cancers

Using the TCGA database, we investigated RGS5 gene variation across tumor types (Fig. 4A). According to the cBioPortal website, we found that RGS5 mutations, primarily amplification, were more common in breast cancer (Fig. 4B). The GSCALite website provided us with the methylation levels of RGS5 in several tumor kinds, and we found that 10 tumor types had variable expression, particularly kidney and lung cancer (Fig. 4C). Additionally, we investigated the frequency of SNV mutations of the *RGS5* gene in 16 different forms of cancer. The highest sample mutation frequency was found in UCEC patients (Fig. 4D). Furthermore, we examined the relationship between RGS5 and pan-cancer CNVs, including none, hete.amp, homo.amp, hete.del, and hete.del. The results suggested that the CNVs mutation of RGS5 was various in each type of cancer, and the main type of CNVs was hete.amp in most cancer types (Fig. 4E).

### Association between RGS5 expression with immune regulation, checkpoints, RNA modification, and drug sensitivity

We investigated the relationship between RGS5 expression and other immunoregulatory genes in various tumor types. In several tumor types, such as WT, ACC, and KIRC, RGS5 expression exhibited strong positive associations with immunoregulatory genes (Fig. 5A). Furthermore, in some tumor types, such as WT, ACC, and KIRC, RGS5 expression demonstrated strong positive relationships with immunoregulatory genes (Fig. 5B). We found that eight different types of immune infiltrating cells in various tumor types had a significant correlation with RGS5 expression. Notably, in a number of tumor forms, including KIRC, ESCA, KIPAN, and TGCT, RGS5 expression was highly connected with endothelial cells and CAF infiltration (Fig. 5C).

Our findings support the idea that RNA modification in a variety of tumor types may be influenced by RGS5 expression. In a variety of tumor types, RGS5

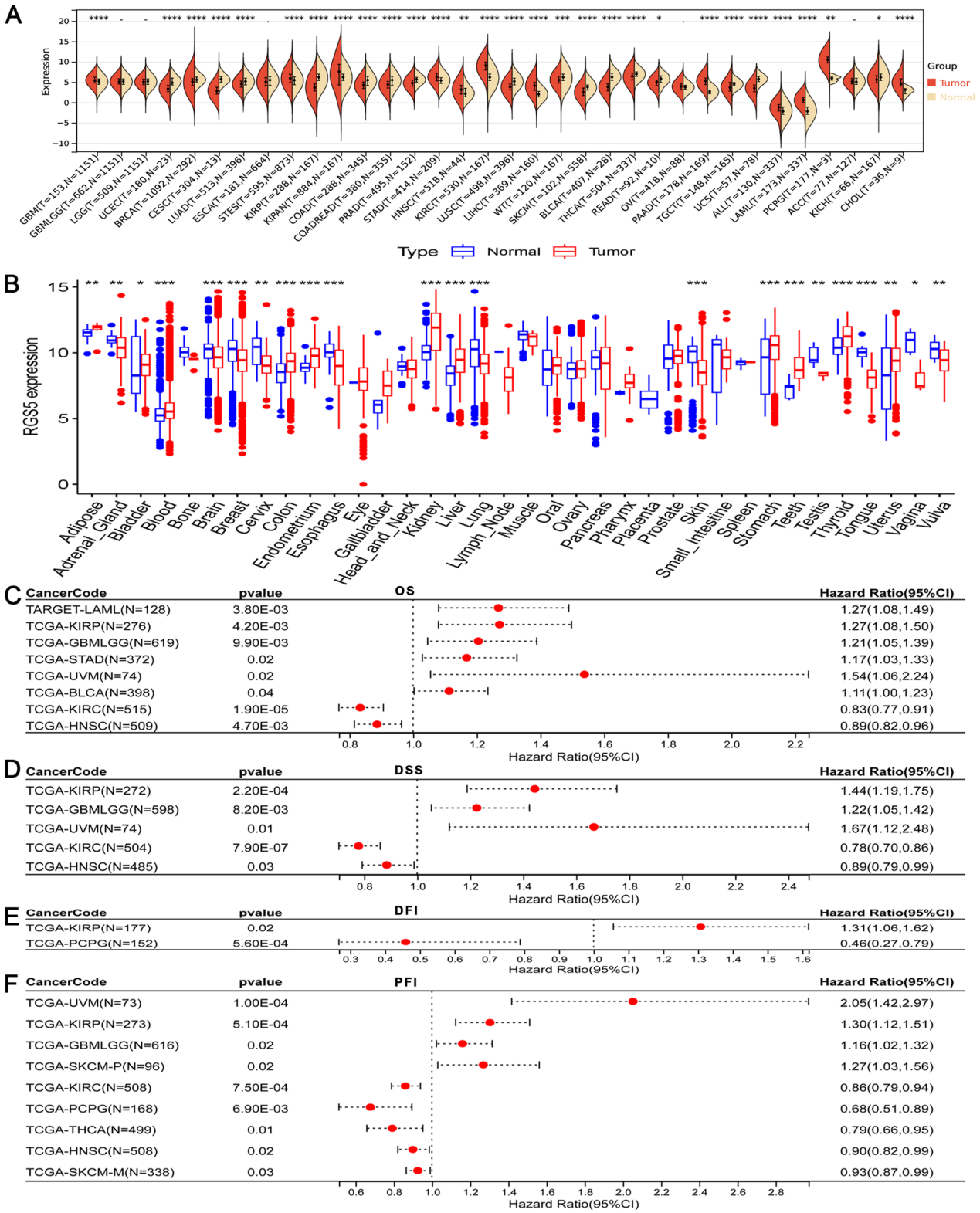


**Fig. 1** RGS5 expression and prognosis analysis in human cancers. **A** Pan-cancer analysis of RGS5 for differential expression between tumor and normal tissues in TCGA cohort. **B** Pan-cancer analysis of RGS5 for differential expression between tumor and normal tissues in GEO cohort. **C** Pan-cancer analysis of RGS5 for overall survival (OS). **D** Pan-cancer analysis of RGS5 for disease-specific survival (DSS). **E** Pan-cancer analysis of RGS5 for disease-free interval (DFI). **F** Pan-cancer analysis of RGS5 for progression-free interval (PFI). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001

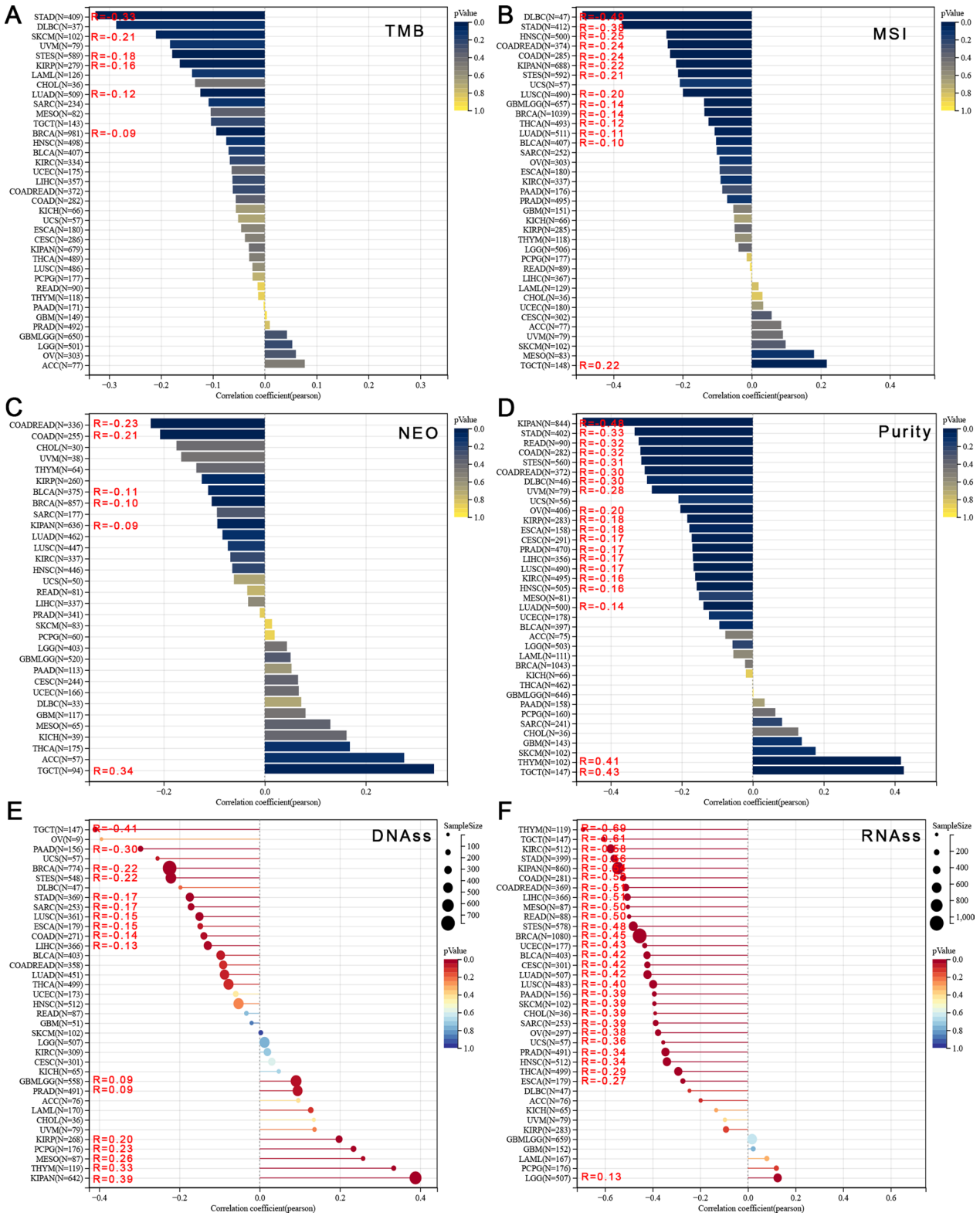


**Fig. 2** The correlation analysis of tumor heterogeneity and stemness in pan-cancer. **A** The correlation between tumor mutation burden (TMB) and RGS5 level. **B** The correlation between microsatellite instability (MSI) and RGS5 level. **C** The correlation between neoantigen (NEO) and RGS5 level. **D** The correlation between tumor purity and RGS5 level. **E** The correlation between DNAss and RGS5 level. **F** The correlation between RNAss and RGS5 level

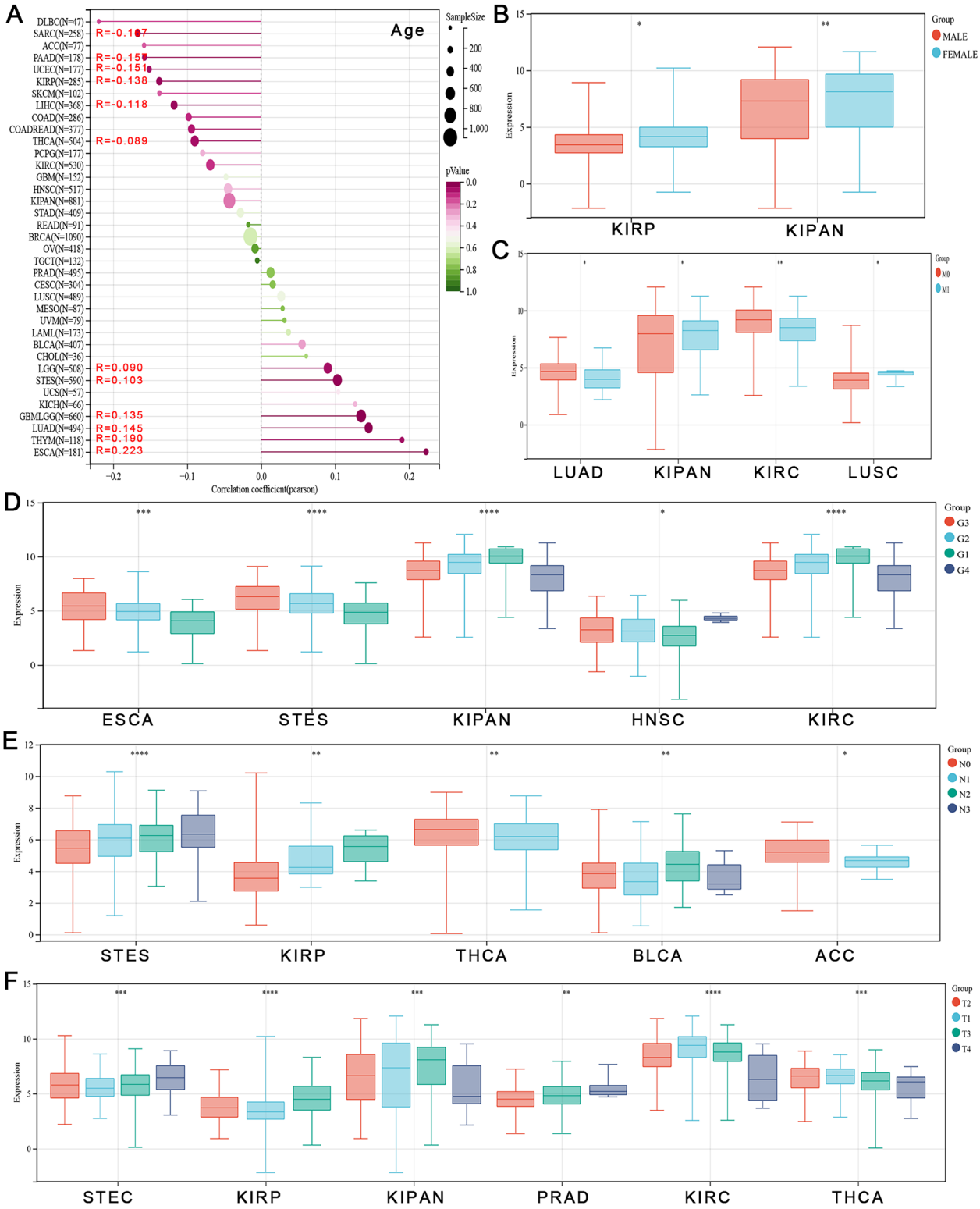


**Fig. 3** The correlation analysis of clinical features in pan-cancer. **A** The correlation between age and RGS5 level. **B** The correlation between gender and RGS5 level. **C** The correlation between M stage and RGS5 level. **D** The correlation between grade and RGS5 level. **E** The correlation between N stage and RGS5 level. (F) The correlation between T stage and RGS5 level. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001

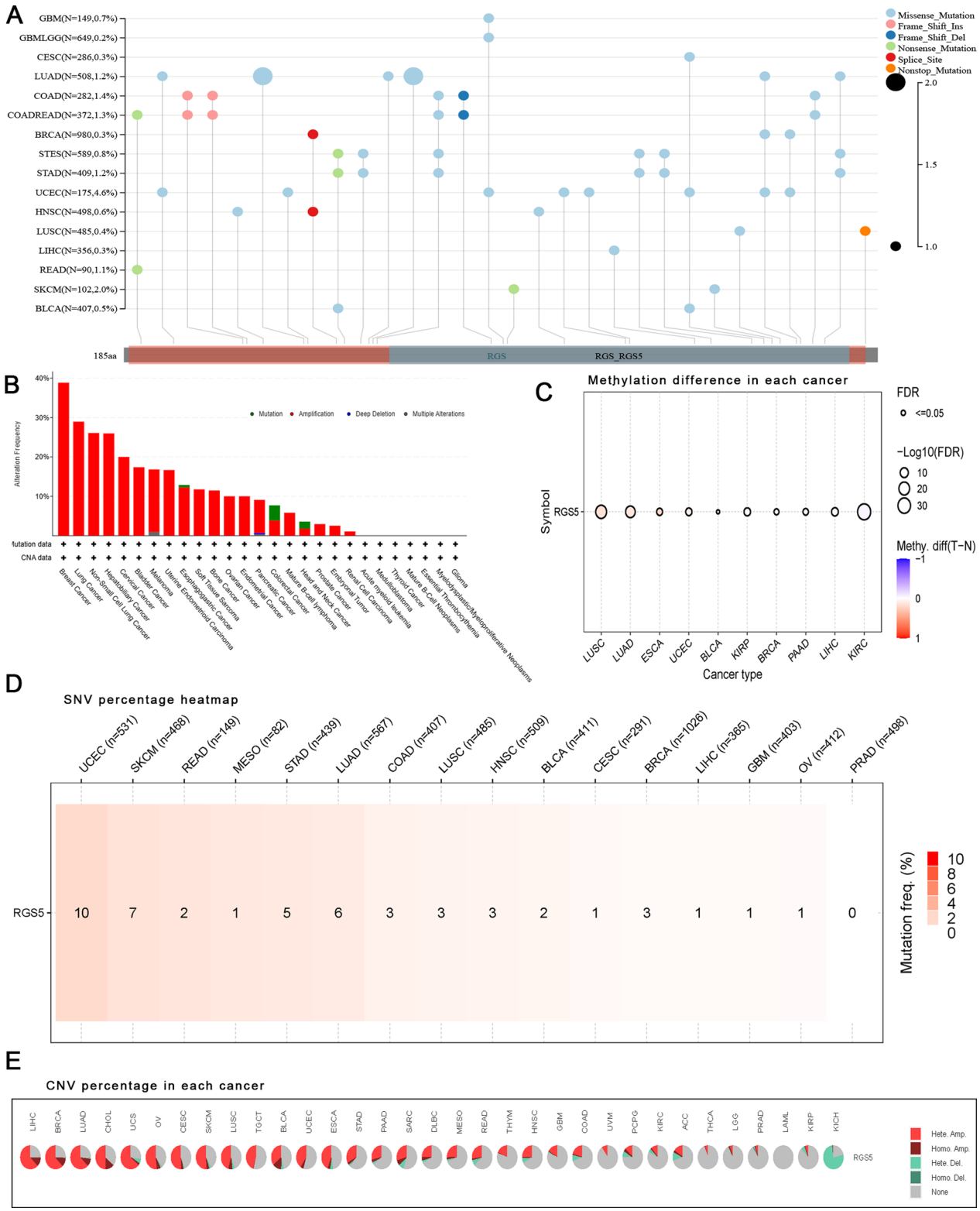


**Fig. 4** The analysis of mutation landscape in pan-cancer. **A** Mutation landscapes of RGS5 for pan-cancer. **B** Mutation frequency of RGS5 for pan-cancer. **C** Methylation difference of RGS5 for pan-cancer. **D** The SNVs mutation type of RGS5 for pan-cancer. **E** The CNVs mutation type of RGS5 for pan-cancer

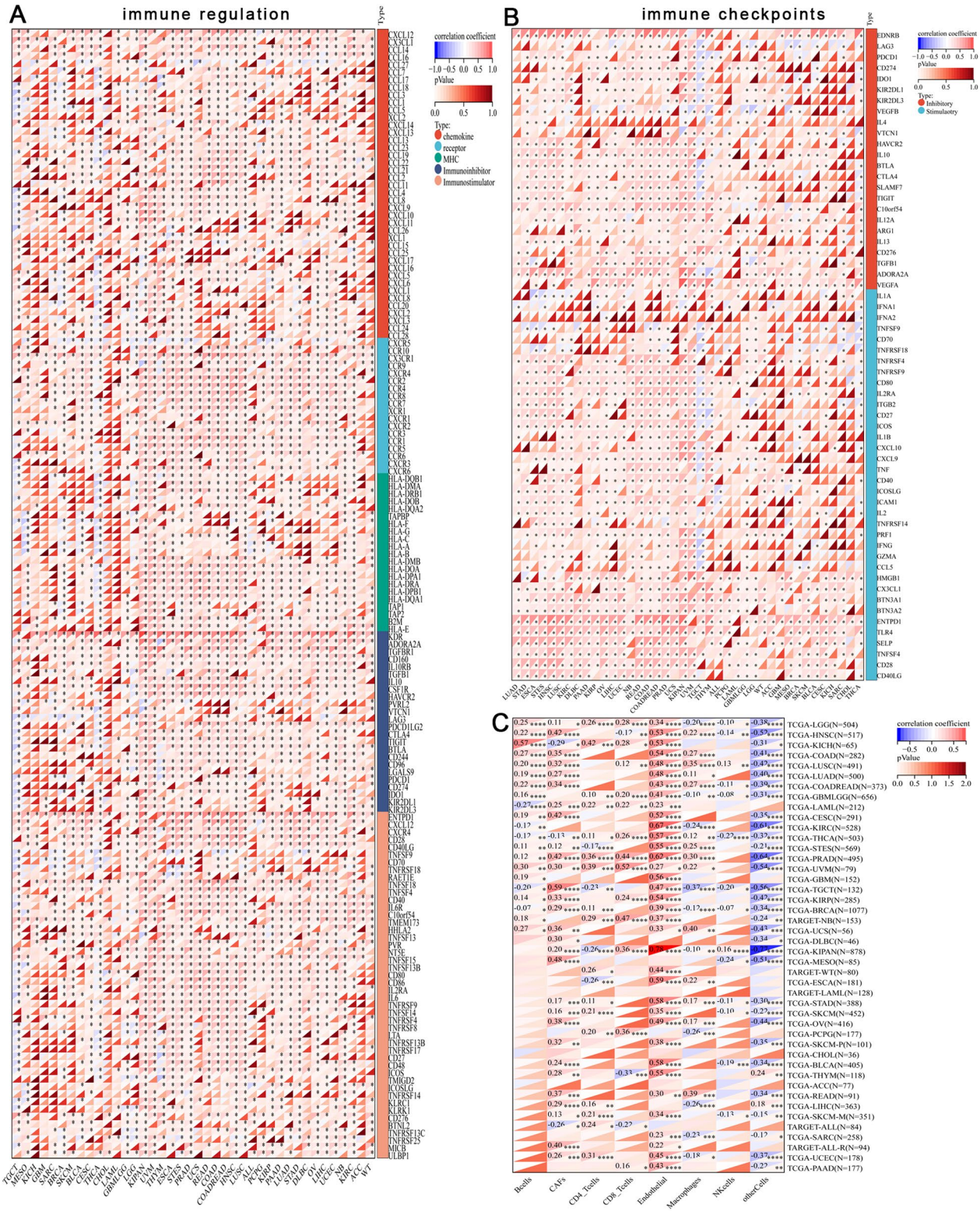


**Fig. 5** Immunological value analysis of RGS5 for pan-cancer. **A** The correlation of RGS5 expression with immune regulatory genes. **B** The correlation of RGS5 expression with immune checkpoint genes. **C** The correlation of RGS5 expression with immune infiltrating cells using TIMER algorithm. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001

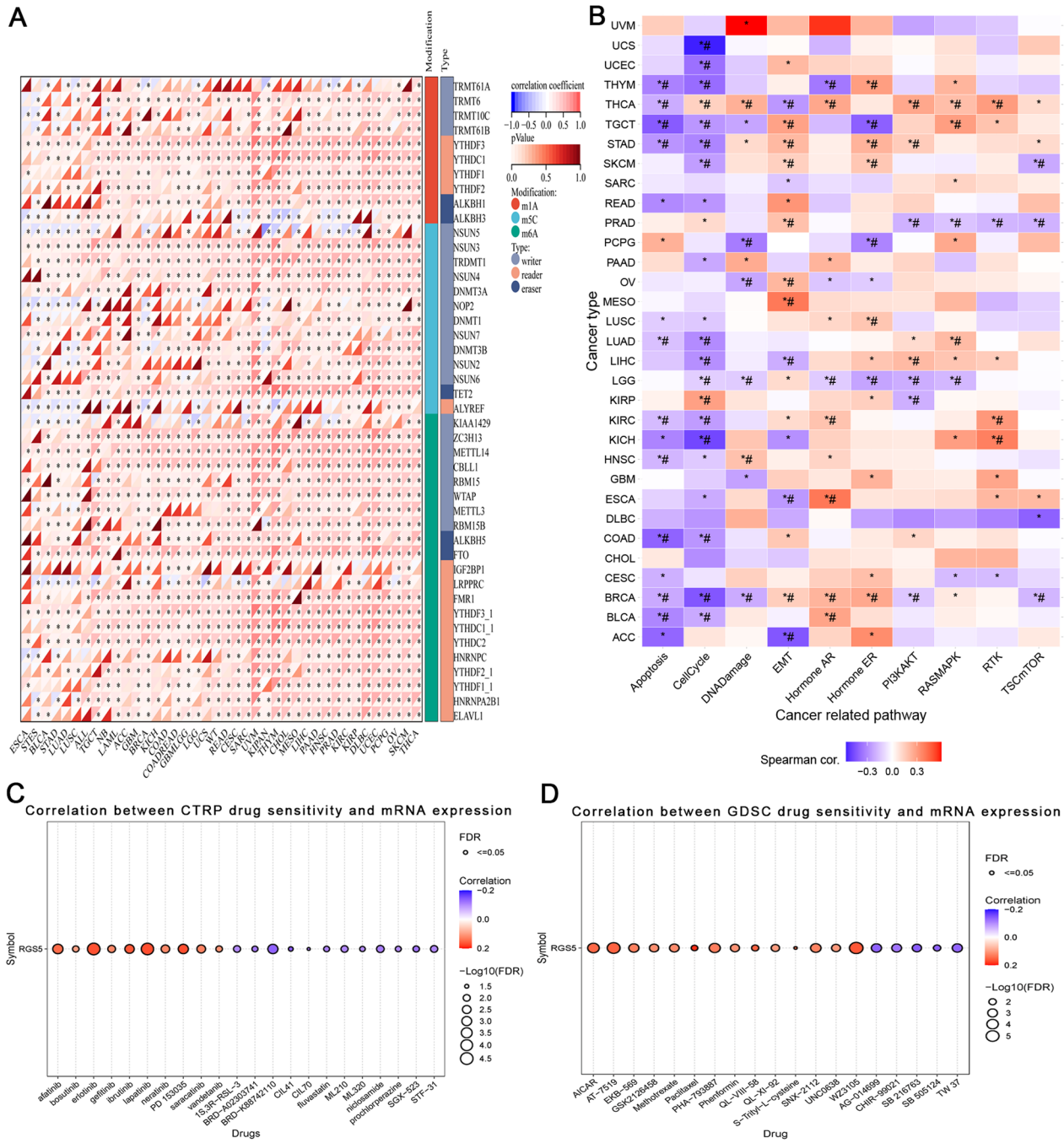


**Fig. 6** The correlation analysis of RGS5 expression and drug sensitivity analysis. **A** The correlation of RGS5 expression with genes of RNA modification. **B** The correlation of RGS5 expression with GSVA. **C** The correlation between gene expression and the sensitivity of GDSC drugs (top 10) in pan-cancer; **D** The correlation between gene expression and the sensitivity of CTRP drugs (top 10) in pan-cancer. *P value ≤ 0.05; #FDR ≤ 0.05

expression showed a favorable correlation with RNA modification genes (m1A, m5C, and m6A) (Fig. 6A). To investigate the biological role of RGS5 in various tumor types, we conducted GSVA analyses. These findings demonstrated that in several tumor species, RGS5 expression was substantially inversely connected with signaling pathways linked to the cell cycle and apoptosis (Fig. 6B). RGS5 expression was favorably connected with CTRP drug sensitivity to cancer medications such as gefitinib, bosutinib, and afatinib (Fig. 6C). Drugs such as AICAR, AT-7519, PHA-793887, and WZ3105

showed favorable relationships with GDSC drug sensitivity (Fig. 6D).

### Expression patterns and experimental verification of RGS5 in ccRCC

We focused on the expression pattern of RGS5 in patients with ccRCC because of previous advances in kidney cancer research. RGS5 was considerably more abundant in tumor tissues than in normal tissues in the TCGA-KIRC cohort (Fig. 7A, B). ROC curve analysis indicated high diagnostic accuracy for RGS5 in ccRCC patients (Fig. 7C). The majority of renal cancer cell lines have increased RGS5 expression, according to the CCLE database (Fig. 7D). High RGS5 expression was associated with favorable outcomes in ccRCC patients for OS, DSS, and PFI (Fig. 7E). We obtained kidney cancer tissue and nearby normal tissue for the IHC assay in order to confirm the aforementioned results. Our findings supported the increased RGS5 protein expression in tumor tissue based on the IHC score (Fig. 7F, G). RGS5 expression was found in several cell subsets, especially fibroblasts, according to an analysis of the TISCH database (Fig. 7H). Existing RGS5 and fibroblast marker (α-SMA) in ccRCC and adjacent tissues were evaluated by IF assay. Co-localization with fibroblast marker α-SMA confirmed cytoplasmic RGS5 expression in ccRCC tissues and its association with fibroblast distribution (Fig. 7I, J).

### Functional enrichment and experimental verification of RGS5 in ccRCC

We identified RGS5 and co-expressed proteins through the GeneMANIA website, where RGS5 was observed to have the strongest correlation with RGS18 (Fig. 8A). Differentially expressed genes based on median RGS5 expression were identified, with a heatmap highlighting the top 50 genes (Fig. 8B). These biological processes are significantly enriched, including nucleosome assembly, nucleosome organization, and protein-DNA complex assembly (Fig. 8C). These cellular components are significantly enriched, including nucleosome, immunoglobulin complex, and CENP-A containing nucleosome (Fig. 8C). These molecular functions were significantly enriched, including structural constituent of chromatin, protein heterodimerization activity, and snRNA binding (Fig. 8C). These signaling pathways are significantly enriched, including Neutrophil extracellular trap formation, Systemic lupus erythematosus, PI3K-Akt signaling pathway, p53 signaling pathway, and IL-17 signaling pathway (Fig. 8D). In addition, we noted that in the rich concentration of cell cycle and cell stroma-related signaling pathways, the groups with high or low expression of RGS5 had significant differences according to the GSVA results, such as olfactory transduction, homologous recombination, and cell adhesion molecules cams (Fig. 8E).

We evaluated RGS5 expression in renal carcinoma cells and tubular epithelial cells (HK2) using WB assays, finding that RGS5 was upregulated in 786-O, ACHN, and Caki-2 (Fig. 9A). RGS5 expression in 786-O and Caki-1 cells was knocked down using siRNA to examine the possible biological role of RGS5 in renal cancer cells. When compared to the siRNA negative control (si-NC), RNA interference dramatically decreased the expression of both RGS5 (Fig. 9B). Transwell experiments showed that the migration and invasion ability of 786-O and Caki-2 cells treated with si-1 or si-2 were weaker than those treated with si-NC (Fig. 9C). Comparing the si-1 or si-2-treated 786-O and Caki-1 cells to the si-NC group, the EdU assay revealed a decrease in EdU-positive cells (Fig. 9D).

## Discussion

Despite significant progress, cancer treatment continues to face numerous challenges, stemming not only from the complexity of tumors themselves but also from the limitations of therapeutic strategies, individual patient variability, and systemic issues within healthcare [43, 44]. Tumor heterogeneity drives the development of secondary drug resistance during treatment, particularly in targeted therapies and immunotherapy [45, 46]. Tumor cells can acquire resistance to drugs such as VEGF inhibitors and EGFR-targeting agents through mechanisms like gene mutations or pathway remodeling [47, 48]. Furthermore, although immune checkpoint inhibitors have revolutionized cancer treatment, their response rates remain

(See figure on next page.)

**Fig. 7** RGS5 expression and prognosis analysis in renal cancer cohort. **A** Unpaired and **B** paired RGS5 expression in KIRC were analyzed. **C** ROC analysis of ANLN expression in the diagnosis of patients with KIRC. **D** RGS5 expression in renal cancer cell line and normal cell line based on the CCLE database. **E** Prognostic analysis of RGS5 in KIRC. **F** Representative pictures of IHC staining of RGS5 in tumor and matched adjacent normal tissues. **G** IHC scores were obtained in 14 cases of renal carcinoma and adjacent tissues. **H** Single-cell analysis of RGS5 in different cell subpopulations was based on the TISCH database. **I** Representative images of RGS5 (red) and α-SMA (green) fluorescence staining in KIRC (three cases of tumor and adjacent tissue) under ×400 magnification. Blue indicates the nucleus. **J** Histochemistry score (H-Score) was obtained from 3 cases of renal carcinoma and adjacent tissues. $^{**}P < 0.01$; $^{***}P < 0.001$

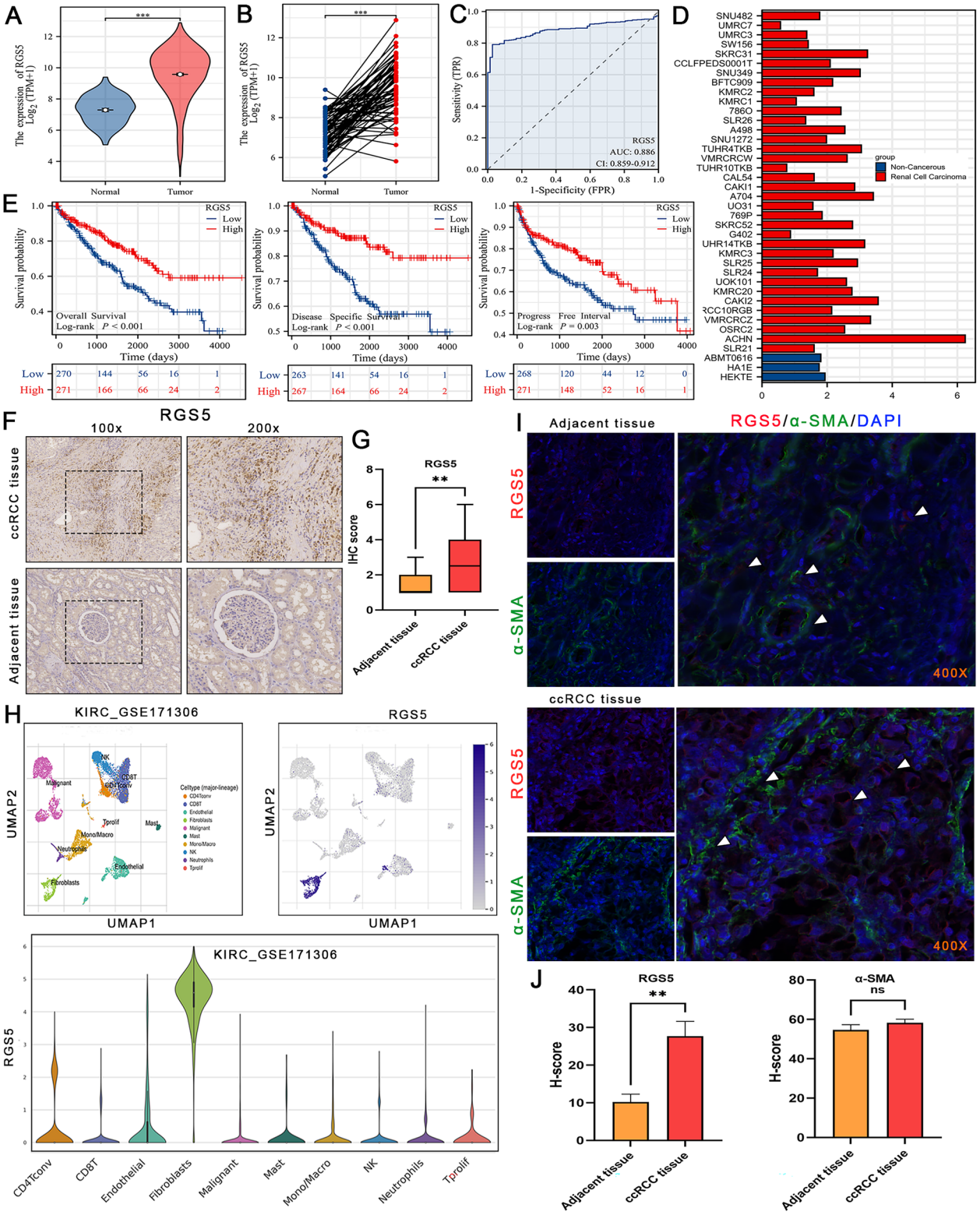


**Fig. 7** (See legend on previous page.)

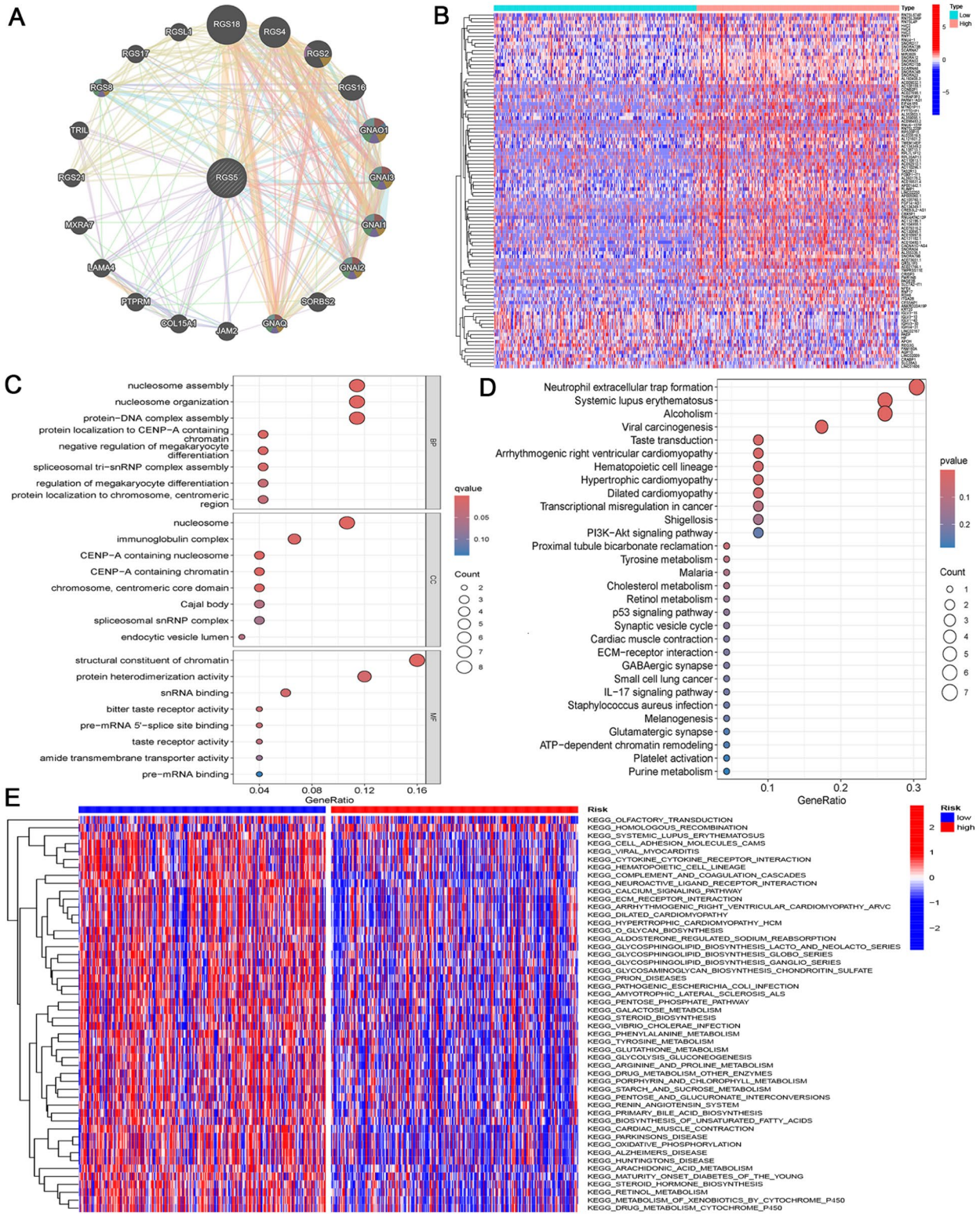


**Fig. 8** Functional enrichment analysis of RGS5 expression in KIRC. **A** The GeneMANIA website identifies RGS5 co-expressed genes. **B** Heatmap showing the top 50 up-regulated genes and top 50 down-regulated genes; red, up-regulated genes; blue, down-regulated genes. **C** GO analysis results of RGS5 expression in KIRC. **D** KEGG analysis results of RGS5 expression in KIRC. **E** GSVA results between RGS5-high and RGS5-low group

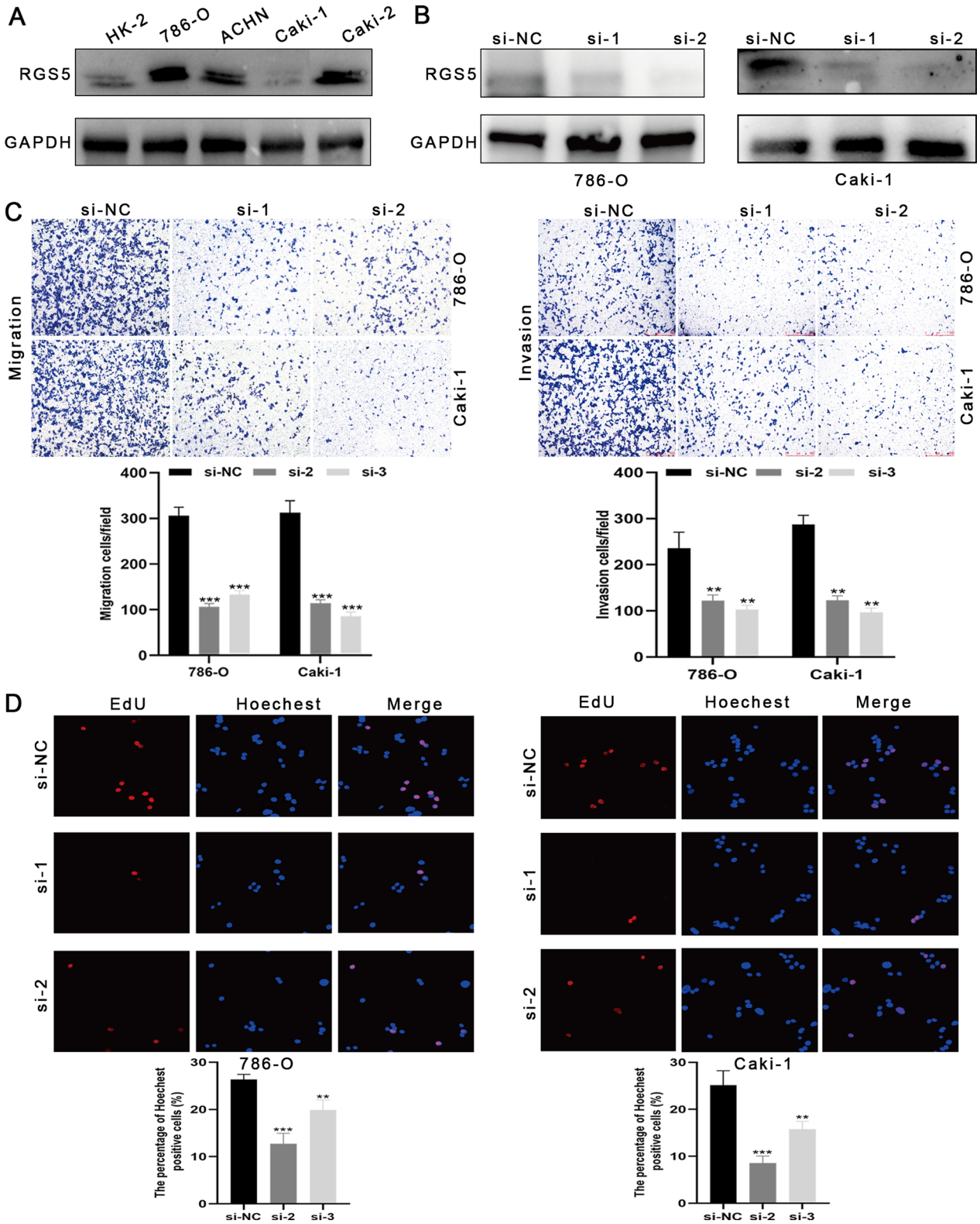


**Fig. 9** Effects of RGS5 silencing on the carcinogenesis of renal cancer cell lines. **A** Protein expression analysis of RGS5 in renal carcinoma cell lines and normal cells by WB assay. **B** RGS5 silencing by targeted siRNA was confirmed using WB assay. **C** Transwell migration and invasion assays for siRNA transfected renal cancer cell line. **D** EdU assay to confirm the proliferation effect of RGS5 expression in renal cancer cell lines. **P < 0.01; ***P < 0.001

low for certain tumor types and are often associated with immune-related adverse effects [49, 50]. Although there are many difficulties, with the advancement of technology and the deepening of multidisciplinary collaboration, the prospect of tumor therapy is still promising [51, 52].

Twenty years ago, a new marker for vascular smooth muscle cells and pericytes was discovered: RGS5, a member of the RGS family of GTPase-activating proteins for G proteins [53]. Beyond its role in cardiovascular disorders, researchers also explored the relationship between RGS5 and cancer [54]. Studies have reported abnormal RGS5 expression in several solid tumors, including gallbladder cancer [55], lung cancer [56], and liver cancers [17]. In our investigation, RGS5 was found to be highly expressed in 12 different cancer types, and IHC analysis confirmed this trend in kidney cancer tissues. Interestingly, while previous studies have shown high RGS5 expression in renal cancer, they also reported a significant decrease in RGS5 levels with increasing tumor grade [13]. This suggests that RGS5 plays a crucial role in the early stages of tumor development, where its loss contributes to pericellular maturation and vascular normalization, thereby reducing tumor hypoxia and angiogenesis [57]. The distribution of CAFs in renal cancer was found to be significantly correlated with RGS5 expression. Consistent with previous findings, RGS5 predominantly localizes to tumor endothelial cells or CAFs, as observed in pancreatic cancer [58], gallbladder cancer [55], cervical cancer [59], and breast cancer [60], while high expression of RGS5 in CAF is often associated with tumor metastasis.

High expression of RGS5 is linked to a better prognosis in non-small cell lung cancer, where it has been demonstrated to have a role in cancer differentiation and metastasis [56]. Our findings suggest that abnormal RGS5 expression, whether elevated or decreased, reflects tumor heterogeneity and disease progression. For instance, high RGS5 expression is linked to early tumor development, while decreased expression in certain high-grade or advanced tumors may indicate a poorer prognosis [13, 56]. Prognostic analysis reveals that high RGS5 expression is associated with favorable outcomes in renal cell carcinoma. Previous studies have also shown that renal cancer patients with higher RGS5 levels experience longer survival times, underscoring its potential prognostic impact [61]. Conversely, in some cancers, including KIPR, BLCA, UVM, LAML, GBM, and STAD, elevated RGS5 expression correlates with poorer outcomes. In these cases, increased RGS5 levels are often associated with aggressive tumor phenotypes characterized by poor cell differentiation and rapid growth [14, 17]. We found that the clinical outcomes of various malignancies may be positively or negatively impacted by RGS5 expression. Furthermore, RGS5 displayed distinct functional patterns in several cancer cell lines [17], which could be connected to the genomic instability and various molecular roles of RGS5 in various tumor types. These findings underscore the prognostic significance of RGS5 in assessing tumor outcomes and provide valuable insights into tumor progression and therapeutic optimization. However, further research is needed to elucidate its precise mechanisms of action and clinical implications across various tumor types.

TMB has become a useful tool for directing immunotherapy, particularly anti-PD-1 and anti-CTLA4 treatments, in the age of precision medicine. Studies have indicated that TMB can function as a biomarker to improve immunotherapy's effectiveness in treating a variety of malignancies, including lung and breast tumors [12–14]. Additionally, in patients with previously treated recurrent or metastatic advanced solid tumors, TMB has demonstrated promise as a prognostic biomarker for response to pembrolizumab monotherapy [15]. RGS5 is mainly linked to the control of the tumor microenvironment and tumor angiogenesis [14]. The hypoxic and immunosuppressive conditions brought on by increased RGS5 expression may theoretically affect the accumulation of mutations in tumor cells, even though there is no concrete evidence connecting RGS5 expression to TMB. Another genetic biomarker for determining which individuals will benefit from immune checkpoint drugs is MSI [16]. High-frequency MSI is an independent predictor of clinical outcomes in endometrial cancer [17]. There is currently no concrete evidence that RGS5 expression and tumor MSI status are related. Nonetheless, it is conceivable that RGS5 indirectly affects the immune response in MSI tumors given the correlation between MSI and elevated neoantigen burden and high mutation rates, as well as RGS5's impact on the tumor immunological landscape. Our research showed that RGS5 expression had a positive correlation with MSI in TGCT but a negative correlation with TMB in six cancer types and MSI in thirteen cancer types.

The trafficking and activities of B and T lymphocytes are disrupted by aberrant production of RGS molecules, which can lead to allergic reactions, lymphoid malignancies, and autoimmune disorders [62, 63]. It has been demonstrated that targeting tumor-specific T cells' regulator of G protein signaling 1 (RGS1) increases the cells' trafficking to breast cancer [64]. Public databases like the TCGA project are constantly evolving and improving, making it possible to discover new immunotherapy targets and assess their relationship to patient outcomes [65]. RGS5 expression is particularly associated with the formation and maintenance of an immunosuppressive environment. Upregulation of RGS5 in tumor vasculature has been observed to promote abnormal angiogenesis,

leading to a hypoxic and immunosuppressive microenvironment [57]. Overall, the RGS family plays a critical role in human immune regulation. Previous research has demonstrated that RGS5 expression and activity in neutrophils and cytotoxic T lymphocytes (CTLs) significantly influence their functions within the complex microenvironment of cancerous or inflamed tissues [66]. These findings suggest that RGS5 expression is closely linked to immune infiltration in tumors, impacts patient prognosis, and identifies potential new targets for immunosuppressant development.

As a useful molecular marker for determining tumor aggressiveness and patient prognosis, we found that increased RGS5 expression is associated with poor clinical outcomes in a variety of tumor types [3]. This insight can aid in guiding individualized treatment strategies. Furthermore, RGS5 may indirectly influence tumor progression by participating in tumor cell senescence pathways, such as DNA damage responses and cell cycle arrest, highlighting its potential research significance in the context of tumor senescence [17, 67]. Although publically accessible datasets support these conclusions, additional in vitro and in vivo research is required to confirm these findings and clarify the underlying mechanisms.

## Conclusion

Aberrant RGS5 expression is frequently linked to clinical outcomes in a range of tumor types, which may establish it as a potential indicator of tumor aggressiveness and patient prognosis. According to the current findings, RGS5 has a significant influence on the immune microenvironment by fostering immunosuppressive circumstances, underscoring its enormous potential as a tumor immunotherapy target.

### Abbreviations

| | |
|---|---|
| GPCR | G protein-coupled receptor |
| scRNA | Single-cell RNA |
| GAPs | GTPase-accelerating proteins |
| RGS | Regulators of G protein signaling |
| TCGA | The Cancer Genome Atlas |
| CAFs | Cancer associated fibroblasts |
| TME | Tumor microenvironment |
| OS | Overall survival |
| DSS | Disease-specific survival |
| DFI | Disease-free interval |
| PFI | Progression-free interval |
| CCLE | Cancer Cell Line Encyclopedia |
| TMB | Tumor mutation burden |
| MSI | Microsatellite instability |
| NEO | Neoantigen |
| GDSC | Drug Sensitivity in Cancer |
| CTRP | Cancer Therapeutics Response Portal |
| IHC | Immunohistochemistry |
| IF | Immunofluorescence |
| ACC | Adrenocortical carcinoma |
| BLCA | Bladder Urothelial Carcinoma |
| BRCA | Breast invasive carcinoma |
| CESC | Cervical squamous cell carcinoma and endocervical adenocarcinoma |
| CHOL | Cholangiocarcinoma |
| COAD | Colon adenocarcinoma |
| COADREAD | Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma |
| DLBC | Lymphoid Neoplasm Diffuse Large B-cell Lymphoma |
| ESCA | Esophageal carcinoma |
| FPPP | FFPE Pilot Phase II |
| GBM | Glioblastoma multiforme |
| GBMLGG | Glioma |
| HNSC | Head and Neck squamous cell carcinoma |
| KICH | Kidney Chromophobe |
| KIPAN | Pan-kidney cohort (KICH + KIRC + KIRP) |
| KIRC | Kidney renal clear cell carcinoma |
| KIRP | Kidney renal papillary cell carcinoma |
| LAML | Acute Myeloid Leukemia |
| LGG | Brain Lower Grade Glioma |
| LIHC | Liver hepatocellular carcinoma |
| LUAD | Lung adenocarcinoma |
| LUSC | Lung squamous cell carcinoma |
| MESO | Mesothelioma |
| OV | Ovarian serous cystadenocarcinoma |
| PAAD | Pancreatic adenocarcinoma |
| PCPG | Pheochromocytoma and Paraganglioma |
| PRAD | Prostate adenocarcinoma |
| READ | Rectum adenocarcinoma |
| SARC | Sarcoma |
| STAD | Stomach adenocarcinoma |
| SKCM | Skin Cutaneous Melanoma |
| STES | Stomach and Esophageal carcinoma |
| TGCT | Testicular Germ Cell Tumors |
| THCA | Thyroid carcinoma |
| THYM | Thymoma |
| UCEC | Uterine Corpus Endometrial Carcinoma |
| UCS | Uterine Carcinosarcoma |
| UVM | Uveal Melanoma |
| OS | Osteosarcoma |
| ALL | Acute Lymphoblastic Leukemia |
| NB | Neuroblastoma |
| WT | High-Risk Wilms Tumor |

## Supplementary Information

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Supplementary Material 1.

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### Author contributions

YZ, HW, FD and KH carried out the interpretation of data and drafted the manuscript. YZ collected the clinical samples. Literature research was conducted by ZT. JW, LB and XC conceived and designed the study. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent for participate

The study was carried out in accordance with the Declaration of Helsinki and was reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Anhui Medical University (No. YX2023-219). After receiving the patients' written informed agreement, we collected tissue samples.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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## Publisher's Note