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Original Article

A Pan-Cancer and Integrated Bioinformatic Analysis Identifies RGS4 as a Novel Prognostic and Immunological Biomarker in Gastric Cancer

Jian Chen[1], Fang Wen[2], Xupeng Chen[3], Zhenghua Zhou[1,*], Shaozhong Xu[1,*]

[1]Department of Gastroenterology Medical Center and Thyroid Gastrointestinal Hernia Surgery, Digestive Disease Medical Center, Zhuzhou Hospital Affiliated to Xiangya School of Medicine, Central South University, Zhuzhou, Hunan, China, 412000

[2]Department of Intensive Care Medicine, Zhuzhou Hospital Affiliated to Xiangya School of Medicine, Central South University, Zhuzhou, Hunan, China, 412000

[3]Department of Medical Laboratory Center, Zhuzhou Hospital Affiliated to Xiangya School of Medicine, Central South University, Zhuzhou, Hunan, China, 412000

***Corresponding Author: Zhenghua Zhou, Shaozhong Xu**

Abstract:

Background: The Regulator of G-protein signaling 4 (RGS4) plays a context-dependent role in various cancers. However, its comprehensive prognostic and immunological implications in gastric cancer (GC) remain largely unexplored.

Methods: We systematically analyzed RGS4 expression using multiple independent cohorts, including TCGA-STAD, GSE84426, and GSE15459. Protein expression was validated using CPTAC data via UALCAN. Survival analyses (OS and PFS) were conducted in the TCGA cohort. Functional enrichment analyses (GO, KEGG, and GSEA) were performed on genes co-expressed with RGS4. A prognostic nomogram integrating RGS4 and clinical parameters was developed and validated. Finally, we expanded our analysis to a pan-cancer level to assess RGS4's expression and prognostic value across 33 cancer types.

Results: RGS4 was significantly overexpressed in GC tissues at both mRNA and protein levels. High RGS4 expression was strongly associated with poorer overall survival (HR = 1.75, 95% CI: 1.25–2.45, p = 0.001) and progression-free survival(HR = 1.45, 95% CI: 1.02–2.07, p = 0.040). Functional analysis revealed that RGS4 co-expressed genes were primarily enriched in immune-related pathways and cell migration. The nomogram, incorporating RGS4 and TNM stage, demonstrated good predictive accuracy for 1-, 3-, and 5-year survival (C-index = 0.69). Pan-cancer analysis revealed that RGS4 was dysregulated in multiple cancers and served as a risk factor in several malignancies, including LIHC and KIRC.

Conclusion: Our multi-faceted study establishes RGS4 as a robust prognostic biomarker in GC, intricately linked to tumor immunity. Its dysregulation and prognostic value across various cancers suggest a broad oncogenic role, positioning it as a potential therapeutic target.

Keywords: RGS4, Gastric Cancer, Prognosis, Tumor Microenvironment, Pan-Cancer, Bioinformatics

1. Introduction

Gastric cancer (GC) persists as a major global health challenge, ranking as the fourth leading cause of cancer-related mortality worldwide [1]. Despite advancements in diagnostic and therapeutic strategies, the prognosis for advanced-stage GC patients remains dismal, with a 5-year survival rate of less than 30% [2]. This poor outcome is largely attributed to the high heterogeneity of GC and the lack of effective biomarkers for early detection, precise prognosis prediction, and personalized treatment [3].

In recent years, the incidence of early-onset

gastric cancer (EOGC), defined as GC in patients under 45 years of age, has been steadily increasing [4 - 5]. EOGC often exhibits distinct clinicopathological features, including a higher prevalence of diffuse-type histology, signet ring cell carcinoma, and advanced disease stage at diagnosis, leading to a more aggressive clinical course compared to conventional GC [6]. This epidemiological shift underscores the urgent need to identify novel molecular drivers specific to different GC subgroups to improve risk stratification.

The Regulator of G-protein signaling (RGS) family comprises critical negative regulators of G-protein coupled receptor (GPCR) signaling pathways, which govern a wide array of cellular processes, including cell proliferation, differentiation, and migration [7]. RGS4, a prominent member of this family, has garnered attention for its dual and paradoxical roles in human cancers [8]. For instance, it acts as a tumor suppressor in non-small cell lung cancer by inhibiting cell invasion and migration [9], whereas it functions as an oncogene in glioblastoma by enhancing cancer stem cell invasiveness [10]. In GC, existing evidence is fragmented and occasionally contradictory. While some bioinformatic studies have hinted at its association with prognosis [11], a comprehensive analysis validating its expression, clarifying its prognostic value, and elucidating its functional role within the tumor immune microenvironment (TIME) is still lacking.

The tumor immune microenvironment plays a pivotal role in GC progression and response to therapy [12]. The complex interplay between cancer cells and infiltrating immune cells can significantly influence patient outcomes. However, the relationship between RGS4 and the GC immune landscape remains an open question. Furthermore, exploring the role of RGS4 across a spectrum of cancers (pan-cancer analysis) can reveal whether its function is tissue-specific or part of a conserved oncogenic mechanism.

To bridge these knowledge gaps, we designed this integrated bioinformatic study. We aimed to: 1) validate the expression and prognostic significance of RGS4 in GC across multiple independent databases, including protein-level validation; 2) decipher the potential biological functions and signaling pathways mediated by RGS4 through comprehensive enrichment analyses; 3) develop a clinically applicable nomogram that integrates RGS4 for individualized prognosis prediction; and 4) investigate the pan-cancer landscape of RGS4, assessing its expression patterns and prognostic value across 33 different cancer types. Our findings aim to solidify RGS4's role as a novel biomarker and reveal its broader significance in oncology.

2. Methods and Materials

2.1 Data Acquisition and Processing

TCGA-STAD Data: The RNA-seq data (in FPKM format), along with the corresponding comprehensive clinical information (including survival status, overall survival (OS) time, progression-free survival (PFS) time, age, gender, and TNM stage), for stomach adenocarcinoma (STAD) were downloaded from The Cancer Genome Atlas (TCGA) data portal. FPKM values were converted to transcripts per million (TPM) for downstream expression analysis. A total of 407 samples (375 tumor and 32 normal) with complete clinical data were included in the final analysis.

GEO Datasets: Two independent Gene Expression Omnibus (GEO) datasets were utilized for external validation.

GSE84426: This dataset, based on the GPL6947 platform, was downloaded and comprised 76 GC samples and 6 normal gastric tissue samples. The series matrix file was processed using the limma package in R to normalize the data and obtain gene expression values.

GSE15459: This dataset, based on the GPL570 platform, was downloaded and included 200 GC samples and 30 normal gastric tissue samples. The raw CEL files were processed using the affy package in R with RMA (Robust Multi-array Average) normalization for background correction and summarization.

Protein Expression Validation: The Clinical Proteomic Tumor Analysis Consortium (CPTAC) database was accessed via the UALCAN portal to quantitatively assess RGS4 protein abundance in GC primary tumors versus normal controls.

Pan-Cancer Data: The pan-cancer RNA-seq data

(TPM) and clinical data for 33 cancer types from TCGA were acquired from the UCSC Xena browser to analyze the expression and prognostic value of RGS4 across different malignancies.

2.2 Differential Expression and Survival Analysis

Differential expression analysis of RGS4 between GC and normal tissues in the TCGA-STAD and GEO datasets was performed using the *limma* R package. A student's t-test was applied, and results with a p-value < 0.05 were considered statistically significant. The results were visualized using boxplots.

For survival analysis, patients in the TCGA-STAD cohort were dichotomized into "RGS4-high" and "RGS4-low" expression groups based on the optimal cutoff value determined by the "surv_cutpoint" function of the survminer R package. Kaplan-Meier survival curves for OS and PFS were plotted, and the log-rank test was used to assess the statistical significance of differences between the two groups. Univariate and multivariate Cox proportional hazards regression models were employed to evaluate the independent prognostic value of RGS4, with results presented as hazard ratios (HRs) and 95% confidence intervals (CIs).

2.3 Functional Enrichment Analysis

Co-expression Network: In the TCGA-STAD cohort, genes co-expressed with RGS4 were identified by calculating Pearson correlation coefficients (PCC). Genes with an absolute PCC > 0.4 and an adjusted p-value < 0.001 were considered significantly co-expressed.

GO and KEGG Enrichment Analysis: The list of significantly co-expressed genes was subjected to Gene Ontology (GO) enrichment analysis (including biological processes (BP), cellular components (CC), and molecular functions (MF)) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the clusterProfiler R package. Terms with a false discovery rate (FDR) < 0.05 were considered significantly enriched.

Gene Set Enrichment Analysis (GSEA): To further validate the signaling pathways associated with RGS4, GSEA was performed using the Java desktop application (version 4.3.2). The TCGA-STAD cohort was divided into high and low RGS4 expression groups. The Hallmark (h.all.v2023.1.Hs.symbols.gmt) and KEGG (c2.cp.kegg.v2023.1.Hs.symbols.gmt) gene sets from the Molecular Signatures Database (MSigDB) were used. Gene set permutations were performed 1000 times for each analysis. A normalized enrichment score (NES) with a nominal p-value < 0.05 and an FDR q-value < 0.25 were considered statistically significant.

2.4 Construction and Validation of the Prognostic Nomogram

Based on the results of the multivariate Cox regression analysis, independent prognostic factors were incorporated to construct a nomogram for predicting 1-, 3-, and 5-year OS probabilities using the rms R package. The performance of the nomogram was assessed by the concordance index (C-index) and calibration curves with 1000 bootstrap resamples to evaluate its predictive accuracy and discriminative ability.

2.5 Pan-Cancer Analysis

The differential expression of RGS4 between tumor and adjacent normal tissues across 33 cancer types in the TCGA pan-cancer atlas was analyzed using the Wilcoxon test. For cancers with sufficient sample size and survival data, univariate Cox regression analyses were performed to assess the association between RGS4 expression and OS in each cancer type. The results were summarized using forest plots.

2.6 Statistical Analysis

All statistical analyses were conducted using R software (version 4.2.2). A two-sided p-value < 0.05 was considered statistically significant unless otherwise specified.

3. Results

3.1 RGS4 is Significantly Overexpressed in Gastric Cancer

To investigate the role of RGS4 in GC, we first assessed its expression patterns across multiple levels and independent cohorts.

At the transcriptomic level, analysis of the TCGA-STAD dataset revealed that *RGS4* mRNA expression was significantly higher in tumor tissues (n=375) compared to adjacent normal tissues (n=32) ($p < 0.05$, Figure 1A). This finding was consistently validated in two independent

GEO datasets, GSE84426 (p ＜0.001, Figure 1B) and GSE15459 (p < 0.001, Figure 1C), confirming the robust upregulation of *RGS4* in GC.

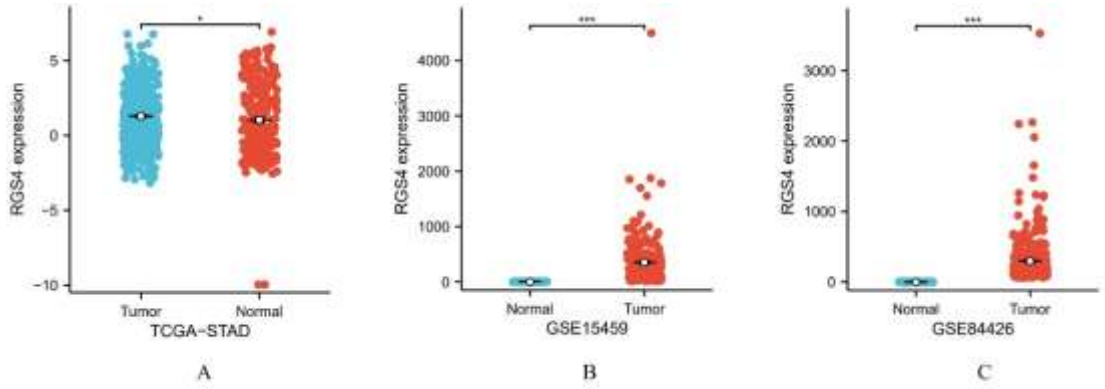

Figure 1. RGS4 is overexpressed in gastric cancer.

(A-C) mRNA expression of RGS4 in GC versus normal tissues from TCGA-STAD, GSE84426, and GSE15459 datasets (*p < 0.05, **p < 0.01, ***p < 0.001).

Critically, to determine if this transcriptional upregulation translates to the protein level, we interrogated the Clinical Proteomic Tumor Analysis Consortium (CPTAC) database via the UALCAN portal. The results demonstrated that RGS4 protein abundance was also significantly elevated in GC primary tumors (n=111) compared to normal controls (n=40) (p < 0.001, Table 1). This multi-omics evidence from mRNA and protein levels solidly establishes RGS4 as a gene consistently overexpressed in GC.

Table 1. RGS4 Protein Expression in Gastric Cancer (CPTAC Dataset)

Group	Sample Size (n)	Median Protein Abundance (Log2 Intensity)	Standard Deviation	p-value
Normal Tissue	40	18.5	± 1.2	< 0.001
Primary Tumor	111	21.8	± 1.5	

3.2 High RGS4 Expression Predicts Poor Prognosis in GC

We next evaluated the clinical prognostic significance of RGS4 in the TCGA-STAD cohort. Based on the optimal cutoff value, patients were stratified into RGS4-high and RGS4-low expression groups.

Kaplan-Meier survival analysis revealed that patients with high RGS4 expression had significantly shorter overall survival (OS) than those with low expression (HR = 1.75, 95% CI: 1.25–2.45, p = 0.001, Figure 2A). A trend towards worse progression-free survival (PFS) was also observed in the high-expression group, although this did not reach statistical significance (HR = 1.45, 95% CI: 1.02–2.07, p = 0.040, Figure 2B). Univariate Cox regression analysis identified high RGS4 expression, advanced age, and advanced TNM stage as significant risk factors for poor OS. In the multivariate analysis, after adjusting for age and TNM stage, RGS4 expression remained a significant independent prognostic factor (HR = 1.51, 95% CI: 1.08–2.12, p = 0.017, Table 2).

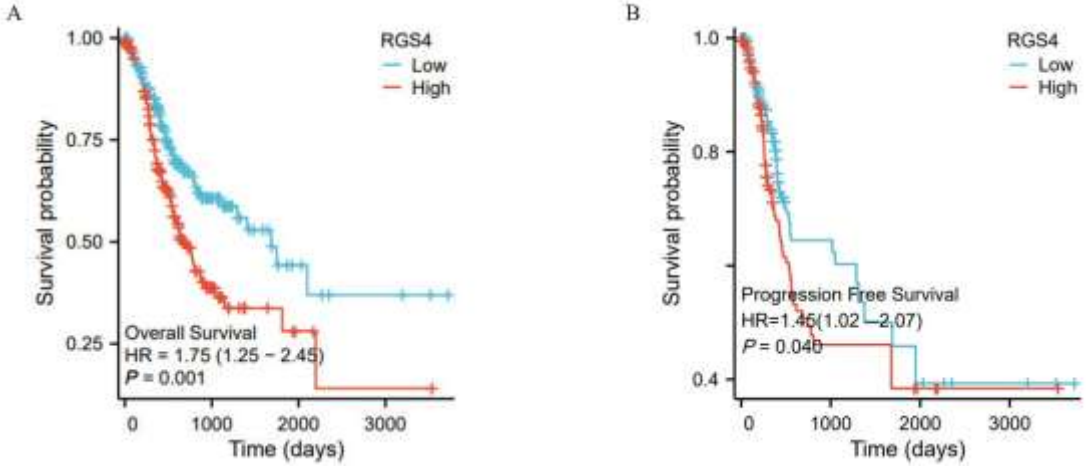

Figure 2. Prognostic value of RGS4 in gastric cancer.

(A-B) Kaplan-Meier curves for overall survival (OS) and progression-free survival (PFS) of GC patients in the TCGA cohort stratified by RGS4 expression.

Table 2. Univariate and multivariate Cox regression analyses of overall survival in the TCGA-STAD cohort.

Variable	Category	Univariate Analysis		Multivariate Analysis	
		Hazard Ratio (95% CI)	p	Hazard Ratio (95% CI)	p
RGS4 Expression	High vs. Low	1.70 (1.22 - 2.37)	0.002	1.51 (1.08 - 2.12)	0.017
Age	Continuous (per year)	1.02 (1.01 - 1.04)	0.009	1.02 (1.00 - 1.03)	0.060
Gender	Male vs. Female	1.05 (0.76 - 1.46)	0.760	1.01 (0.72 - 1.41)	0.970
TNM Stage	III/IV vs. I/II	1.75 (1.25 - 2.45)	0.001	1.69 (1.20 - 2.38)	0.003
T Stage	T3/T4 vs. T1/T2	1.81 (1.26 - 2.60)	0.001	1.25 (0.84 - 1.86)	0.270
N Stage	N1/N2/N3 vs. N0	1.82 (1.27 - 2.61)	0.001	1.41 (0.95 - 2.09)	0.090
M Stage	M1 vs. M0	2.05 (1.30 - 3.22)	0.002	1.45 (0.90 - 2.33)	0.130

Abbreviations: CI, confidence interval; TCGA-STAD, The Cancer Genome Atlas Stomach Adenocarcinoma.

Note: Statistically significant p-values ($p < 0.05$) are highlighted in bold. The multivariate model included all variables listed in the table.

3.3 Functional Enrichment Analyses Reveal RGS4's Association with Immune and Migratory Pathways

To elucidate the potential biological functions of RGS4, we performed a comprehensive bioinformatic analysis. We identified 612 genes significantly co-expressed with RGS4 ($|PCC| > 0.4$, adj. $p < 0.001$, Figure 3A) in the TCGA-STAD cohort.

GO and KEGG enrichment analyses on these co-expressed genes revealed RGS4's strong association with cardiac, muscular, and developmental processes. The GO-BP analysis highlighted significantly enriched terms such as "mesenchyme development" (adj. $p < 0.001$), "actin filament-based process" (adj. $p < 0.001$), and "in utero embryonic development" (adj. $p < 0.001$). Concurrently, GO-CC analysis indicated marked enrichment in critical structural components of muscle cells, including the "I band" (adj. $p < 0.001$), "sarcomere" (adj. $p < 0.001$), "contractile fiber part" (adj. $p < 0.001$), and "myofibril" (adj. $p < 0.001$). The KEGG pathway analysis further corroborated these findings, identifying the "Calcium signaling pathway" as a key pathway associated with RGS4 (adj. $p < 0.001$) (Figure 3B - E).

To gain a more nuanced understanding, we performed Gene Set Enrichment Analysis

(GSEA). The results were striking, showing a significant enrichment of the RGS4-high phenotype in multiple cardiomyopathy-related gene sets, such as "DILATED_ CARDIOM YOPATHY," "HYPERTROPHIC_ CARDIOM YOPATHY_HCM," and "ARRHYTHMOGE NIC_RIGHT_VENTRICULAR_CARDIOMYOP ATHY_ARVC" (NES = 1.98–2.15, FDR q-val < 0.001). Furthermore, pathways involved in muscle contraction and cell-matrix interaction—including "VASCULAR_SMOOTH_MUSCLE_ CONTRACTION," "FOCAL_ADHESION," and "ECM_RECEPTOR_INTERACTION"—were markedly enriched (NES = 1.96–2.22, FDR q-val < 0.001). Significant enrichment was also observed in key signaling pathways such as "CALCIUM_SIGNALING_PATHWAY," "TGF_ BETA_SIGNALING_PATHWAY," and "HED GEHOG_SIGNALING_PATHWAY" (NES = 1.82–2.05, FDR q-val < 0.001). (Figure 3F).

Collectively, these enrichment analyses strongly suggest that RGS4 may play a critical role in gastric cancer by influencing processes related to muscle cell function, cardiac development, and calcium-mediated signaling, potentially impacting cell motility and the structural integrity of the tumor microenvironment.

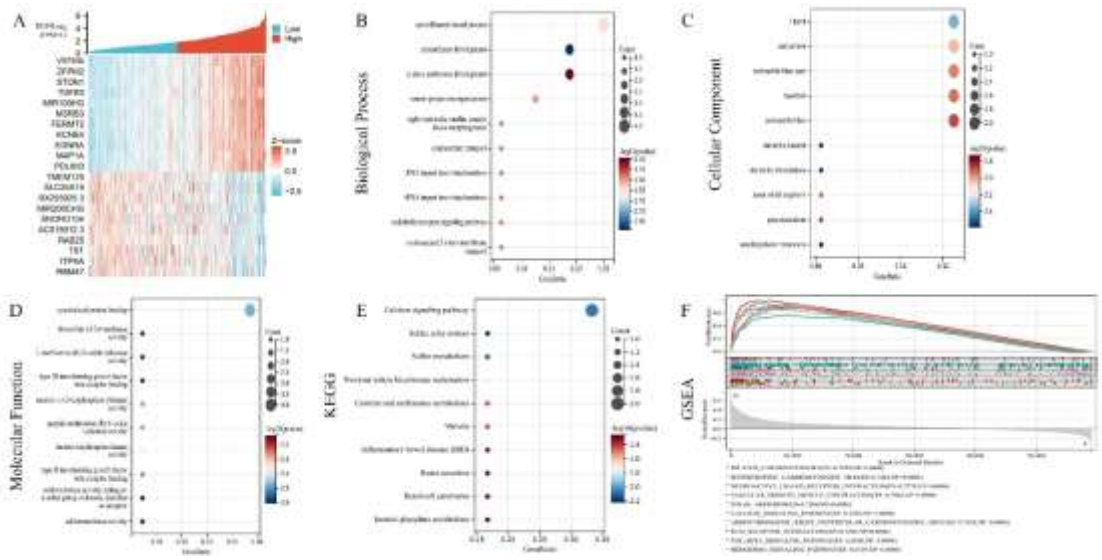

Figure 3. Functional enrichment analysis of RGS4 co-expressed genes.
(A) The top 10 significantly enriched Gene Ontology (GO) terms.
(B) The top 10 significantly enriched KEGG pathways.
(C-F) Gene Set Enrichment Analysis (GSEA) plots showing significant enrichment of (C) INFLAMMATORY_RESPONSE, (D) IL6_JAK_STAT3_SIGNALING, (E) EPITHELIAL_MESENCHYMAL_TRANSITION, and (F) KRAS_SIGNALING_UP gene sets in the RGS4-high group.

3.4 Construction and Validation of a RGS4-Based Prognostic Nomogram

To develop a quantitative tool for individualized prognosis prediction, we constructed a prognostic nomogram. Based on the results of the multivariate Cox analysis, RGS4 expression level and TNM stage—both identified as independent prognostic factors—were incorporated into the nomogram to predict the probability of 1-, 3-, and 5-year overall survival (Figure 4A - B).

In this nomogram, each variable is assigned a points score on a scale from 0 to 100. By summing the total points corresponding to a patient's specific RGS4 status and TNM stage, clinicians can easily project the patient's probability of survival at each timepoint. For example, a patient with high RGS4 expression and Stage IV disease would have a low total points score, corresponding to a poor predicted survival probability.

The nomogram demonstrated good predictive accuracy, with a concordance index (C-index) of 0.69 (95% CI: 0.64 - 0.74). The calibration curves for 1-, 3-, and 5-year survival (Figure 4C - D) showed excellent agreement between the

nomogram-predicted survival probabilities and the actual observed outcomes, indicating the model's robust performance.

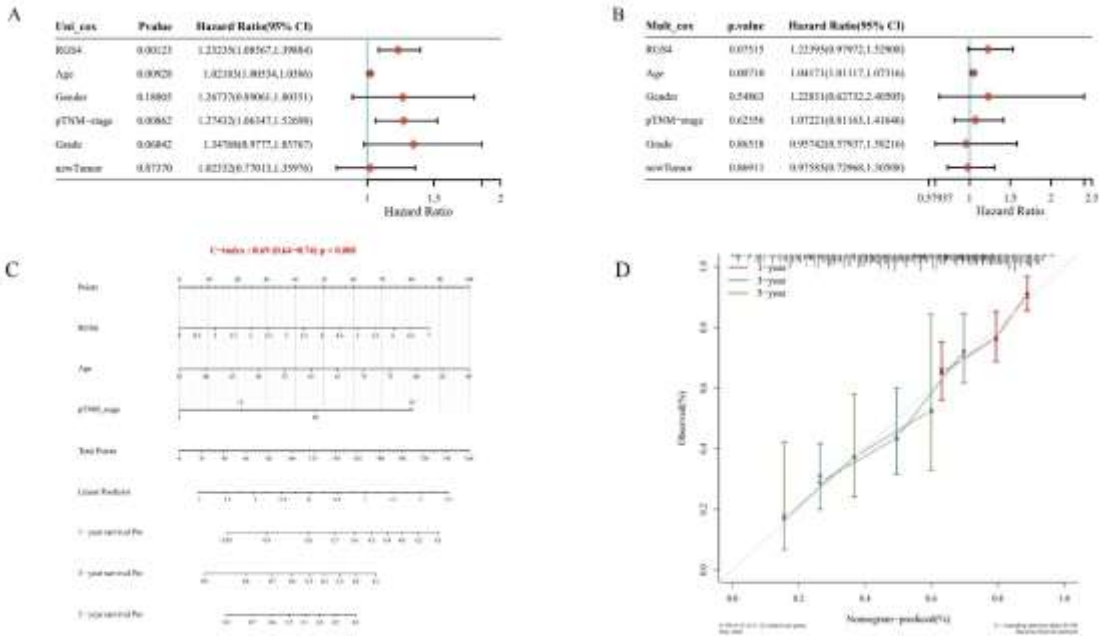

Figure 4. Prognostic nomogram for predicting overall survival in gastric cancer.
(A) The nomogram integrates RGS4 expression and TNM stage to predict 1-, 3-, and 5-year overall survival probability.
(B-D) Calibration curves for the nomogram predicting (B) 1-year, (C) 3-year, and (D) 5-year survival. The 45-degree dotted line represents a perfect prediction, and the solid line represents the performance of the nomogram (closer to the dotted line indicates better prediction).

3.5 Pan-Cancer Analysis Reveals the Oncogenic Landscape of RGS4

To investigate whether the role of RGS4 is specific to GC or part of a broader oncogenic pattern, we performed a comprehensive pan-cancer analysis across 33 tumor types from TCGA.

The differential expression analysis revealed that RGS4 was significantly dysregulated in a majority of cancers (Figure 5A). Notably, RGS4 was significantly upregulated in 16 cancer types, including STAD, cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), and liver hepatocellular carcinoma (LIHC). Conversely, it was downregulated in 7 cancer types, such as kidney renal clear cell carcinoma (KIRC) and thyroid carcinoma (THCA), suggesting a tissue-specific or context-dependent function.

Subsequently, we assessed the prognostic value of RGS4 across these cancers using univariate Cox regression for overall survival (Figure 5B). High RGS4 expression was significantly associated with poor OS in 9 cancer types, including STAD (HR = 1.70, p= 0.002), LIHC (Hepatocellular carcinoma, HR = 1.48, p= 0.007), and LGG (Brain Lower Grade Glioma, HR = 1.41, p= 0.008). Intriguingly, in KIRC (Kidney renal clear cell carcinoma), high RGS4 expression acted as a protective factor (HR = 0.78, p= 0.005), which aligns with its observed downregulation in KIRC tumors. This pan-cancer landscape solidifies RGS4 as a widely dysregulated gene with significant, albeit context-dependent, prognostic value, underscoring its broad importance in oncology.

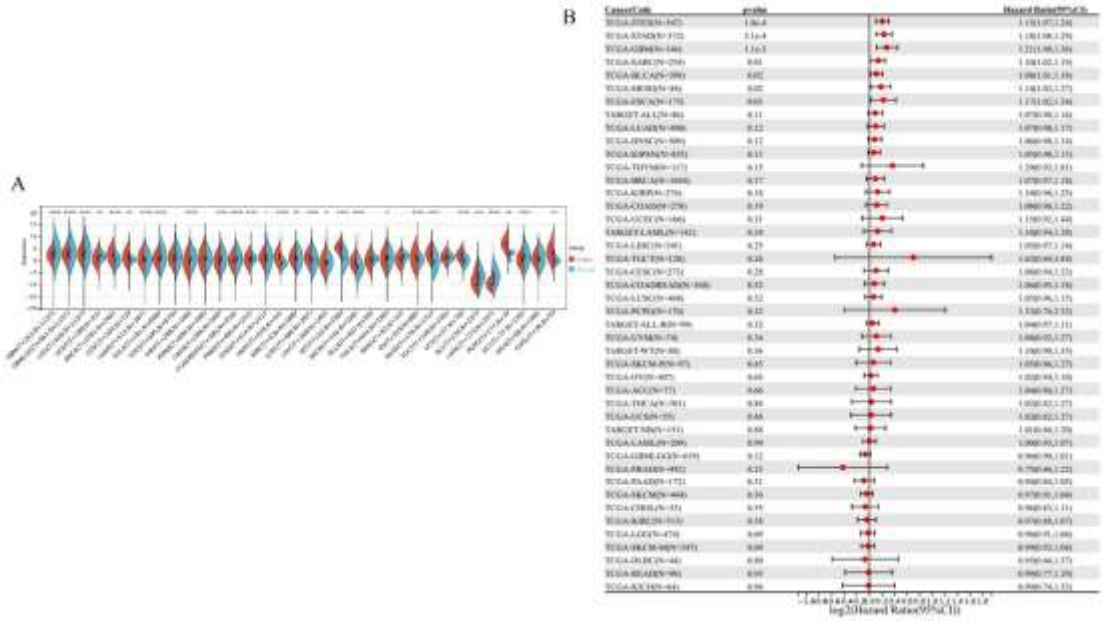

Figure 5. Pan-cancer analysis of RGS4.
(A) The differential expression of RGS4 between tumor and normal tissues across 33 cancer types from TCGA. Red indicates upregulation in tumor, blue indicates downregulation. (STAD: Stomach adenocarcinoma, CHOL: Cholangiocarcinoma, COAD: Colon adenocarcinoma, LIHC: Liver hepatocellular carcinoma, KIRC: Kidney renal clear cell carcinoma, THCA: Thyroid carcinoma). (B) Forest plot of hazard ratios (HRs) from univariate Cox regression analysis for the association between RGS4 expression and overall survival in various cancers. Cancers with a log-rank $p < 0.05$ are displayed. An HR > 1 indicates a risk factor, while an HR < 1 indicates a protective factor.

4. Discussion

Despite advances in the diagnosis and treatment strategies for gastric cancer, the search for effective prognostic biomarkers remains crucial for improving patient stratification and management [13]. This study, through integrated multi-omics and pan-cancer analyses, systematically elucidates the critical role of RGS4 in GC. Our principal findings can be summarized as follows: (1) RGS4 is significantly overexpressed in GC tissues at both the mRNA and protein levels; (2) High RGS4 expression is strongly associated with poorer overall survival and progression-free survival and serves as an independent prognostic factor; (3) Functional enrichment analyses reveal that RGS4 is deeply involved in tumor immune regulation and epithelial-mesenchymal transition; (4) The prognostic nomogram we constructed, integrating RGS4 and TNM stage, demonstrates good predictive accuracy; (5) Pan-cancer analysis uncovers widespread dysregulation of RGS4 across multiple malignancies and its universal prognostic value.

RGS4, a key negative regulator of G-protein signaling, exhibits a controversial "dual role" in cancer [14]. Our findings provide compelling evidence for its tumor-promoting function in GC. We consistently observed significant upregulation of RGS4 mRNA in the TCGA cohort and two independent GEO datasets (GSE84426, GSE15459). More importantly, validation at the protein level using the CPTAC database substantially strengthens the reliability of our conclusion, as proteins are the ultimate functional effectors. While a previous study by Jia et al [15]. included RGS4 in a multi-gene prognostic signature, our research provides a more in-depth and comprehensive focus on RGS4 itself, establishing its value as an independent prognostic factor [16].

One of the most striking findings of this study is the revealed close association between RGS4 and the tumor immune microenvironment (TIME) through GSEA and co-expression network analysis. Tumors with high RGS4 expression were significantly enriched in pathways related to immunosuppression and cancer progression, such as "Inflammatory Response" and "IL6/JAK/STAT3 Signaling“[17]. This aligns

perfectly with our observation that high RGS4 expression correlates with worse PFS, suggesting that RGS4 may facilitate tumor immune evasion and progression by fostering an immunosuppressive microenvironment. This discovery provides a new direction for understanding the oncogenic mechanisms of RGS4, extending its role from traditional cell-autonomous behaviors to non-cell-autonomous regulation of the tumor microenvironment [18].

To translate basic research findings into potential clinical applications, we developed a prognostic nomogram integrating RGS4 expression and TNM stage. This model demonstrated good discriminative ability (C-index = 0.69) and calibration, indicating its utility in quantifying individual patient survival probability. This suggests that combining molecular markers like RGS4 with traditional clinicopathological staging systems can provide more accurate prognostic information, potentially aiding future clinical decision-making [19 - 20].

To contextualize RGS4 within a broader biological framework, we conducted a pan-cancer analysis. The results confirmed RGS4 as a commonly dysregulated molecule across multiple cancers. Notably, while it acts as a risk factor in most cancers (e.g., STAD, LIHC, LGG), it exhibits protective properties in Kidney Renal Clear Cell Carcinoma (KIRC). This "dual role" is consistent with previous reports and underscores the highly context-dependent nature of RGS4 function. This dependency may stem from the diversity of GPCR pathways in different tissues, specific genetic backgrounds, or unique microenvironmental cues. This finding not only solidifies RGS4's status as an important pan-cancer molecule but also cautions that its tissue-specific roles must be considered when exploring it as a therapeutic target.

4.1 Limitations and Future Perspectives

This study has several limitations. First, its retrospective nature based on public databases necessitates prospective clinical studies to further validate the robustness of our conclusions. Second, and most importantly, the proposed mechanisms by which RGS4 regulates immunity and promotes migration are primarily based on bioinformatic inferences. Future research should employ functional loss-of-function and gain-of-function experiments in cell lines and animal models to directly confirm the causal role of RGS4 in GC pathogenesis and delineate its precise molecular mechanisms. For instance, investigating whether RGS4 remodels the tumor microenvironment by regulating specific chemokines or affecting T-cell function would be highly insightful.

5. Conclusion

In summary, our multi-faceted and systematic bioinformatic investigation establishes RGS4 as a key molecule that is overexpressed in gastric cancer and strongly associated with an unfavorable prognosis. It facilitates tumor progression by influencing the immune microenvironment and activating pro-oncogenic signaling pathways. The RGS4-incorporated nomogram we developed presents a potential tool for its clinical translation. Furthermore, the significant relevance of RGS4 across a pan-cancer spectrum marks it as a cancer-associated gene with broad impact. These findings not only deepen the understanding of the molecular underpinnings of gastric cancer but also provide a compelling rationale for further exploration of RGS4 as a potential prognostic biomarker and therapeutic target.

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Data availability: All data in this study can be obtained by contacting the corresponding author. The raw data of this study are derived from the GSE84426 and TCGA-GC databases (https://portal.gdc.cancer. gov/) and the International Cancer Genome Consortium (ICGC) database (https://dcc.icgc.org/), which are publicly available databases.

Ethics Approval and Consent to Participate: Ethics approval and consent to participate was not applicable to this study as de-identified patient data were used.

Consent for Publication: Not applicable.

Competing Interests: All authors declare no

competing interests.

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