Journal of Oncology Research and Therapy
Yi C, et al. Oncol Res Ther 8: 10168.
www.doi.org/10.29011/2574-710X.10168
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Research Article

# Depression-related RGS2 Gene Serves as a Novel Molecular Biomarker of Stomach Adenocarcinoma Prognosis and Immune Infiltration

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**Citation:** Yi C, Yang J, Hong J, Zhang T, Qin L, et al. (2023) Depression-related RGS2 Gene Serves as a Novel Molecular Biomarker of Stomach Adenocarcinoma Prognosis and Immune Infiltration. J Oncol Res Ther 8: 10168. DOI: 10.29011/2574-710X.10168

**Received Date:** 25 April, 2023; **Accepted Date:** 05 May, 2023; **Published Date:** 08 May, 2023

## Abstract

**Background:** The occurrence of depression in stomach adenocarcinoma (STAD) may indirectly affect the survival rate of patients, but the specific mechanism of depression in STAD patients was still unclear, so it is of great significance to study the depression-related genes for the diagnosis and prognosis of STAD. G protein signaling regulator 2 (RGS2), a depression-related gene, can inhibit the invasion and migration of tumor cells by consuming itself. Therefore, RGS2 may be a potential target for the treatment of STAD. This study aims to explore the relationship between depression and STAD patients and whether there is a poor prognostic effect in patients with both depression and STAD by clarifying the value of RGS2 in the diagnosis and prognosis of STAD and analyzing its immune infiltration and other related factors.

**Methods:** Firstly, the pan-cancer analysis of RGS2 was performed in the TIMER database. Then, the expression and prognostic value of RGS2 in STAD were analyzed by The Cancer Genome Atlas (TCGA) database and The Gene Expression Omnibus (GEO) database. The obtained expression data and clinical data were analyzed by univariate and multivariate COX analysis, as well as the immune infiltration of RGS2 was analyzed based on the obtained immune cell data set. STRING database, GeneMANIA database and Gene Expression Profiling Interactive Analysis (GEPIA) database was combined to analyze and construct PPI networks, enrichment analysis was conducted based on Gene Ontology (GO), Gene Set Enrichment Analysis (GSEA) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Finally, the mutation of RGS2 was analyzed by cBioPortal database.

**Results:** The expression of RGS2 was significantly low in 17 cancers, and this low expression was closely related to the RGS2 depletion to inhibit the invasion and metastasis of tumor cells. And the STAD patients with higher RGS2 expression had a poor prognosis. The expression of RGS2 could accurately identify tumors in normal tissues (AUC = 0.816). The ROC values of 1-year, 3-year and 5-year survival rates were above 0.5, especially the ROC value of 1-year survival rate was 0.65. RGS2 was negatively related to immune cell infiltration and immune checkpoints (memory activated CD4 T cells and regulatory T cells) in STAD. The KEGG enrichment analysis showed that RGS2 played an important regulatory role in DNA transcription and cGMP-PKG pathway, and co-regulated cGMP-PKG pathway with PRKG1. In addition, the RGS2 genetic alteration rate was 2.5%.

**Conclusion:** We validated the value of RGS2 in the diagnosis and prognosis of STAD, and the patients with STAD were more likely to suffer from depression. It was also found that if STAD patients have depression, it will lead to a poorer prognosis for STAD patients.

**Keywords:** Stomach adenocarcinoma; TCGA; RGS2; Immune correlation; Depression

## Introduction

There were more than 1 million new cases of STAD and about 769,000 deaths in 2020. Its incidence rate ranked among the top 5 and its mortality ranked among the top 4 in the world [1]. The clinical detection for STAD is histological diagnosis after endoscopic biopsy, due to the highly heterogeneous molecular phenotype of STAD [2], patients with STAD often being found at an advanced stage, which is one of the reasons for the high death rate of STAD [3]. Therefore, it is particularly important to explore new molecular biomarkers and construct prognostic models.

In addition, previous studies have found that depression and higher levels of depression will increase cancer mortality [4]. Statistics show that the currently available antidepressants only have a certain effect on about 50% of patients with depression [5], as well as the risk of depression in the cancer patients was higher than in the general [6], which would lead to a lower willingness for treatment in STAD patients. The occurrence of depression made STAD more intractable. Therefore, studying the genes associated with depression for diagnosis and prognosis of STAD can greatly alleviate and improve the patient's emotional willingness for treatment, which is helpful to improve their survival rates.

RGS2, related to the depression [7], is mainly used to regulate the G protein-coupled receptor signaling cascade response. Many studies have confirmed the value of this gene. Nguyen et al. found that this gene binds to EIF2B5 and blocks its activity, thereby inhibiting the transcription of mRNA into proteins [8]. Subsequently, Phan et al. found that RGS2 is involved in the negative regulation of angiotensin-activated signaling pathway [9]. Furthermore, considering that this gene has shown good diagnostic and prognostic value in other diseases, which associated with the nervous [10], the heart [11], the blood vessels [12], we wondered whether this gene also played a relevant role in STAD.

The aim of this paper was to analyze the expression of RGS2 in STAD through TCGA and GEO databases, and to explore the survival and prognostic value of this gene combined with clinical information. Meanwhile, relevant immune infiltration analysis and enrichment analysis were performed to understand the changes in the immune microenvironment of STAD patients and the key pathways affected by this gene. Finally, related genes were obtained through the construction of PPI network, and the mutation of RGS2 in STAD patients was also analyzed. Figure 1 shows the complete analysis process of this study.

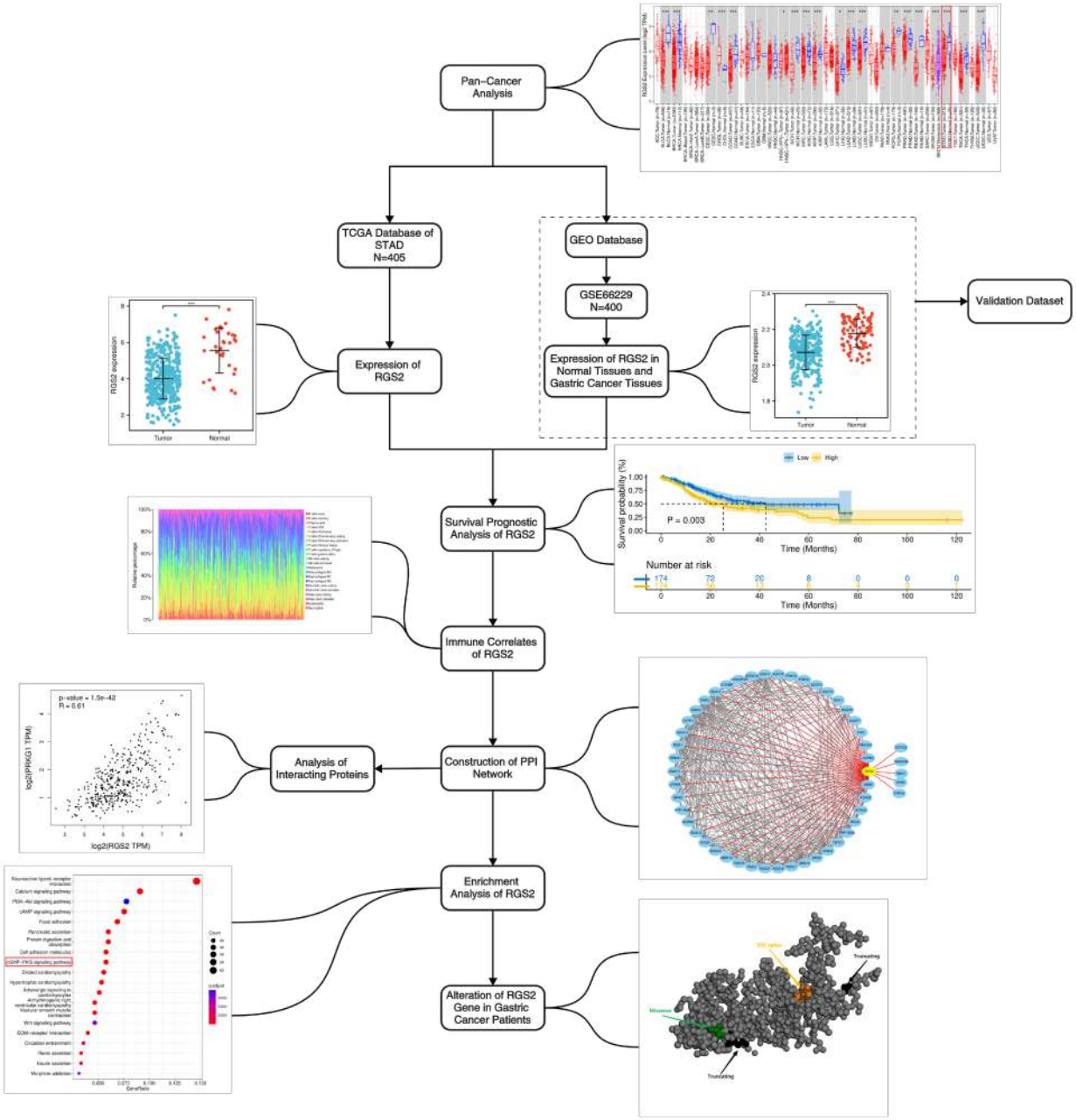


**Figure 1:** Specific analysis process of this study.

## Methods

### Data Acquisition

TCGA data were accessed using the UCSC Xena Browser (https://xenabrowser.net). Transcriptome profiling data and clinical data were obtained from the TCGA database. Transcriptome profiling data contained 373 tumor tissue samples and 32 normal tissue samples. GSE66229 dataset was obtained from the GEO database (Home - GEO - NCBI (nih.gov)) for validation. It contained that the GSE66229 microarray was Affymetrix Human Genome U133 Plus 2.0 Array. The microarray data was generated on the GPL570 platform. The GEOquary package was used to obtain the expression matrix and the hgu133plus2.db package was used for gene annotation.

### Differentially Expressed Genes (DEGs) Screening

DEGs between tumor and normal types were obtained by using the R package DEGeq2 [13] . Samples were grouped based on high and low RGS2 expression with the median expression of RGS2 as the threshold. DEGs between high and low RGS2 expression groups were obtained. For the GSE66229 dataset, the R package limma was used for data correction and DEGs screening. |log2FoldChang|>1 and padj<0.05 were used as the cutoff threshold.

### Analysis of RGS2 Expression Level.

Pan-cancer analysis was performed through the TIMER database(timer.comp-genomics.org). Univariate and multifactorial cox regression analysis was performed on the obtained RGS2 expression data and clinical data in STAD by using the R packages survival and survminer.

### Survival Analysis.

We used the median expression of RGS2 in 348 STAD patients as the threshold to divide the data into high expression group and low expression group, and the OS survival curve was plotted by survival package and survminer package. The Time-Dependent ROC was analysis of by using the R package time ROC.

### Immune Infiltration Analysis

The immune cell data "LM22" [14] and integrated with the expression data of STAD patients, and the data was also integrated with "parallel package", "e1071 package", "preprocessCore package" and CIBERSORT algorithm [15]. Then, the integrated data was computed 1000 times, and finally, the computed data was used to cluster the immune cells.

The above data was integrated with the expression data of RGS2 in tumor tissue through R language, and the median expression of RGS2 in STAD patients was used as the threshold to divide the obtained data into high and low expression groups, and used the "ggsci package", "tidyr package package", "ggpubr package" to draw cibersort boxplots, while importing the cellMarker dataset and integrating it with the expression data of STAD patients through "data.table package" with "GSVA package" [16], finally, ssGSEA boxplots was drawn by the above R package. At the same time, the survival data was integrated with the above calculated results through R language, and then integrated with the expression data of STAD patients, then divided it into high and low expression groups by using the median of the target immune cell data as the threshold, and the immune cell survival curves with significant differences in cibersort were plotted by the "survival package". The immune cell survival curves with significant differences in cibersort were plotted using the "survival package". And the TIMER database was used to analyze the tumor purity and immune infiltration of STAD.

### Construction of PPI Network

The corresponding PPI networks were constructed by STRING database (https://cn.string-db.org), GEPIA database (GEPIa.cancer-PKU.cn) and GENEMANIA database (Genemania.org) respectively. In the STRING database, the medium confidence value was 0.4, and the intersection gene of the three database -- PRKG1(PKG) was obtained. The correlation between RGS2 and PRKG1 was analyzed through GEPIA database.

### Enrichment Analysis

We used the DEGs between high and low RGS2 expression groups to perform GO enrichment , KEGG enrichment(www.kegg, JP) and GSEA enrichment by using the R package clusterProfiler [17]. Finally, the cGMP-PKG SIGNALING PATHWAY plot was obtained by analyzing RGS2 by the KEGG database.

### Mutations in the RGS2 Gene in STAD Patients

Mutations status of RGS2 in a total of 1,328 STAD patients from six datasets were analyzed through the cBioPortal for Cancer Genomics database(www.cbioportal.org) - including OncoSG, 2018; TMUCIH, PNAS 2015; TCGA, Firehose Legacy; Pfizer and UHK, Nat Genet 2014; U Tokyo, Nat Genet 2011 and UHK, Nat Genet 2011.

### Statistical Analysis.

R (v.4.2.0) was used for statistical analysis. Differences between groups were compared by Wilcoxon rank sum test, One-way ANOV and T test. Correlations were determined by Spearman correlation test. Kaplan-Meier plots were created and log-rank tests were performed to determine the significance of the difference between survival curves, where P<0.05 was statistically significant. Cytoscape software was used to beautify the PPI network that had been gotten.

## Results

### Expression of RGS2 mRNA in a Variety of Tumor Tissues.

To explore the effect of RGS2 on tumor cells, this study first analyzed its expression in 33 human cancers. As shown in Figure 2, RGS2 was significantly under expressed in 16 cancer types compared to corresponding normal tissues(P<0.05), and significantly overexpressed in cholangiocarcinoma (CHOL) (P<0.05).

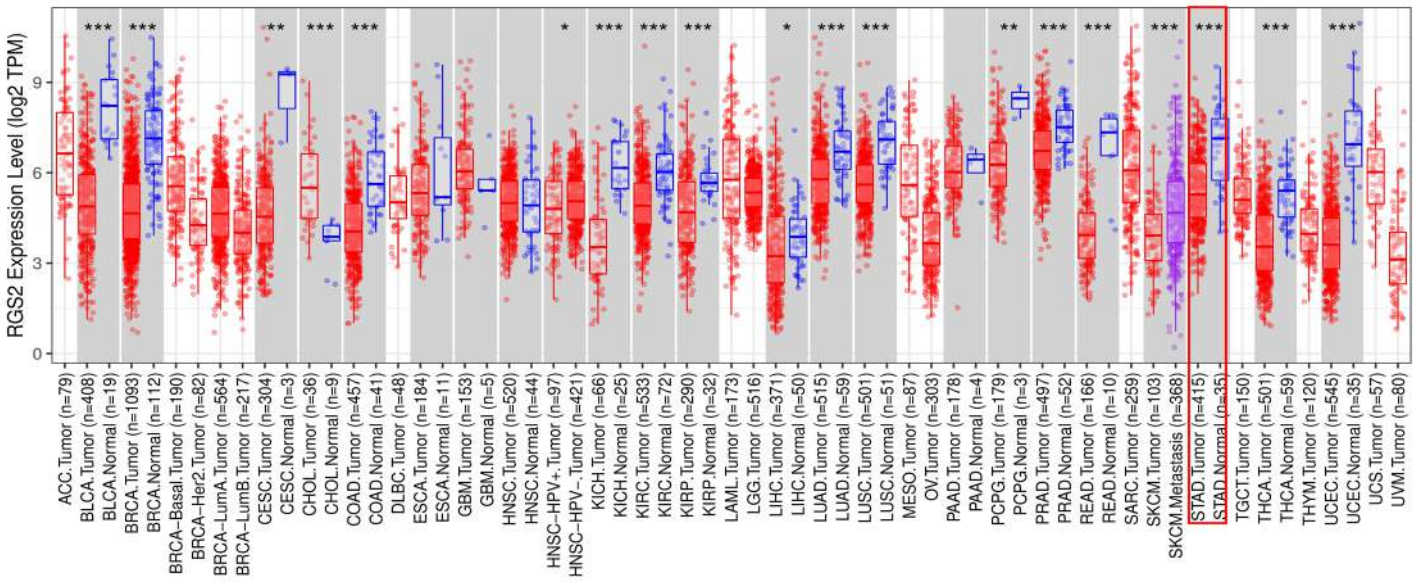


**Figure 2:** The expression of RGS2 in pan-cancer, * P<0.05, ** P<0.01, *** P<0.001.

### The Expression Level of RGS2 in STAD Tissues is Lower than that in Noncancerous Tissues

In order to further study the effect of RGS2 expression in STAD patients, the expression of RGS2 in STAD patients was firstly analyzed by TCGA and GEO database. Under expression of RGS2 was observed in a comparative study of normal and STAD tumor tissues based on the TCGA dataset (P<0.001) (Figure 3A), and it was also observed in GSE66229 dataset (P<0.001) (Figure 3C). The expression level of RGS2 can accurately identify STAD tumor and normal tissue (AUC = 0.816) (Figure 3B).

DEGs analysis was performed based on the TCGA data, including 2133 up-regulated genes and 2349 down-regulated genes. And RGS2 belonged to the low expression group in STAD tissue cells (Figure 4).

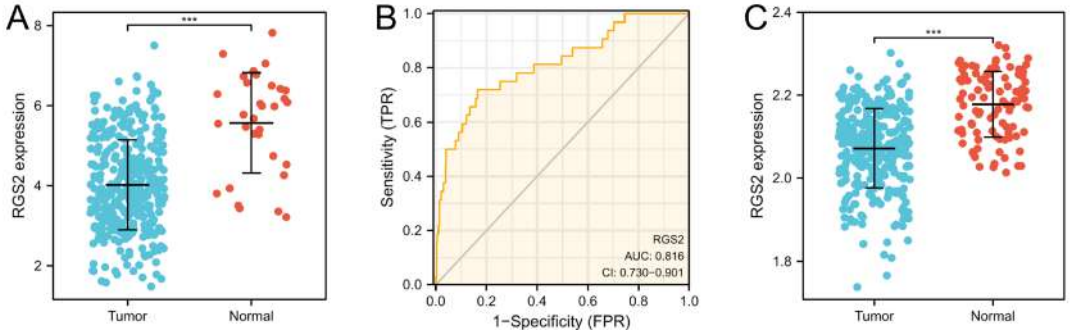


**Figure 3:** Expression of RGS2 in STAD. **(A)** RGS2 expression in normal versus tumor tissues-TCGA. **(B)** ROC curves of RGS2 in STAD tumor tissues versus normal tissues. **(C)** RGS2 expression in normal versus tumor tissues-GSE66229. * P<0.05, ** P<0.01, *** P<0.001.

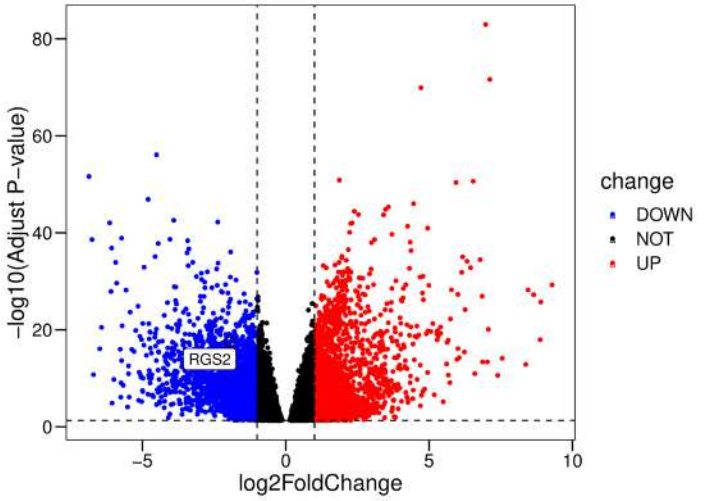


**Figure 4:** The volcano map of difference analysis.

**Survival Prognosis of RGS2 in STAD.**

To understand whether RGS2 has a survival prognostic value for STAD patients, the survival analysis was performed. Combining univariate and multivariate cox regression analysis, it can be seen that the expression of RGS2 in STAD patients was an independent prognostic factor (Supplementary Table S1).

To further understand the effect of RGS2 expression on the survival and prognosis of STAD patients, the survival curve and ROC curve were drawn. According to the Kaplan-Meier survival curve, STAD cases with lower RGS2 expression showed higher OS ($P<0.01$) (Figure 5A). Time-dependent survival ROC curves for RGS2 to predict 1-, 3-, and 5-year survival rates were created by the clinical survival data. All of these AUC values were above 0.55, this situation was considered suitable for forecasting, especially forecasts within 1 year had an AUC value of 0.65 (Figure 5B, 5C, 5D).

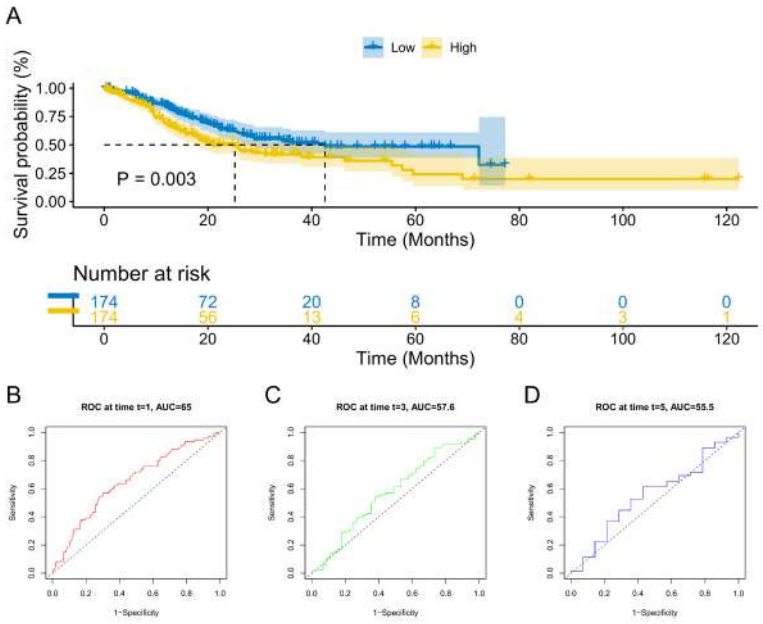


**Figure 5:** Survival prognosis of RGS2. **(A)** Survival analysis-OS. **(B)** ROC curve-1 year. **(C)** ROC curve-3 years. **(D)** ROC curve-5 years.

### Expression of RGS2 in Different Clinical States.

Clinical features were important for the diagnosis and treatment of STAD. Table S2 has showed the relevant clinical data of patients with STAD and the result of the T test has also been showed. The expression of RGS2 was significantly different between normal tissues and different age (Figure 6A), histological grade (Figure 6B), pathology M (Figure 6C), pathology N (Figure 6D), pathology T (Figure 6E), gender (Figure 6F) and tumor stage (Figure 6G) tumor tissues of STAD patients (P<0.05).

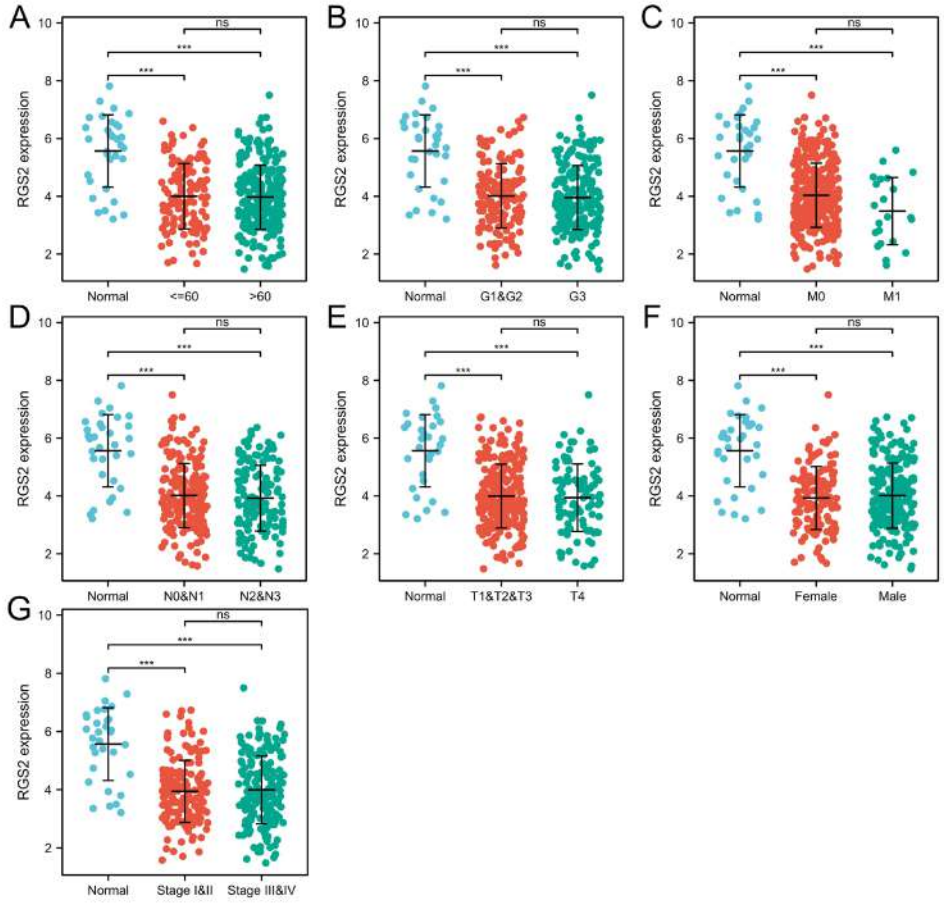


**Figure 6:** The relationship between RGS2 expression and clinical features. **(A)** Age. **(B)** Histological grade. **(C)** Pathology M. **(D)** Pathology N. **(E)** Pathology T. **(F)** Gender. **(G)** Tumor stage (red: Stage I & II, green: Stage III & IV). ns: not significant, * P<0.05, ** P<0.01, *** P<0.001.

### The Relationship Between the RGS2 and Immune Cell Infiltration in STAD

To explore the relationship between immune infiltration and RGS2 expression, the relationship between the under-expression of RGS2 and immune infiltration were analyzed. The relative percentage of 22 immune cells in STAD tumor tissue was analyzed (Figure 7). And the results derived from the cibersort algorithm showed that the RGS2 expression was significantly different (P<0.05) (Figure 8A), and the proportion of activated memory CD4 T cells, follicular helper T cells, regulatory T cells and M0 Macrophages was lower in STAD tumor tissue when RGS2 was under expression. When RGS2 was highly expressed, M2 Macrophages, resting Dendritic cells and resting Mast cells accounted for a higher proportion in STAD tumor tissue. But survival analysis of them revealed that none of them significant prognostic significance (P>0.05) (Figure S1), but these survival analysis results still had other value, such as activated memory CD4 T cells and regulatory T cells, their survival time was shorter in the under expression of RGS2 patients compared to the high ones.

The results derived from the ssGSEA algorithm showed that the expression of RGS2 was significantly different in activated B cell, activated dendritic cell, CD56bright natural killer cell, CD56dim natural killer cell, central memory CD8 T cell, effector memeory CD4 T cell, effector memory CD8 T cell, eosinophil, gamma delta T cell, immature B cell, immature dendritic cell, macrophage, mast cell, MDSC, monocyte, natural killer cell, natural killer T cell, neutrophil, plasmacytoid dendritic cell, regulatory T cell, T follicular helper cell, type 1 T helper cell and type 2 T helper cell ($P<0.05$) (Figure 8B). Among them, activated dendritic cell and type 17 T helper cell were significantly overexpressed in STAD tumor tissue when RGS2 was low-expressed. The other cells were significantly under expressed.

In order to further study the relationship between the expression of RGS2 in STAD tumor tissue and immune cells, the relationship between RGS2 expression and immune cell infiltration purity was analyzed by TIMER database (Figure 9). memory B cell、naive B cell、Eosinophil、M2 Macrophage、activated Mast cell、Monocyte、resting Myeloid dendritic cell and gamma delta T cell were significantly positively correlated, but M0 Macrophage、resting Mast cell、activated memory CD4 T cell、follicular helper T cell and regulatory T cell (Tregs) were significantly negatively correlated. The expression of RGS2 in immune cells were significantly different in STAD patients ($P<0.05$).

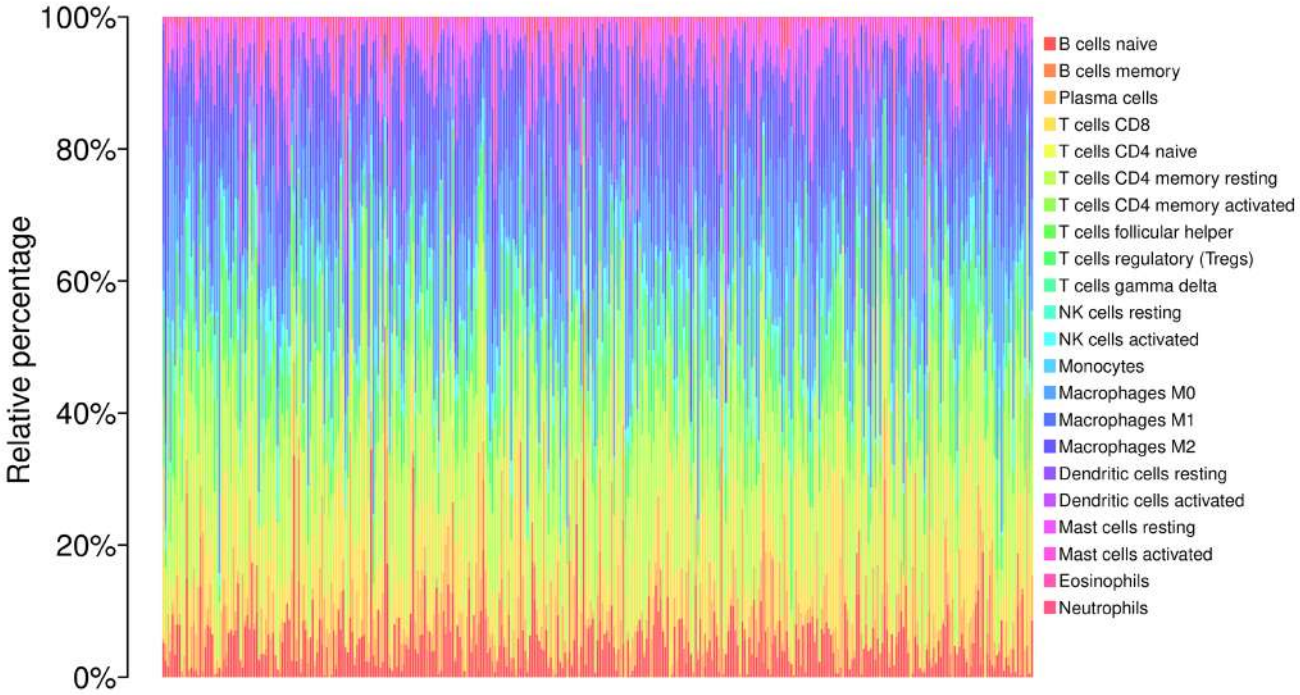


**Figure 7:** Clustering of immune cells in STAD tissue.

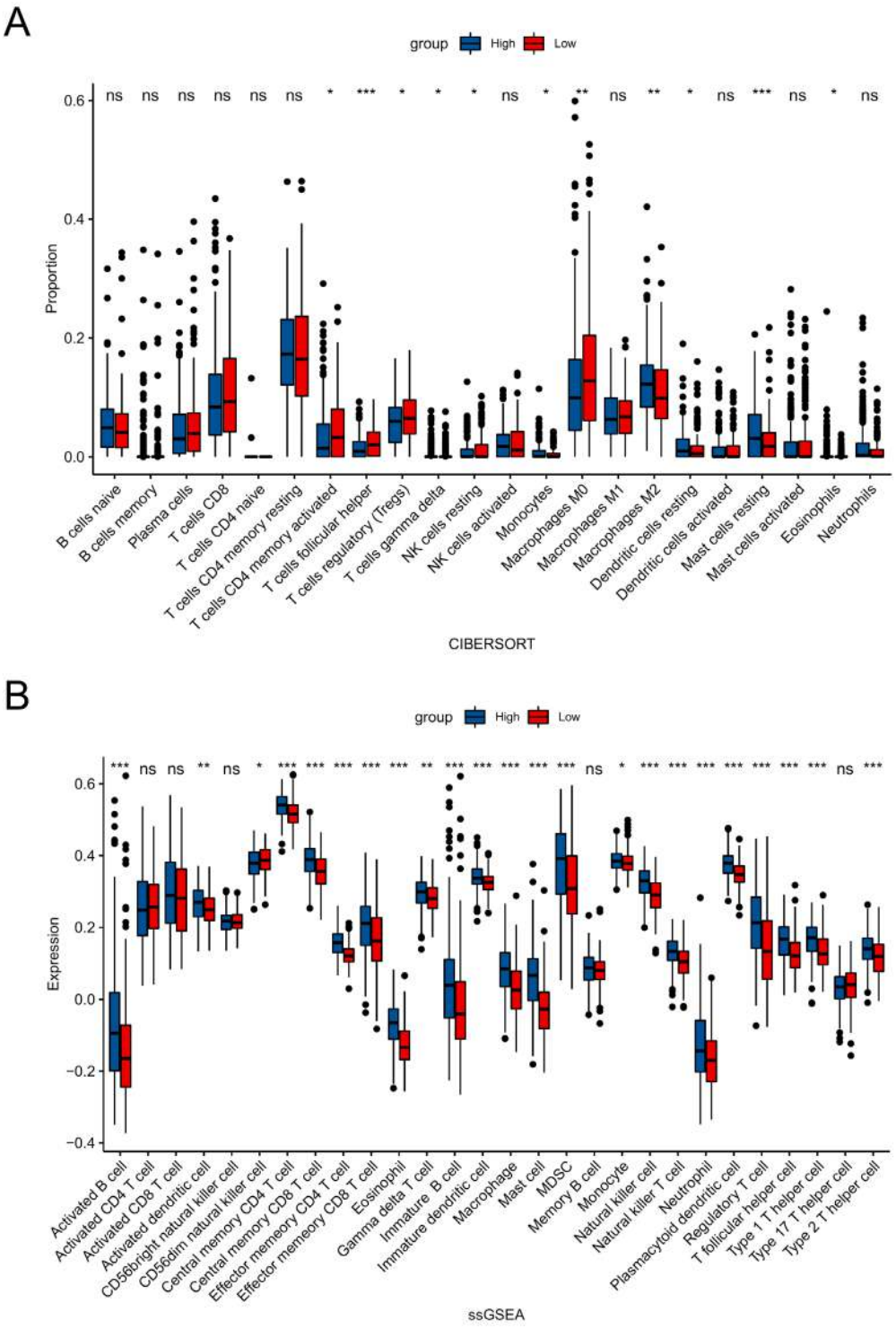


**Figure 8:** Analysis of immune cells by cibersort and ssGSEA algorithm. **(A)** Cibersort analysis. **(B)** ssGSEA analysis. ns: not significant, * P<0.05, ** P<0.01, *** P<0.001.

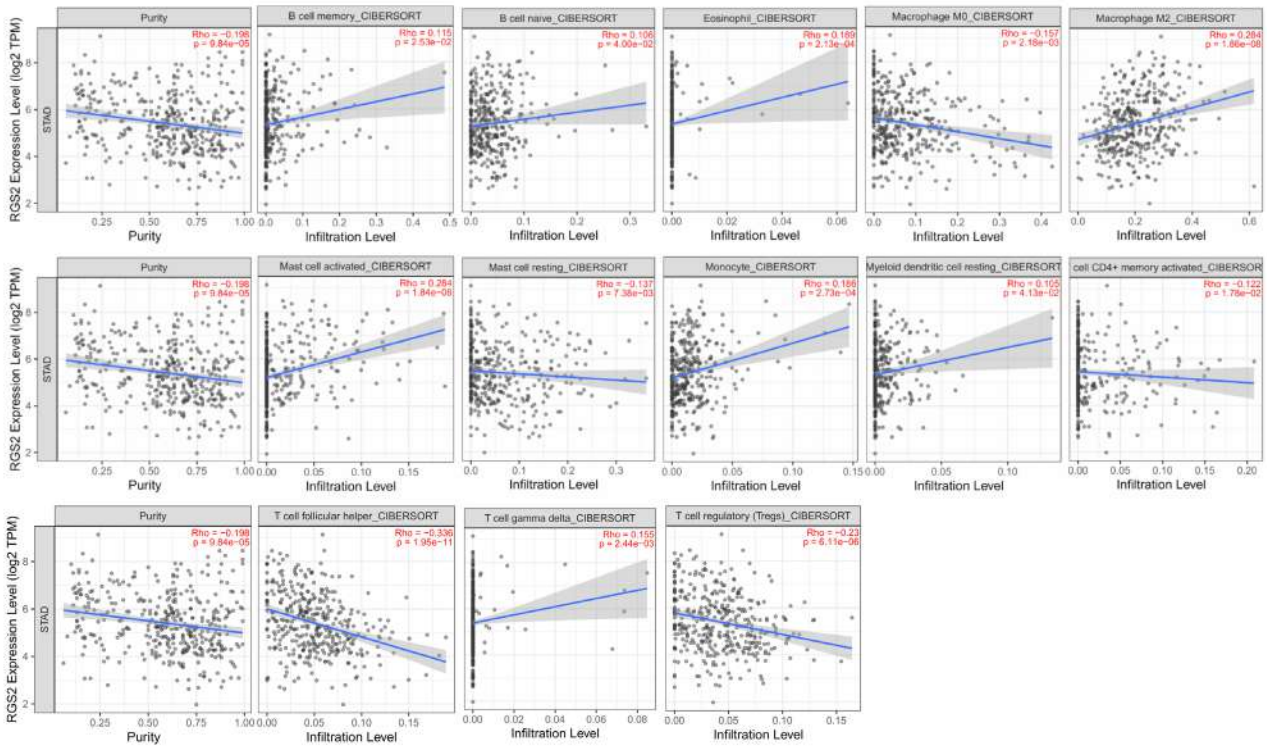


**Figure 9:** The analysis of immune infiltration and tumor purity from TIMER database.

## Correlation Analysis of PPI Networks and Interacting Proteins

To explore related gene of RGS2 in patients with STAD, the PPI network of RGS2 was analyzed. The result showed that RGS2 has many interacting proteins. For example, in the GeneMANIA database, there were 19 interacting proteins of RGS2 (Figure 10C). And there also have constructed the PPI network by GEPIA (Figure 10B). Another PPI network constructed by the STRING database, it can be found that the network has 51 nodes and 348 interaction relationships (Figure 10A). And the three databases were crossed to one gene of PRKG1 (Figure 11A).

The protein encoded by PRKG1 mainly has the functions of relaxing smooth muscle tone, preventing platelet aggregation and regulating cell growth, and the gene is most strongly expressed in all types of smooth muscle, platelets, cerebellar purkinje cells, hippocampal neurons and the lateral amygdala. The function of PRKG1 gene was similar to that of RGS2, and there was a close correlation between PRKG1 and RGS2 ($R=0.61$, $P<0.05$) (Figure 11B). And the heatmap obtained from unsupervised clustering results also showed that PRKG1 and RGS2 was closely related (Figure 11C).

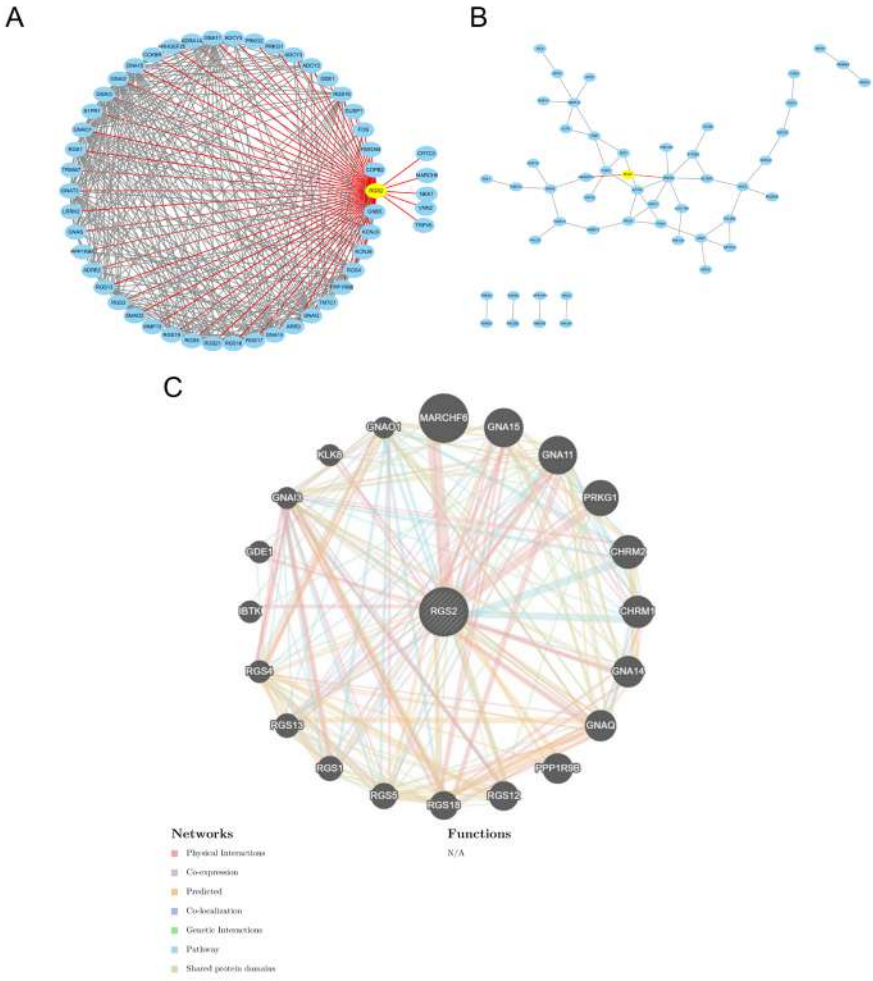


**Figure 10:** PPI network. **(A)** PPI network from STRING database. **(B)** PPI network from GEPIA database. **(C)** PPI network from GeneMANIA database.

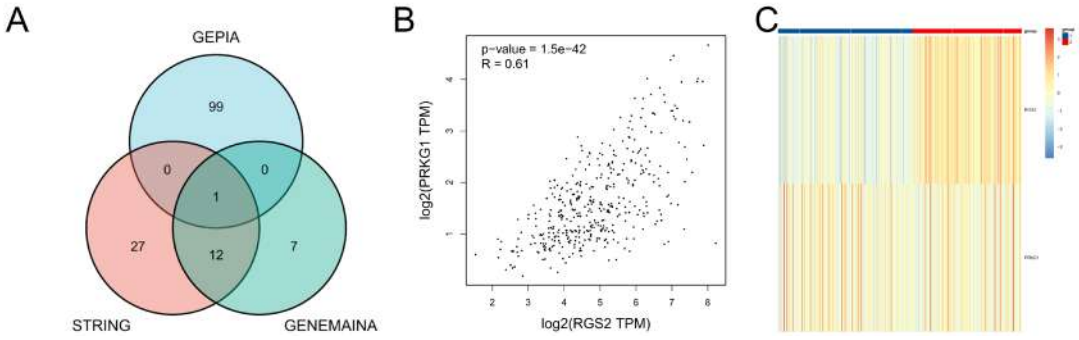


**Figure 11:** Correlation analysis of RGS2 and PRKG1. **(A)** Venn diagram. **(B)** Correlation scatter plot of RGS2 and PRKG1. **(C)** Unsupervised clustering heatmap.

## GO/KEGG/GSEA Enrichment Analysis with cGMP-PKG SIGNALING PATHWAY.

In order to further explore the relationship between the expression of RGS2 and the mechanism in STAD patients, the enrichment analysis of RGS2 was carried out. GO enrichment analysis was performed on biological processes (BP), cellular components (CC) and molecular functions (MF) of STAD patients, among which the expression of RGS2 was significantly different from BP in muscle system process, muscle contraction, regulation of membrane potential, axon development, regulation of blood circulation, muscle organ development, heart contraction, heart process, vascular process in circulatory system and regulation of heart contraction. For CC, there were significant difference with the expression of RGS2 in collagen-containing extracellular matrix, neuronal cell body, synaptic membrane, contractile fiber, myofibril, sarcomere, postsynaptic membrane, I band, Z disc and sarcolemma. At the same time, for MF, receptor ligand activity, signaling receptor activator activity, channel activity, passive transmembrane transporter activity, ion channel activity, actin binding, gated channel activity, extracellular matrix structural constituent, glycosaminoglycan binding and hormone activity (Figure 12A).

And based on the results of GSEA enrichment analysis of RGS2, there were 48 physiological mechanisms, these mechanisms had significant differences with the expression of RGS2. Among them, 46 physiological mechanisms were significantly different from overexpression of RGS2, and the top ten were shown here (Figure 12D). At the same time, DNA binding transcription factor activity and sequence specific DNA binding were significantly different from the under-expression of RGS2 (Figure12B,12C).

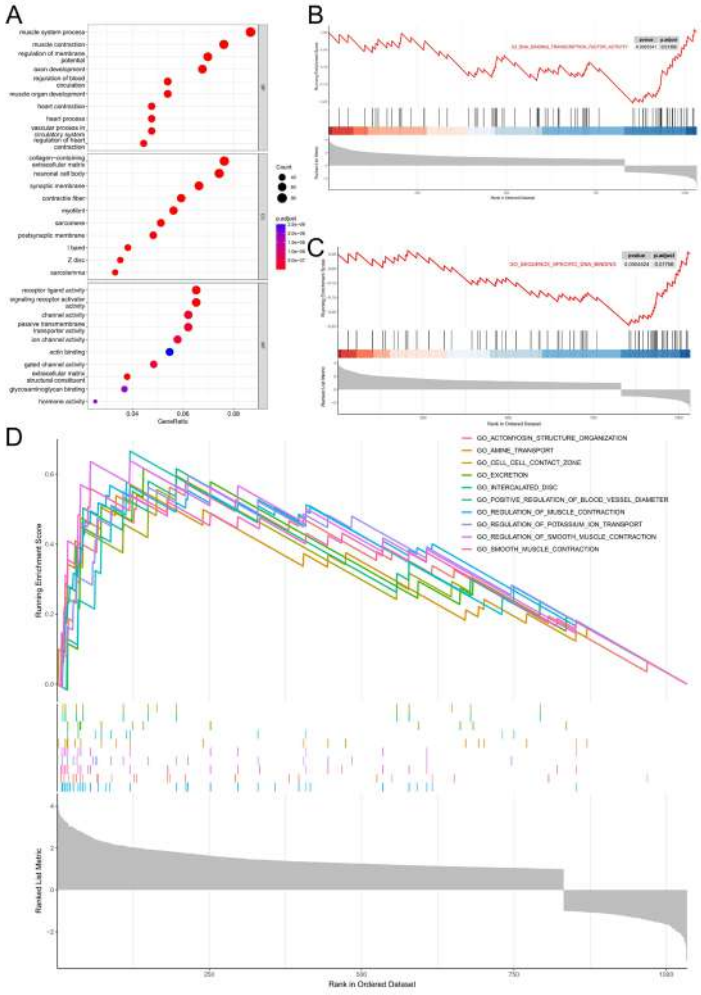


**Figure 12:** GO enrichment analysis and GSEA enrichment analysis. **(A)** GO enrichment analysis. **(B)** DNA binding transcription factor activity-GSEA. **(C)** Sequence specific DNA binding-GSEA. **(D)** GSEA enrichment analysis-high expression enrichment-top ten.

When exploring the correlation between PRKG1 and RGS2, we found PRKG1 and RGS2 were related to cGMP-PKG pathway. In order to further explore the relationship between PRKG1 and RGS2, KEGG enrichment analysis was performed on RGS2. The results of KEGG enrichment analysis showed that the expression of RGS2 was significantly different (P<0.05) in many biochemical response pathways, such as cAMP signaling pathway and cGMP-PKG signaling pathway (Figure 13A).

From the specific action map pf cGMP-PKG pathway, it can be found that RGS2 can be produced by the phosphorylation of PRKG1, both of which play a crucial role in the cGMP-PKG signaling pathway (Figure 13B). It has also been suggested that this pathway may be involved in many diseases.

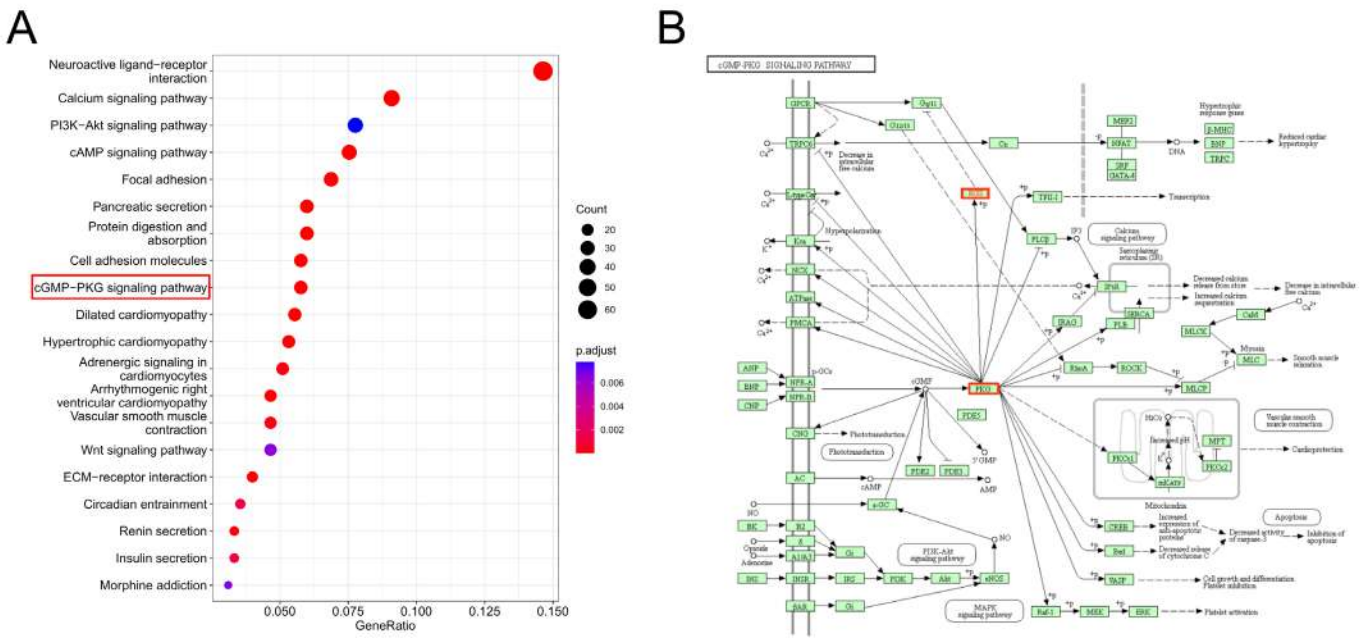


**Figure 13:** KEGG analysis. **(A)** KEGG enrichment analysis. **(B)** cGMP-PKG signaling pathway.

**Mutations in RGS2 in Patients with STAD.**

In order to understand the impact of RGS2 gene mutation in STAD patients, its mutation status in STAD patients was analyzed. The mutation status of RGS2 in STAD patients from six datasets were analyzed by the cBioPortal database. The percentage of RGS2 gene mutation in STAD was 2.5% (Figure 14D), and its mutation rate ranged from 2.51% to 4.28% (Figure 14A). The resulting of scatterplot showed that the main mutation types of RGS2 in STAD patients, its Spearman coefficient was 0.12 and P<0.05, and its Pearson coefficient was 0.06 and P=0.0652 (Figure 14B). According to its mutation site map, it can be seen that there were 7 mutation sites (VUS) on the DNA fragment where RGS2 is located, among which there were three mutation sites (VUS) in the RGS2 gene-X92_ splice, Truncating and Missense (Figure 14E), and by the 3D structural model of RGS2 can visually see its mutation site location (Figure 14C). And the Kaplan-Meier plot and the log-rank test indicated it was no significant difference in OS (P=0.656) (Figure 14F) and disease-free survival (P=0.109) (Figure 14G) between patients with and without the RGS2 mutation.

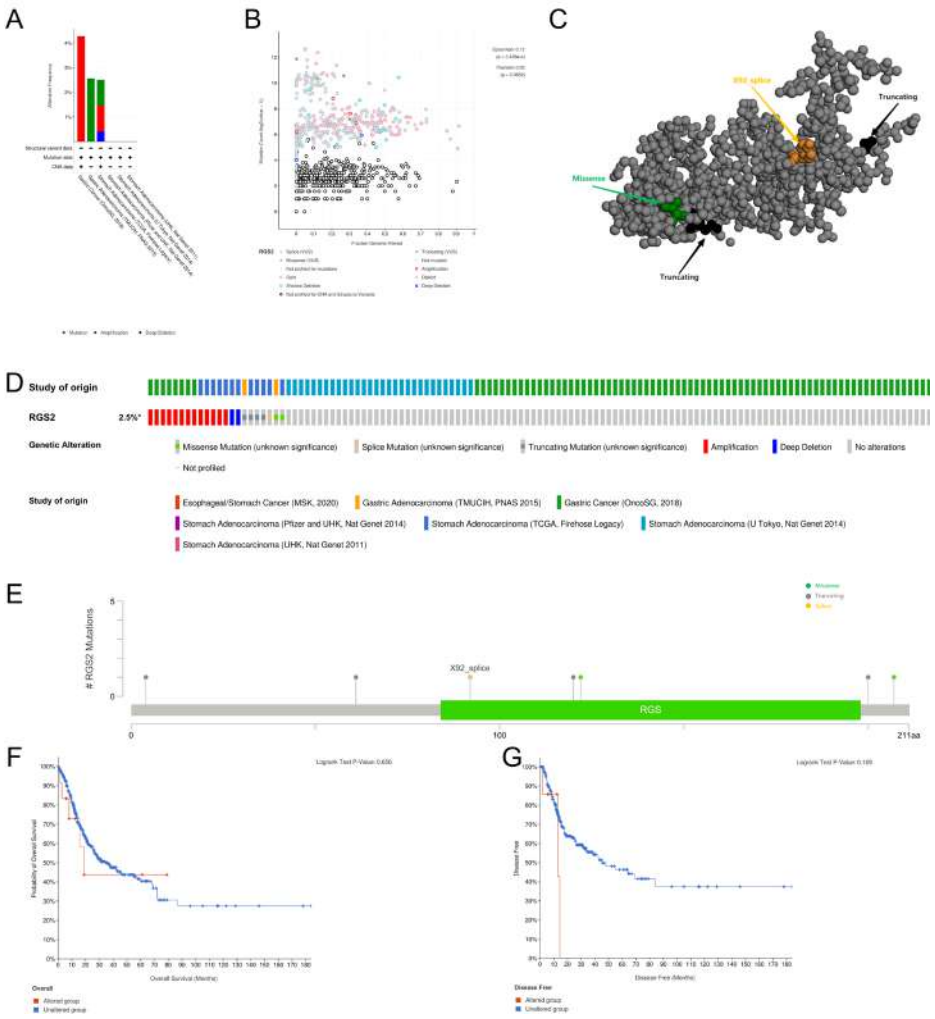


**Figure 14:** The mutation status analysis of RGS2 in STAD. **(A)** Mutation rate of RGS2 in different datasets. **(B)** Scatter plot of RGS2 mutation types. **(C)** 3D model of RGS2 mutation loci. **(D)** RGS2 mutation probability. **(E)** RGS2 mutation loci. **(F)** RGS2 mutation survival analysis-OS. **(G)** RGS2 mutation survival analysis-DF.

## Discussion

Due to the high mortality of STAD [18], it is necessary to find more accurate biomarkers to detect at an early stage and monitor disease progression. According to the prior studies, RGS2 is low-expression in various disease types [19], and identified as a prognostic factor [20]. In our research, we expected to explore the potential mechanism of RGS2 in promoting STAD and its viability as a molecular biomarker.

Pan-cancer analysis certified that RGS2 was downregulated in most cancer types. Further exploration revealed that higher RGS2 expression was associated with reduced overall survival (OS) in STAD patients. We performed T test to evaluate the relationship between the expression of RGS2 and the Clinicopathological characteristics of STAD. The result showed that the expression of RGS2 was significantly different between normal tissues and different age, histological grade, pathology M, pathology N, pathology T, gender and tumor stage tumor tissues of STAD patients. Besides, univariate and multivariate Cox analysis have indicated this gene was an independent factor to predict prognosis of STAD patients. All these foresaid results and ROC analysis suggested that RGS2 maybe a promising prognostic biomarker for STAD.

The tumor microenvironment (TME), consisted of various types of immune cells, played a crucial role in tumor progression, metastasis, and treatment resistance [21]. The composition of tumor-infiltrating immune cells strongly influenced the tumor microenvironment and the development of the tumor [22]. We have confirmed that RGS2 expression correlated with immune cell infiltration in the previous analysis. Therefore, we inferred it may affect the tumor microenvironment by changing proportions of specific immune cell types, thereby promoting tumor progression and metastasis. In fact, it was the case that RGS2 has recently been shown to be an important component in maintaining the tumor microenvironment [23]. Our work demonstrated the significant negative correlation in the activated memory CD4 T cells and the regulatory T cells related to the expression of RGS2. The activated memory CD4 T cells and the regulatory T cells were important components of the tumor microenvironment, and played complex roles in cancer pathophysiology [24]. Previous studies have found that impaired CD4+ memory T cell response to H. pylori antigen observed in the peripheral blood of H. pylori positive patients of STAD could be restored by depletion of CD4+ CD25 high Treg cells [25]. And about 5% of all CD4+ T cells in gastric and duodenal mucosa of H. pylori positive patients were CD25 high cells, whereas only 1% to 2% of CD4+ cells in uninfected gastric and duodenal mucosa were CD25 high cells. But the majority of the cells within the mucosal CD4+ CD25 high cell population were indeed Treg cells, and that the infected antral and duodenal mucosa had an increased frequency of CD4+ CD25 high cells compared to uninfected mucosa, was thus enriched in Treg cells. Meanwhile, in asymptomatic individual, Treg cells may help to maintain a balance between bacterial colonization and inflammation, preventing disease development, and any alteration in Treg cell activity may disturb this balance and contribute to disease [26]. Our results were supported by the findings of similar studies about this topic [27]. Definitely, the tumor microenvironment had a high level of complexity in its regulation. And other immune cell types in the tumor microenvironment may also influence tumor cell survival, including activated memory CD4 T cells and regulatory T cells. Future studies were needed to further explore the relationship between RGS2 expression and these cells.

In the PPI network results, we observed a strong correlation between RGS2 and PRKG1. The protein encoded by PRKG1 mainly functions to relax smooth muscle tone [28], prevent platelet aggregation [29] and regulat cell growth [30]. In addition, RGS2 was functionally similar to the protein encoded by PRKG1. Therefore, further investigation into the specific mechanisms of these proteins in tumor cells could yield new insights for improved STAD treatment strategies.

In our enrichment analysis, we observed significant differences in RGS2 expression related to various physiological mechanisms, such as DNA-binding transcription factor activity and sequence-specific DNA binding, based on the results of GO enrichment. Relevant research on DNA-binding transcription factor activity has been reported in lung cancer, with TTF-1 DNA-binding activity being a potential predictor of lung cancer metastasis and survival. In lung adenocarcinoma tissue, the TTF-1 DNA-binding activity was significantly higher (172+/-23.2) than that of other cases, including small cell carcinoma (141+/-16.3) and squamous cell carcinoma (122+/-13.6) ($P<0.05$). Moreover, a higher level of TTF-1 DNA-binding activity was associated with low overall survival and disease-free survival [31]. The results of GSEA enrichment analysis also indicated a significant association between under expression of RGS2 and DNA-binding transcription factor activity. Furthermore, RGS2 depletion could potentially inhibit DNA-binding transcription factor activity and thereby inhibit the metastasis and survival of STAD tumor cells. Additionally, previous studies have demonstrated the potential of sequence-specific DNA binding molecules to trigger the release of immunogenic signals and enhance phagocytosis in cancer cells. For instance, exposure to polyamide 1 resulted in increased phagocytosis rates of macrophages in patients [32]. In our KEGG enrichment analysis results, RGS2 exhibited significant differential expression in numerous pathways. Notably, RGS2 and PRKG1 interacted in the cGMP-PKG signaling pathway, and RGS2 could be produced via phosphorylation of PKG (PRKG1). Further investigation into the role of this pathway in STAD led us to uncover experimental evidence linking the inducible nitric oxide synthase (iNOS) and the nitric oxide (NO) / soluble guanylyl cyclases (sGC) / cyclic guanosine monophosphate (GMP) / protein kinase G (PKG) pathway to cognitive deficits and depression [33].

cGMP-PKG signaling pathway was also associated with various diseases, such as prostate cancer [34], nasopharyngeal carcinoma [35], numerous cardiac disease [36], cervical cancer [37]. In order to conduct in-depth research on the specific role of this pathway in STAD, we found the literature on the specific mechanism of action of this pathway in other cancers for analogy. In renal cancer, decreasing levels of cyclin D1 and increasing levels of p21 could inhibit cancer cells proliferation and promote cancer cells apoptosis through the cGMP-PKG pathway in OS-RC-2 cells transfected with PDE5 siRNA [38]. And for colon cancer, the cGMP-PKG pathway inhibited colon tumor cell growth that involves the transcriptional suppression of β-catenin to inhibit Wnt /β-catenin T cell factor transcriptional activity, leading to downregulation of cyclin D1 and surviving [39].

## Conclusion

In summary, we demonstrated that RGS2 expression was downregulated in STAD and significantly associated with good survival outcomes. At the same time, patients with STAD were more likely to suffer from depression. The RGS2 gene interacted with PRKG1 in STAD patients, and low expression of RGS2

reduced the expression level of PRKG1, which affected the cGMP-PKG signaling pathway. Additionally, low expression of RGS2 may resist the proliferation, migration, and invasion of STAD tumor cells. However, excessive depletion of RGS2 did not affect tumor cells. Furthermore, low expression of RGS2 affected the expression of memory activated CD4 T cells and regulatory T cells, leading to decreased expression levels in STAD patients, thereby increasing the risk of death in patients who simultaneously suffered from STAD and depression.

### Acknowledgments

We gratefully acknowledge the financial supports from the PhD fellowship of Nanchang Hangkong University (EA202008259), Natural Science Foundation of Hubei Province (2019CFB391).

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