Original Article

Comprehensive analysis of *GINS* subunit expression, prognostic value, and immune infiltration in clear cell renal cell carcinoma

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Background: In recent decades, there has been increasing evidence that Go-Ichi-Nii-San (*GINS*) subunits play an important role in the development and progression of various tumors. However, little research has been conducted on the role of *GINS* subunits in clear cell renal cell carcinoma (ccRCC). This study sought to explore the differential expression, prognosis, and immunological significance of *GINS* subunits in ccRCC.

Methods: We used various analysis packages of R (version 3.6.3), the University of ALabama at Birmingham CANcer (UALCAN) data analysis portal, the Cancer Cell Line Encyclopedia (CCLE), the cBio Cancer Genomics Portal (cBioPortal), and the Tumor Immune Estimation Resource (TIMER) to study the gene expression, promoter methylation level, gene mutations, prognostic and diagnostic value, immune infiltration, pathway enrichment, and other aspects of the *GINS* subunits. Next, the genes related to the *GINS* subunits were analyzed using the STRING and GeneMANIA platforms, and the correlation between GINS subunits and the functions involved were investigated.

Results: The expression level of *GINS1/2/3/4* was significantly higher in ccRCC tumor tissues than normal tissues, and was significantly related to tumor grade and stage. The expression of *GINS1/2/4* may be related to the methylation degree of the promoter region. The prognostic and diagnostic analyses showed that the increased expression of *GINS1* was associated with various poor prognoses and had diagnostic value. The *GINS* subunit mutation also significantly affected the clinical prognosis of ccRCC patients. Finally, the correlation analysis of the immune infiltration level, co-expression, and enrichment of related genes indicated that *GINS* subunit expression was associated with different levels of ccRCC immune infiltration.

Conclusions: The analysis results showed that the differential expression of *GINS* subunits in ccRCC, which had prognostic and diagnostic value, was correlated with clinicopathological stage, immune infiltration, and other related aspects. *GINS1* may serve as a new potential prognostic biomarker for ccRCC patients and be used to guide treatment.

Keywords: Go-Ichi-Nii-San subunits (GINS subunits); clear cell renal cell carcinoma (ccRCC); bioinformatics analysis; biomarker; prognosis

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most common histological type of renal cell carcinoma (RCC), and accounts for a high proportion of cancer-related deaths (1,2). According to the latest cancer statistics (3), in the United States, there are more than 65,000 new cases and almost 15,000 ccRCC-related deaths each year, and it is the sixth most common cancer in men. Currently, surgery is the main treatment for ccRCC, but there is still a high recurrence rate after radical surgery, and if distant organ metastasis occurs after surgery, the mortality rate is very high (4). As more and more cancer genome changes are sequenced, we are able to better understand the underlying molecular mechanisms of ccRCC (5,6). Therefore, it is of great importance to identify new biomarkers for the prognostic value analysis and clinical treatment of ccRCC.

Go-Ichi-Nii-San (*GINS*) subunits are a protein complex composed of *GINS1/2/3/4* in the human genome (7). GINS subunits act as a replication helicase to unwrap double-stranded DNA before replicating forks during chromosome replication (8). The upregulation or downregulation of *GINS* complex members has been reported to play an important role in the progression and prognosis of various cancers, including lung adenocarcinoma, adrenal cortical adenocarcinoma, breast cancer, hepatocellular carcinoma, cervical cancer, endometrial cancer, and bladder cancer (9-14).

In recent years, a number of studies have shown that GINS1 expression is upregulated in breast cancer, synovial sarcoma, liver cancer, pancreatic cancer, and other cancers, and the knockdown of the *GINS1* gene can inhibit the proliferation of pancreatic cancer cells (15). *GINS2* is also a promising biomarker. GINS2 expression is closely related to methyltransferase and the MMR protein, and thus affects the tumor immune microenvironment and immune invasion (16). It has been reported that *GINS3* has an effect on the prognosis of pancreatic cancer patients, and the expression of *GINS3* is related to the grade of pancreatic cancer (15). In addition, *GINS4* is highly expressed in esophageal squamous-cell carcinoma (ESCC), and promotes the growth, migration, and invasion of cancer cells. Jin *et al.* suggested that *GINS4*, which is regulated by the HCP5/miR-17-5p axis, could serve as a novel immune-related prognostic gene and therapeutic target for ESCC (17). Unfortunately, the role and value of *GINS* in ccRCC has not been thoroughly studied.

In this study, various analysis packages for R (version 3.6.3), the University of ALabama at Birmingham CANcer (UALCAN) data analysis portal, the cBio Cancer Genomics Portal (cBioPortal), and the Tumor Immune Estimation Resource (TIMER) were used to analyze *GINS* subunit expression, gene mutation, prognostic and diagnostic value, immune infiltration, pathway enrichment, and other aspects. In addition, we used the STRING and GeneMANIA platforms to analyze the genes associated with the *GINS* subunits and demonstrate their association with the related functions. In conclusion, *GINS1* may serve as a new potential prognostic biomarker for ccRCC patients and be used to guide treatment. We present this article in accordance with the REMARK reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-24-95/rc).

Highlight box

Key findings

- The increased expression of Go-Ichi-Nii-San (*GINS*) subunits may play an important role in the occurrence and progression of cancer in clear cell renal cell carcinoma (ccRCC) patients. Thus, *GINS1* may serve as new survival biomarker or potential therapeutic target for patients with ccRCC.

What is known, and what is new?

- *GINS* subunits are differentially expressed in the tumor tissue and normal tissue of multiple cancers, and play a role in the development and progression of tumors.
- *GINS* subunits are differentially expressed in tumor tissues and normal tissues of ccRCC patients, and are closely correlated with the prognosis and immunity of tumor patients.

What is the implication, and what should change now?

- *GINS1* may serve as a new survival biomarker or potential therapeutic target for patients with ccRCC. Our findings could improve the accuracy of prognostic prediction in patients with ccRCC.

Methods

GINS subunit mRNA and cell level expression profiles

R (version 3.6.3) (18) was used to conduct a relatively comprehensive analysis of the expression level of the *GINS* subunits in different tumor tissues and normal tissues in The Cancer Genome Atlas (TCGA) database and normal tissues in the Genotype-Tissue Expression (GTEx) database. The "ggplot2" package was used for the visualization. The Wilcoxon signed-rank test was used to evaluate statistical

significance (19). In the Cancer Cell Line Encyclopedia (CCLE) database (https://portals.broadinstitute.org/ccle/), the Kruskal-Wallis rank test was used to calculate the expression profile of *GINS* subunits in different tumor cell lines. The *GINS* subunit expression levels in the tumor tissues and normal tissues of ccRCC patients was analyzed by the UALCAN platform (http://ualcan.path.uab.edu) (20), an interactive web portal, which comprises the clinical data of 31 cancer types. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Correlation analysis of the clinicopathological parameters and promoter methylation levels of GINS subunits in ccRCC

First, we used the UALCAN database to analyze differences in *GINS* subunit expression in ccRCC patients with different pathological stages and grades. Subsequently, we used the Kruskal-Wallis test to analyze the RNA-sequencing and clinical data retrieved from TCGA to explore the relationship between *GINS* subunit expression and other histological parameters, including T stage, N stage, and M stage (parameter setting: $P<0.05$). Finally, the UALCAN platform was used to detect the relationship between *GINS* subunits and promoter methylation levels in ccRCC.

Prognostic and diagnostic value analysis

Based on the differences in the expression of the *GINS* subunits, a survival analysis was also performed using R (version 3.6.3). Specifically, the "survMiner" package (version 0.4.9) was used for the visualization, and the "survival" package (version 3.2-10) was used to conduct the statistical analysis of the survival data. Kaplan-Meier curves were used to demonstrate the overall survival (OS) and disease-specific survival (DSS) of ccRCC patients with high levels of GINS subunit expression. In addition, the UALCAN database was used to explore the effect of *GINS* subunit expression on survival in ccRCC patients. The receiver operating characteristic (ROC) curve was constructed, the area under the curve (AUC) value was calculated, and the diagnostic value of the GINS subunit in ccRCC and its relationship with the clinicopathological grade and stage of ccRCC patients were analyzed.

Genetic mutation analysis

Using the cBioPortal, TCGA (provisional) dataset was analyzed for alterations, including mutations and putative copy-number alterations (21). The sample comprised 510 patients with ccRCC. Using these patients from the TCGA database, we investigated the genetic mutations of *GINS* subunits and their relationship with OS, DSS, disease-free survival (DFS), and progression-free survival (PFS) in ccRCC patients. The results are shown in Kaplan-Meier curves. A P value <0.05 was considered statistically significant (22).

Analysis of immune infiltration level

The TIMER is a comprehensive web server for the systematic analysis of immune infiltrations across different cancer types (23). Using this online platform, we analyzed the *GINS* subunits to estimate the abundance of six immune cell types (B cells, CD4 T cells, CD8 T cells, neutrophils, macrophages, and dendritic cells) (24).

Correlation analysis

We evaluated the correlation among the *GINS* subunits using Pearson correlation (25) coefficients, and conducted a comprehensive statistical and graphic analysis using R (version 3.6.3). The results were visualized using "ggplot2". A P value <0.05 was considered statistically significant. Using the R (version 3.6.3) "stat" package (26), we obtained the top 20 ccRCC genes most associated with *GINS* subunit expression from TCGA database. A total of 80 genes related to *GINS1/2/3/4* were identified. After duplicates were removed, 55 genes remained that were used for the protein-protein interaction (PPI) and enrichment analyses.

PPI and enrichment analysis

STRING (https://string-db.org/) (27) is a website of PPIs that aims to establish a comprehensive and objective global network. It can also be used to retrieve known proteins and PPIs. We conducted a PPI network analysis to examine the different expressions and interactions of the genes related to the *GINS* subunits. GeneMANIA (http://www.genemania.org/) (28) is a resource rich website containing genetic information. It can be used to undertake analyses of gene lists and can prioritize gene function with high accuracy using prediction algorithms. We used it to identify the predictive genes associated with each *GINS* subunit, and elucidate the relationships and functions of these genes. In addition, we performed Gene Ontology (GO)

and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses (29). GO terms can be divided into the following three gene-set libraries: biological processes (BPs), molecular functions (MFs), and cellular components (CCs). Finally, the "clusterProfiler" package (version 3.14.3) of R (version 3.6.3) software was used to identify the enriched BPs, CCs, MFs, and KEGG pathways that had the strongest interactions with the *GINS* subunit proteins, and the results were visualized.

Statistical analysis

R (version) 3.6.3 software (The R Foundation for Statistical Computing, Vienna, Austria) and the Gene Expression Profiling Interactive Analysis (GEPIA), UALCAN, TIMER, STRING, and GeneMANIA databases were used for the analysis. The Wilcoxon test was used to compare differences between two groups. A P value <0.05 was considered statistically significant.

Results

Differential expression of GINS subunits in pancancer and ccRCC

The analysis of gene expression levels showed that the *GINS* subunits were upregulated or downregulated in tumors in pancancer. Compared with paracancer tissue and normal tissue, *GINS1/3/4* expression was significantly increased in 27 tumor tissues, while *GINS2* expression was significantly increased in 28 tumor tissues (*Figure 1*). Notably, *GINS1/2/3/4* expression was significantly higher in the kidney clear cell carcinoma (KIRC) tissues than the paracancer tissues and normal tissues. Based on these results, we can conclude that the differential expression of the *GINS* subunits is closely related to the occurrence and progression of human cancers, notably including ccRCC. Meanwhile, the *GINS* subunit expression levels in KIRC tissues and normal tissues were analyzed using the UALCAN platform and the GEPIA dataset, respectively. The results again showed that the expression of *GINS1*, *GINS2*, *GINS3*, and *GINS4* was higher in the KIRC samples than the normal kidney tissues (*Figure 2*).

GINS subunit expression at the cellular level and promoter methylation level

Figure 3A-3C show the relative GINS1/2/4 expression levels in the ccRCC cell lines in the CCLE. The results showed that *GINS1/2/4* was expressed in different ccRCC cell lines. The methylation level of the promoter region plays an important role in the genesis and progression of tumors. Therefore, we analyzed the methylation levels in the *GINS1/2/4* promoter regions of ccRCC tissues (*Figure 3D-3F*). We found that the promoter methylation levels of *GINS1/4* were significantly lower in the ccRCC tissues than the normal tissues, and the promoter methylation levels of *GINS2* were significantly higher in the ccRCC tissues than the normal tissues. These results suggest that the expression of *GINS1/4* may be related to the degree of methylation of the promoter region.

Correlation between GINS subunit expression and clinicopathological parameters in ccRCC patients

We then analyzed the correlation between the messenger RNA (mRNA) expression of the *GINS* subunits and tumor stage and grade in ccRCC patients. As *Figure 4A* shows, the high expression of *GINS1/2/3/4* mRNA in tumor tissues was correlated with tumor stage, and *GINS1/2/4* mRNA expression levels were the highest in tumor stage IV. Figure S1 shows the relationship between T stage, N stage, and M stage, and the mRNA expression levels of the *GINS* subunits and tumor stage in more detail. As *Figure 4B* shows, the high expression of *GINS1/2/3/4* mRNA in tumor tissues was correlated with tumor grade. Moreover, the mRNA expression level of *GINS2* was the highest in grade IV tumor tissues. In conclusion, the mRNA differential expression of *GINS* subunits was significantly correlated with the grade and stage of ccRCC patients.

Prognostic and diagnostic role of GINS subunit expression in ccRCC patients

Figure 5 shows the correlation between the differential mRNA expression of the *GINS* subunits and the clinical prognosis of ccRCC patients. A high expression of *GINS1* (P<0.001) was significantly correlated with poor OS in ccRCC patients, while the high expression of *GINS1* (P=0.02), *GINS2* (P=0.02), and *GINS3* (P=0.02) was significantly correlated with poor DSS in ccRCC patients. To further examine the clinical factors associated with survival, we first performed a univariate logistic regression analysis, and found that age [hazard ratio (HR) =1.02909, 95% confidence interval (CI): 1.01633–1.042, P<0.001], T stage (HR =1.91232, CI: 1.62501–2.25043, P<0.001), N

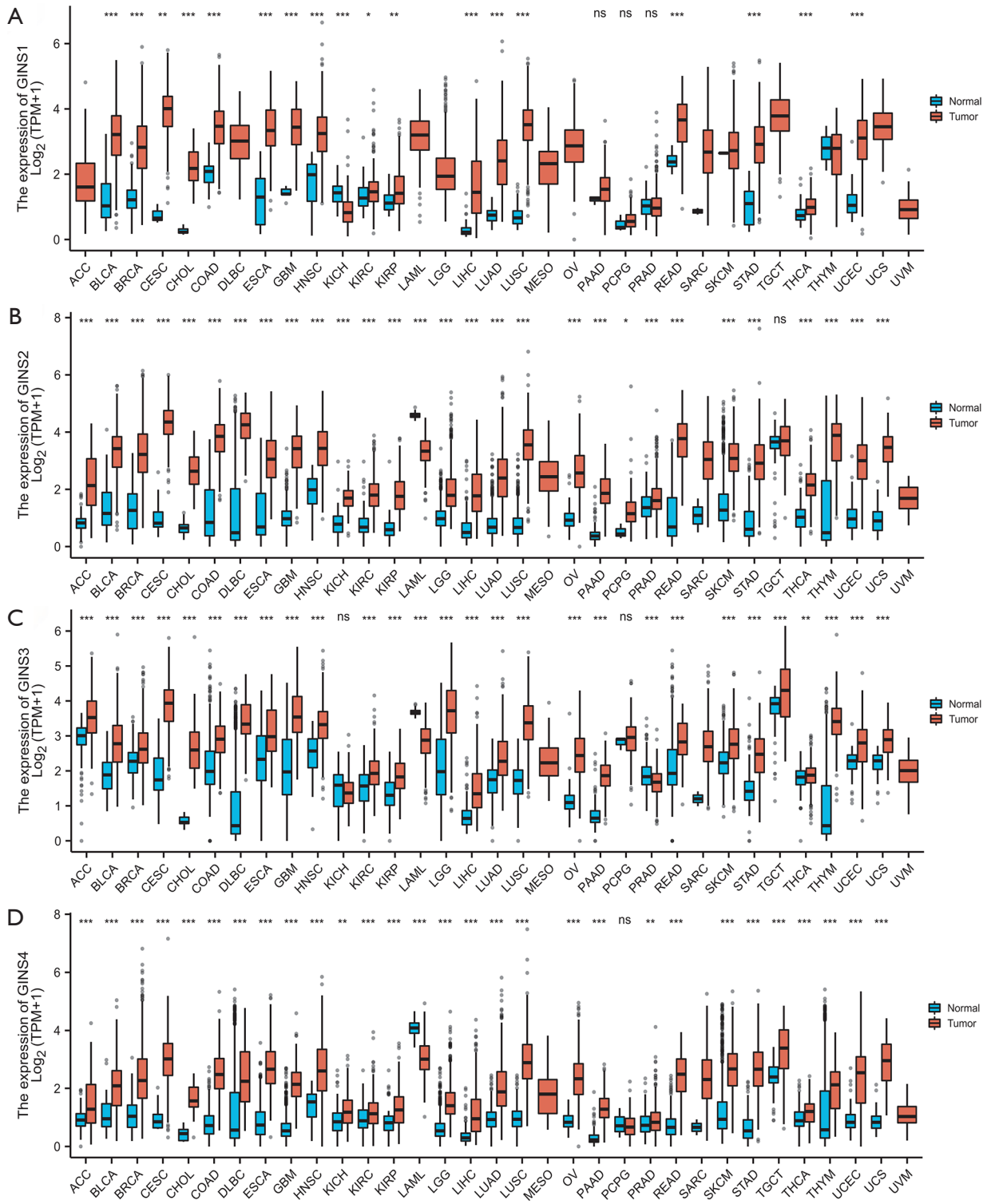

Figure 1 The expression mRNA levels of the *GINS* subunits in different tumor tissues and in TCGA database and normal tissues in the GTEx database. The expression mRNA levels of (A) *GINS1*, (B) *GINS2*, (C) *GINS3*, and (D) *GINS4*. ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001. TPM, transcripts per million; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS3*, Go-Ichi-Nii-San3; *GINS4*, Go-Ichi-Nii-San4; *GINS*, Go-Ichi-Nii-San; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression.

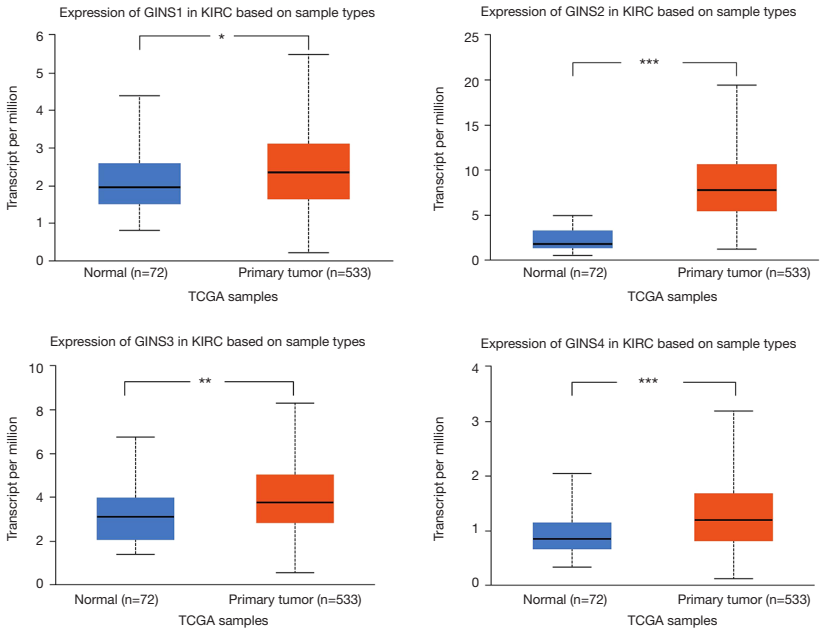

Figure 2 Expression of *GINS* subunits in KIRC in the UALCAN database. *, P<0.05; **, P<0.01; ***, P<0.001. TCGA, The Cancer Genome Atlas; KIRC, kidney clear cell carcinoma; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS3*, Go-Ichi-Nii-San3; *GINS4*, Go-Ichi-Nii-San4; *GINS*, Go-Ichi-Nii-San; UALCAN, The University of ALabama at Birmingham CANcer data analysis Portal.

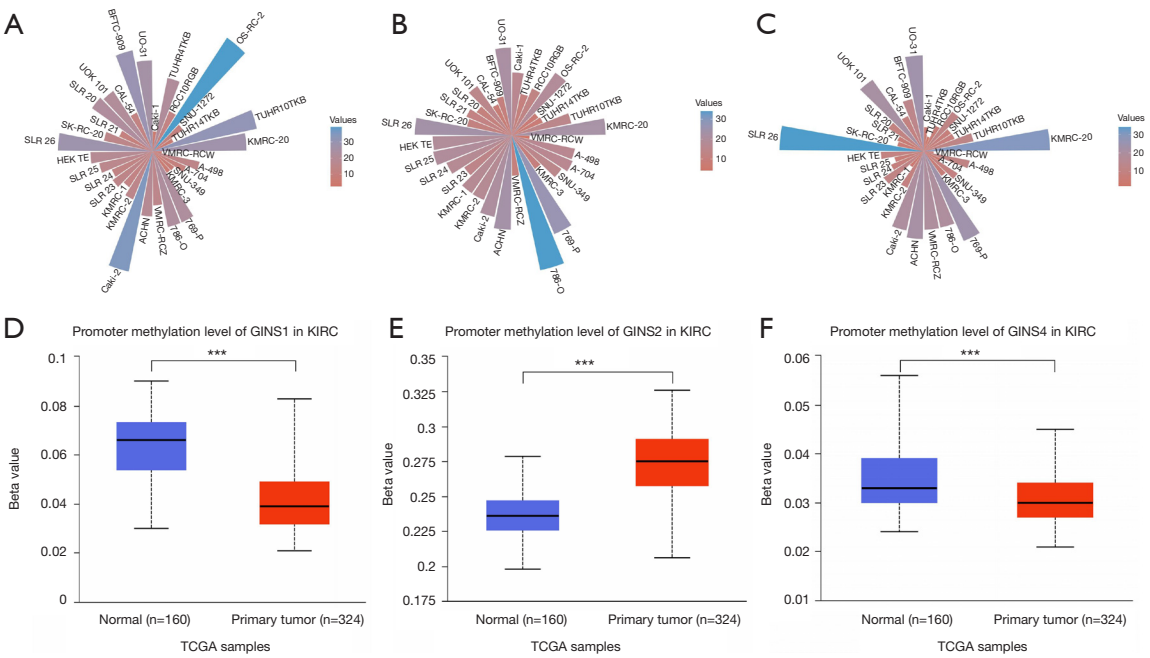

Figure 3 *GINS* subunit expression at the cellular level and promoter methylation level. (A) Expression of *GINS1* in ccRCC cell lines. (B) Expression of *GINS2* in ccRCC cell lines. (C) Expression of *GINS4* in ccRCC cell lines. (D) Promoter methylation level of *GINS1* in KIRC. (E) Promoter methylation level of *GINS2* in KIRC. (F) Promoter methylation level of *GINS4* in KIRC. ***, P<0.001. TCGA, The Cancer Genome Atlas; KIRC, kidney clear cell carcinoma; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS4*, Go-Ichi-Nii-San4; *GINS*, Go-Ichi-Nii-San; ccRCC, clear cell renal cell carcinoma.

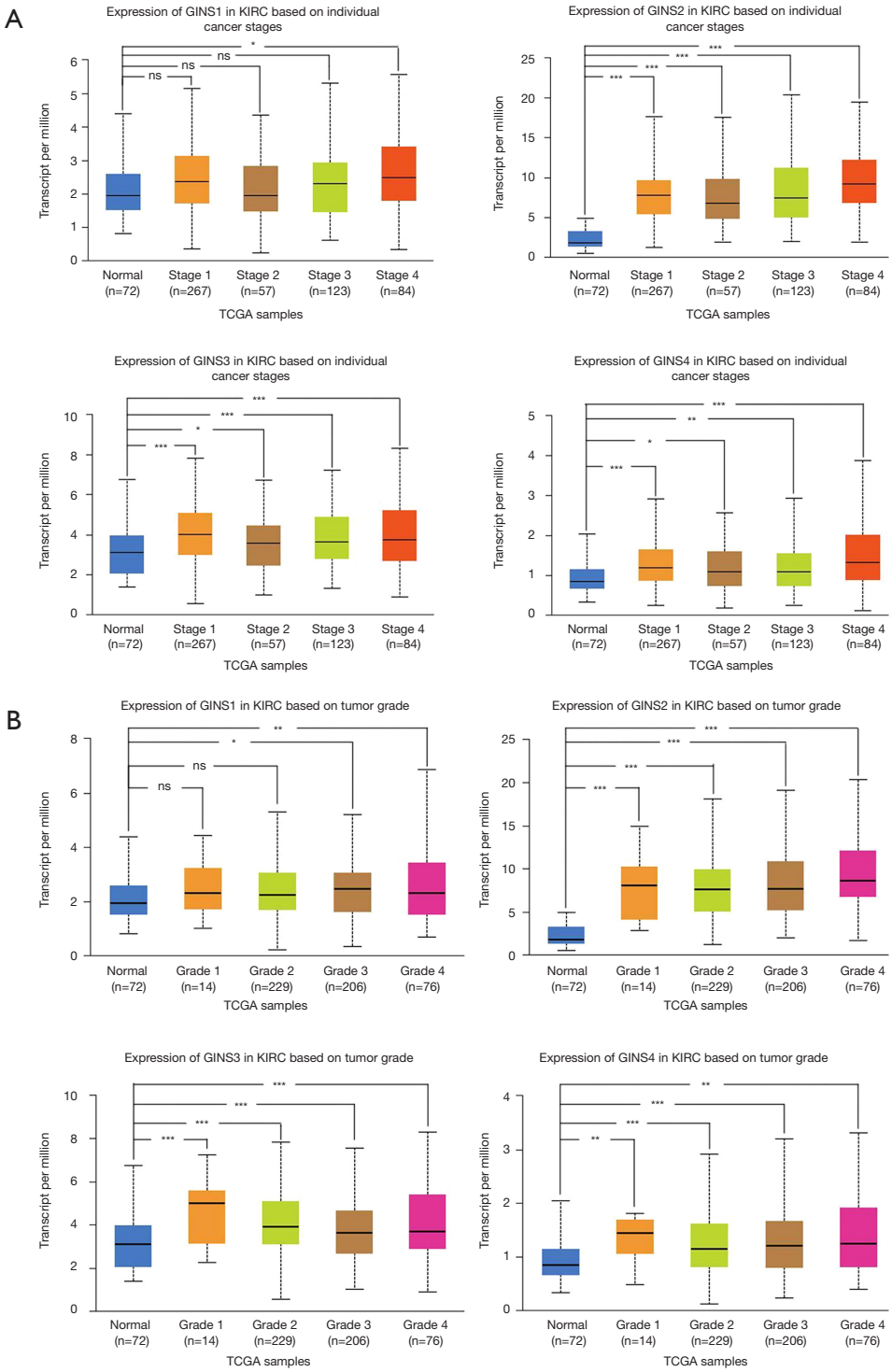

Figure 4 The relationship between the mRNA expression of different *GINS* subunits in TCGA database and the cancer stage and grade of ccRCC patients (including data from 72 normal subjects and 531 ccRCC patients). (A) The mRNA expression of the *GINS* subunits (*GINS1/2/3/4*) was correlated with the cancer stage of patients. (B) The mRNA expression of the *GINS* subunits (*GINS1/2/3/4*) was correlated with the cancer grade of patients. ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001. TCGA, The Cancer Genome Atlas; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS3*, Go-Ichi-Nii-San3; *GINS4*, Go-Ichi-Nii-San4; *GINS*, Go-Ichi-Nii-San; ccRCC, clear cell renal cell carcinoma.

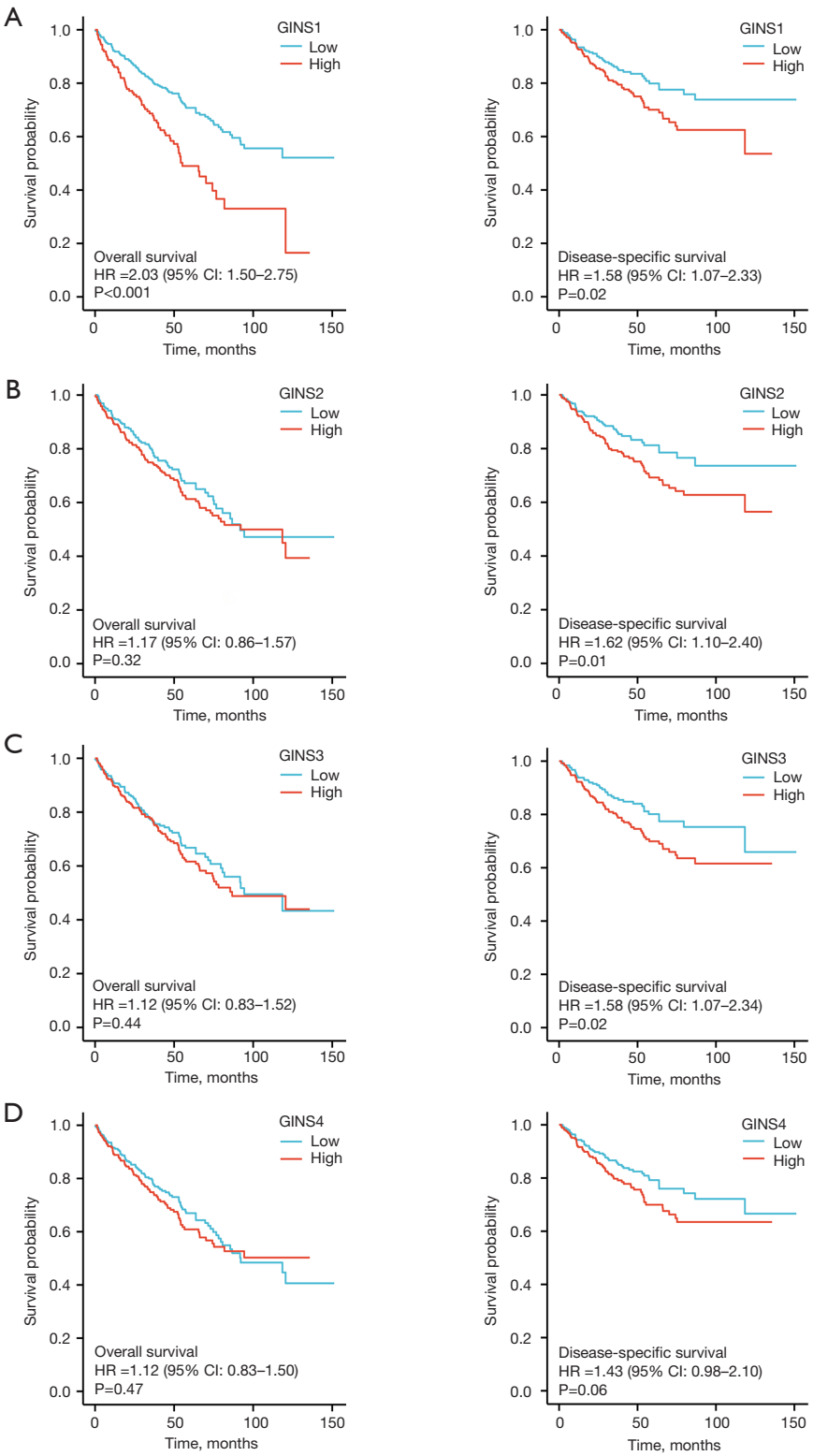

Figure 5 Relationship between the expression level of *GINS* subunits and the prognosis of ccRCC patients. (A) *GINS1*; (B) *GINS2*; (C) *GINS3*; and (D) *GINS4*. HR, hazard ratio; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS3*, Go-Ichi-Nii-San3; *GINS4*, Go-Ichi-Nii-San4; *GINS*, Go-Ichi-Nii-San; ccRCC, clear cell renal cell carcinoma.

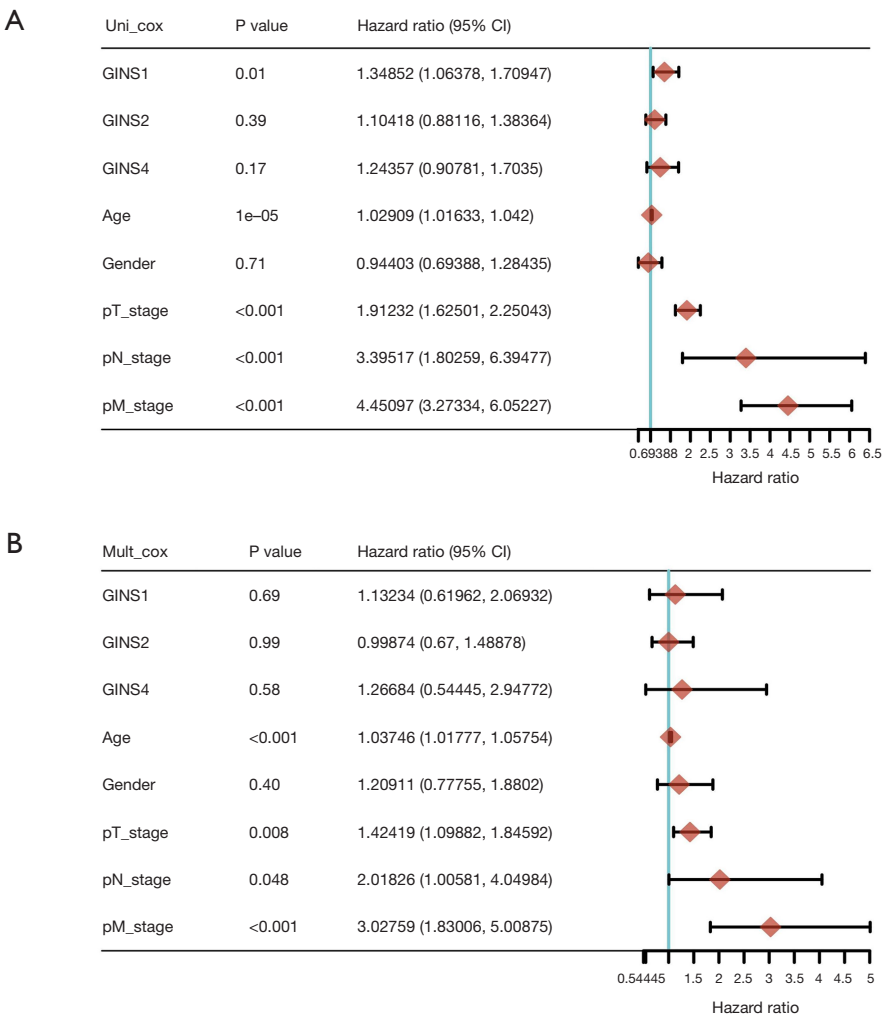

Figure 6 Univariate logistic and multivariate Cox regression analyses showing the hazard ratios for different clinical variables. (A) Univariate analysis. (B) Multivariate analysis. CI, confidence interval; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS4*, Go-Ichi-Nii-San4.

stage (HR =3.39517, 95% CI: 1.80259–6.39477, P<0.001), M stage (HR =4.45097, 95% CI: 3.27334–6.05227, P<0.001), and *GINS1* expression level (HR =1.34852, 95% CI: 1.06378–1.70947, P=0.01) were significantly associated with OS (*Figure 6A*). The multivariate Cox regression analysis showed that age (HR =1.03746, 95% CI: 1.01777–1.05754, P<0.001), T stage (HR =1.42419, 95% CI: 1.09882–1.84592, P=0.00754), N stage (HR =2.01826, 95% CI: 1.00581–4.04984, P=0.048), and M stage (HR =3.02759, 95% CI: 1.83006–5.00875, P<0.001) were independent prognostic factors for ccRCC (*Figure 6B*). Meanwhile, data from the UALCAN platform also showed that high *GINS1* expression was significantly associated with poor OS in ccRCC patients (Figure S2). In addition, the diagnostic value of the differential mRNA expression of *GINS1/2/4* was also examined in the study. The larger the AUC value, the higher the diagnostic value. The results showed that the expression of *GINS2* had high diagnostic value in all patients (AUC =0.932), patients with various pathological stages (AUC =0.931), and patients with various tissue grades (AUC =0.932) (*Figure 7*).

Gene mutation of GINS subunits and their correlation with prognosis in ccRCC patients

As *Figure 8A* shows, among 99 samples of 510 patients

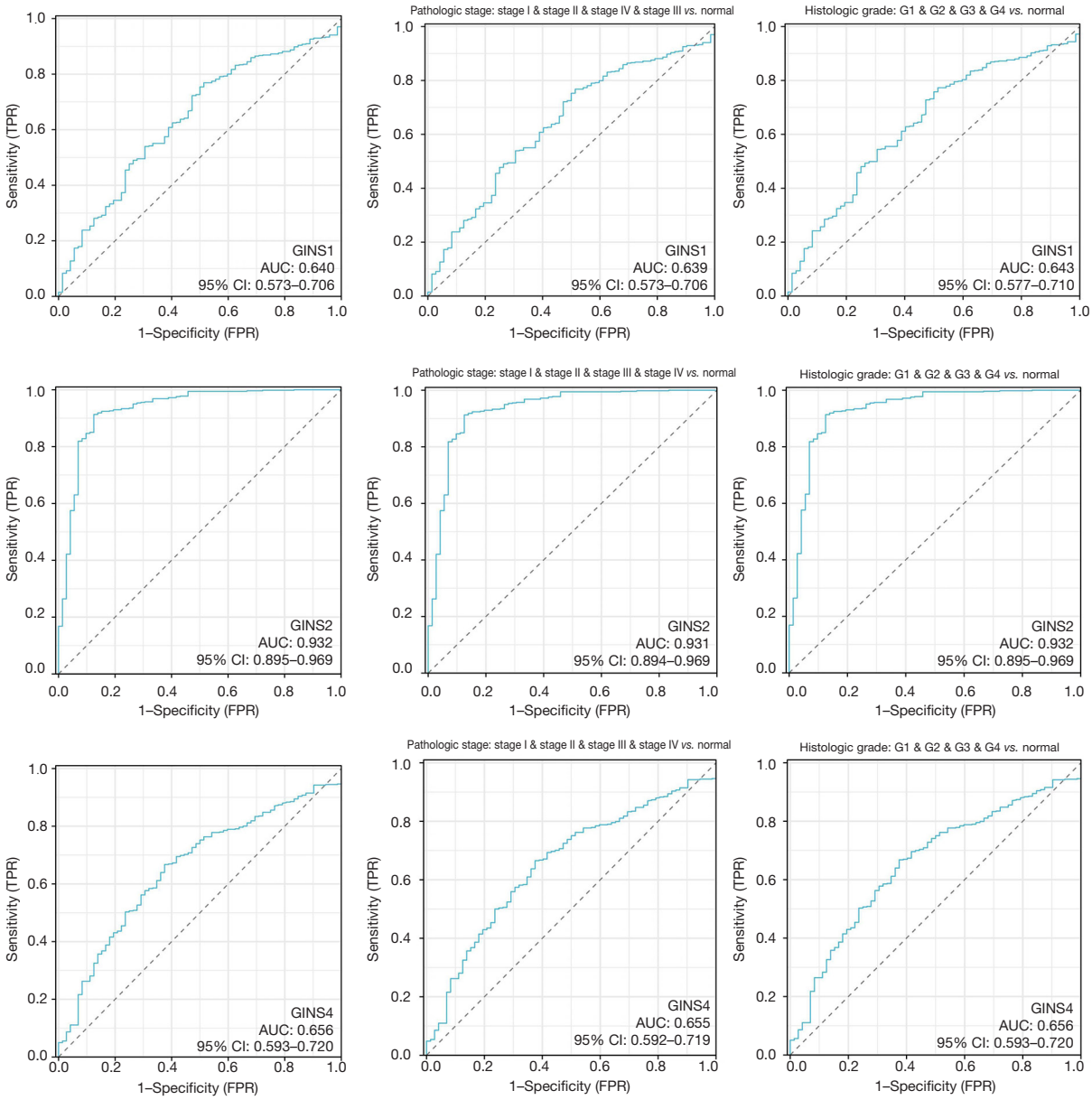

Figure 7 The diagnostic value of *GINS1/2/4* in ccRCC patients. TPR, true positive rate; AUC, area under the curve; CI, confidence interval; FPR, false positive rate; ROC, receiver operating characteristic; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS4*, Go-Ichi-Nii-San4; *GINS*, Go-Ichi-Nii-San; ccRCC, clear cell renal cell carcinoma.

(19%), the mutation rates of *GINS1*, *GINS2*, *GINS3* and *GINS4* were 8%, 7%, 7%, and 8%, respectively. The mRNA expression z-scores of the *GINS* subunits relative to the normal samples were also determined. In addition, log-rank tests and Kaplan-Meier plots of the cBioPortal platform were used to analyze the relationship between the *GINS* subunit gene mutations and the clinical prognosis of ccRCC patients. According to the results, the *GINS* subunit gene mutations were significantly associated with poor OS (P=6.414e−4), DSS (3.907e−5), and PFS (P=0.01) in ccRCC patients. Conversely, the mutations were not associated with DFS in ccRCC patients (P=0.15) (*Figure 8B*). Therefore, gene changes in *GINS* subunits also have a statistically significant effect on the clinical prognosis of ccRCC patients.

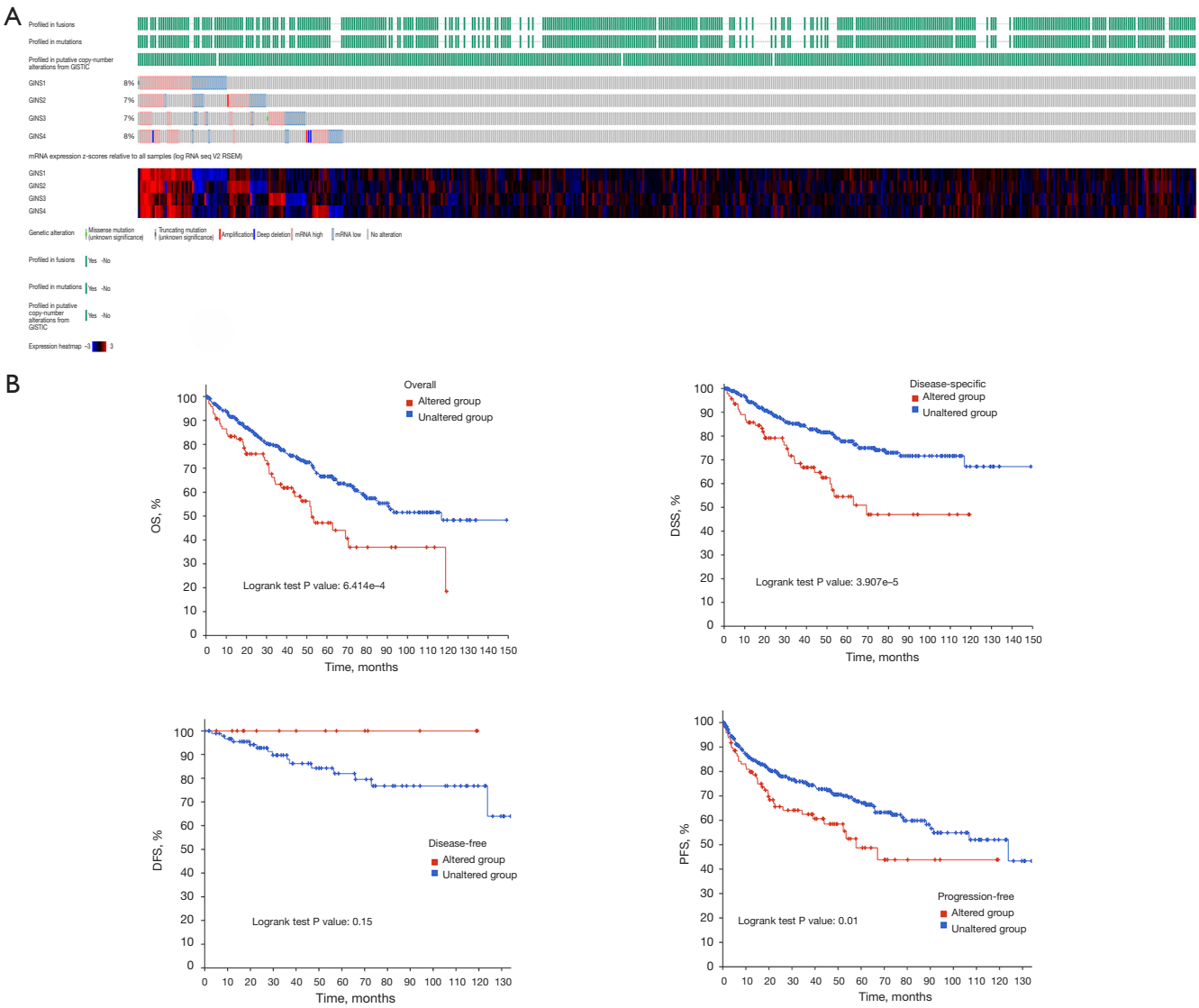

Figure 8 Genetic mutations in *GINS* subunits and their association with the OS, DSS, DFS, and PFS of ccRCC patients. (A) The mutation rates of *GINS1*, *GINS2*, *GINS3*, and *GINS4* were 8%, 7%, 7% and 8%, respectively, and the mRNA expression z-scores of *GINS* subunits relative to the normal samples. (B) Genetic mutations in *GINS* subunits were associated with OS, DSS, DFS, and PFS in ccRCC patients. *GINS1*, Go-Ichi-Nii-*San1*; *GINS2*, Go-Ichi-Nii-San2; *GINS3*, Go-Ichi-Nii-San3; *GINS4*, Go-Ichi-Nii-San4; GISTIC, genomic identification of significant targets in cancer; OS, overall survival; DSS, disease-specific survival; DFS, disease-free survival; PFS, progression-free survival; *GINS*, Go-Ichi-Nii-San; ccRCC, clear cell renal cell carcinoma.

Correlation between GINS subunits mRNA expression and immune infiltration levels in ccRCC patients

Based on the above analysis results of the *GINS* subunits in ccRCC patients, we further analyzed the correlation of the *GINS1/2/3/4* subunits with tumor immune cells in ccRCC patients. Six common tumor immune cells were included in the analysis (i.e., B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells). We found that the four subunits were significantly negatively correlated with tumor purity in ccRCC patients. The expression level of *GINS1* was positively correlated with the levels of infiltrating B cells (r=0.268, P=5.78e−09), $CD8^+$ T cells (r=0.15, P=0.002), $CD4^+$ T cells (r=0.228, P=7.51e−07), macrophages (r=0.375, P=2.10e−16), neutrophils (r=0.383,

P=1.80e−17), and dendritic cells (r=0.324, P=1.31e−12) (*Figure 9A*). The expression level of *GINS2* was positively correlated with the levels of infiltrating B cells (r=0.136, P=0.003), $CD8^+$ T cells (r=0.093, P=0.051), macrophages (r=0.047, P=0.32), neutrophils (r=0.095, P=0.04), and dendritic cells (r=0.139, P=0.003), but was negatively correlated with $CD4^+$ T cells (r=−0.031, P=0.501) (*Figure 9B*). The expression level of *GINS3* was positively correlated with infiltrating B cells (r=0.111, P=0.02), $CD8^+$ T cells (r=0.164, P=5.55e−04), $CD4^+$ T cells (r=0.342, P=4.20e−14), macrophages (r=0.354, P=1.20e−14), neutrophils (r=0.39, P=4.58e−18), and dendritic cells (r=0.289, P=3.51e−10) (*Figure 9C*). The expression level of *GINS4* was positively correlated with infiltrating B cells (r=0.228, P=7.96e−07), $CD8^+$ T cells (r=0.105, P=0.03), $CD4^+$ T cells (r=0.276, P=1.81e−09), macrophages (r=0.34, P=1.34e−13), neutrophils (r=0.361, P=1.45e−15), and dendritic cells (r=0.318, P=3.74e−12) (*Figure 9D*). These results suggest that the expression of some *GINS* subunits is associated with different levels of immune infiltration in ccRCC. To ensure the accuracy of the results, we also used the Microenvironment Cell Populations-Counter (MCP-Counter) deconvolution method in the TIMER tool to analyze the correlation between *GINS1/2/4* expression and immune infiltration (*Figure 10*).

Co-expression and correlation analyses

After exploring the gene change patterns and immune infiltration levels of the *GINS* subunits in ccRCC patients, we further analyzed the potential co-expression of differential genes in ccRCC. Notably, as *Figure 11A* shows, the expression of *GINS1*, *GINS2*, *GINS3*, and *GINS4* showed a medium-high positive correlation. Subsequently, we selected the top 20 genes with the highest correlation with each *GINS* subunits, and the results are shown in *Figure 11B*.

PPI and enrichment analyses

In addition, we performed a PPI network analysis using the STRING platform of the differentially expressed *GINS* subunits to explore their potential interactions (*Figure 12A*). The GeneMANIA results also showed that the main functions of the differentially expressed *GINS* subunits and their associated molecules (e.g., *CDC45*, *MCM7*, *MCM2*, *WDHD1*, and *EXO1*) included DNA-dependent DNA replication, the DNA replication preinitiation complex, recombinational repair, nuclear DNA replication, and the protein-DNA complex (*Figure 12B*). We used R (version 3.6.3) to analyze the GO and KEGG pathways of the 55 genes that interact most with the *GINS* subunits proteins (*Figure 12C*). The GO analysis showed that the significantly enriched BP terms included "DNA conformational change", "DNA-dependent DNA replication" and "DNA replication", the significantly enriched CC terms mainly included "chromosome", "central region", "chromosome region", and "concentrated chromosome", and the significantly enriched MF terms mainly included "catalytic activity", "action on DNA", "DNA helicase activity", and "DNA replication origin binding". The KEGG pathway analysis showed that the target genes were mainly related to the "Fanconi anemia pathway", "cell cycle", and "DNA replication".

Discussion

GINS1 is a member of the *GINS* complex, which is reported to be involved in the initiation and elongation processes of DNA replication (30). Related study has shown that *GINS1* not only participates in early embryogenesis under physiological conditions, but also participates in tumor genesis under pathological conditions (31). *GINS1* has been found to be highly expressed in lung cancer, and it is associated with increased tumor proliferation and the poor prognosis of patients (32-34). Research (1) has also shown its overexpression by promoter hypermethylation in breast cancer, which suggests that the *GINS1* has potential diagnostic and prognostic value.

In recent years, immunotherapy has become a hot research direction in oncotherapy. Immunotherapy alone or in combination has been shown to suppress progression in cancer patients with Eastern Cooperative Oncology Group performance status scores of 0 and 1 (35). The tumor mutation burden and gene expression profile can be used as predictors of the immune checkpoint inhibitor response (36). In addition, the expression level of *GINS1* has been shown to be positively correlated with $CD8^+$ T cell infiltration, which suggests that immunotherapy could be groundbreaking in the treatment of KIRC (37).

Many studies have reported that *GINS1* is related to the proliferation of tumor cells. Tang *et al.* found that the knockdown of *GINS1* can significantly inhibit its abnormally high expression, which in turn can inhibit tumor cell proliferation and promote cell apoptosis; thus, *GINS1* may be a potential target for inhibiting the proliferation of

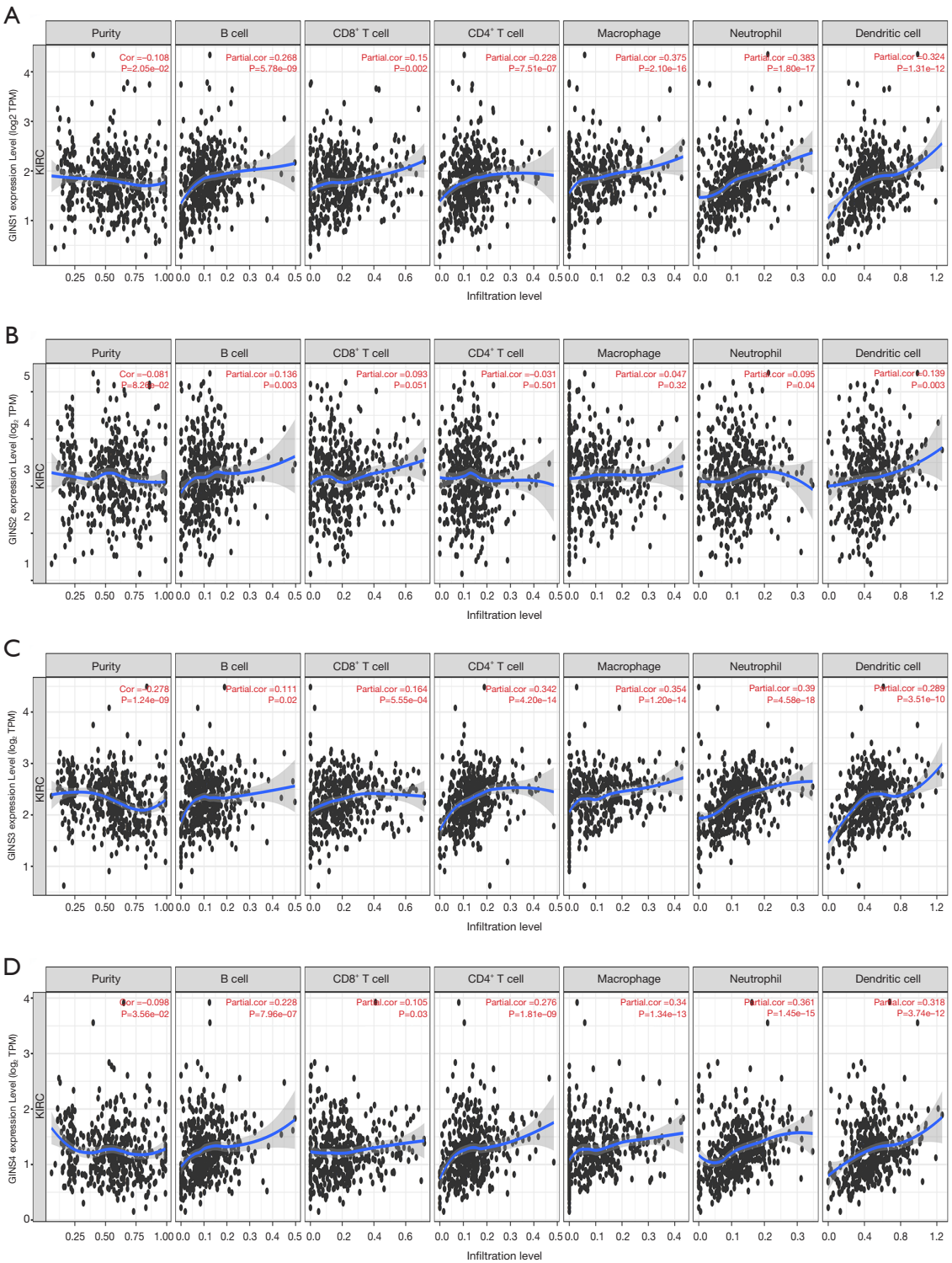

Figure 9 The correlation between the mRNA expression levels of the *GINS* subunits (*GINS1/2/3/4*) and the level of immune infiltration in ccRCC. TPM, transcripts per million; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS3*, Go-Ichi-Nii-San3; *GINS4*, Go-Ichi-Nii-San4; *GINS*, Go-Ichi-Nii-San; ccRCC, clear cell renal cell carcinoma.

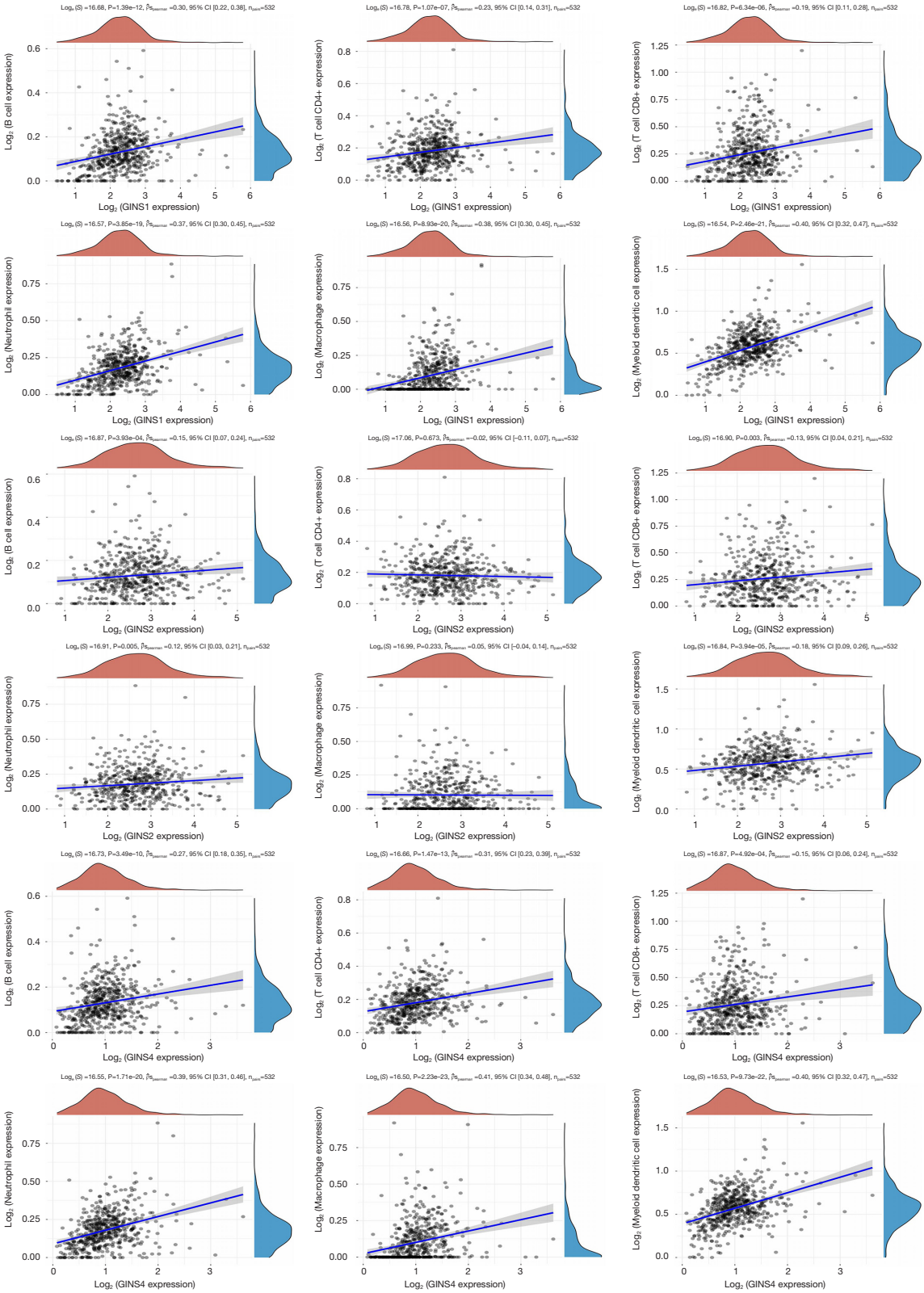

Figure 10 Comparison of differentially expressed *GINS1/2/4* with MCP-counter scores of immune cells. *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS4*, Go-Ichi-Nii-San4; TPM, transcripts per million; MCP, microenvironment cell population.

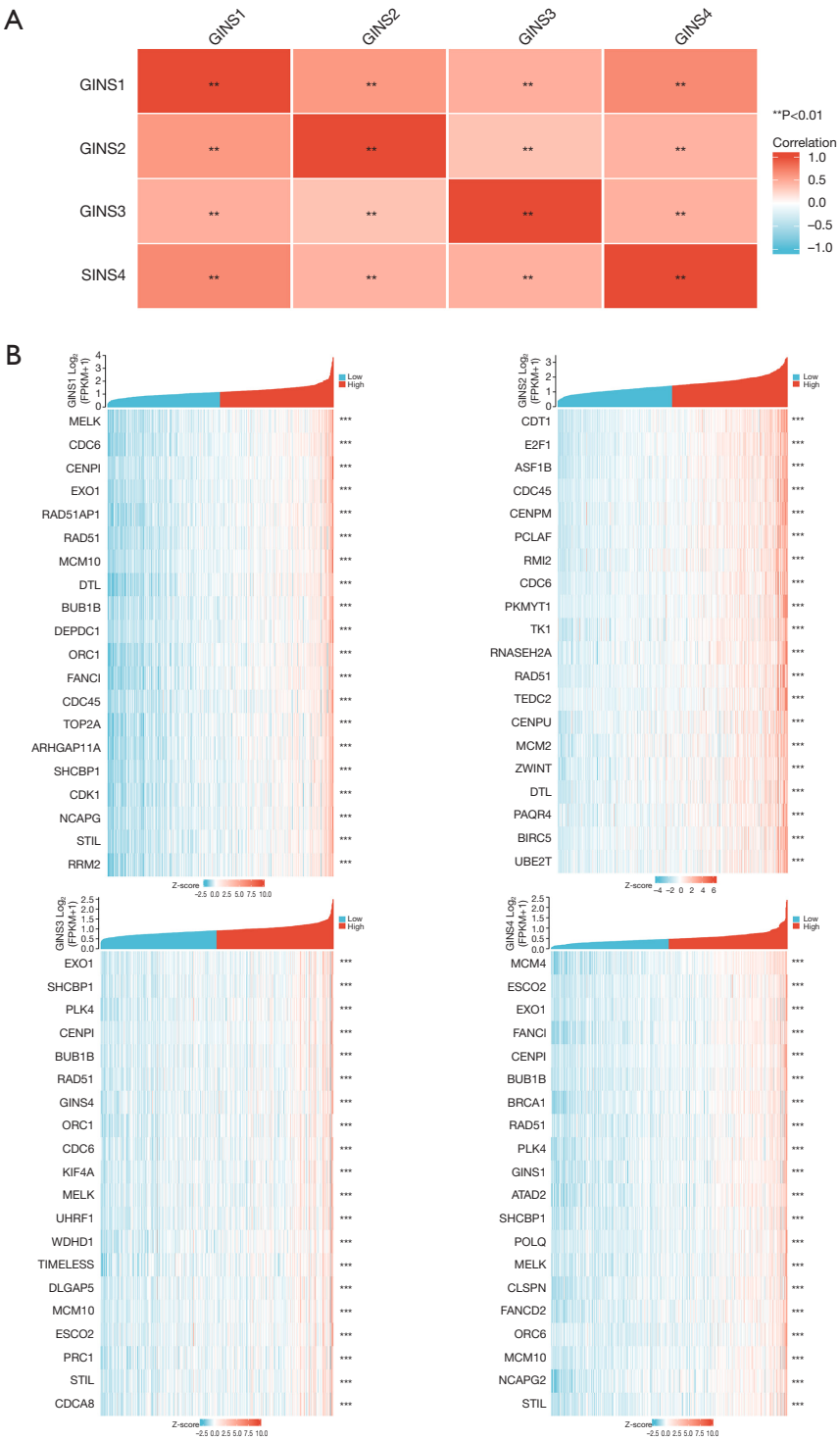

Figure 11 Correlation heatmaps of the *GINS* subunits and their most correlated genes in ccRCC. (A) Correlation heatmap of the differentially expressed *GINS* subunits in ccRCC. The red and blue cells indicate co-occurrence and mutual exclusivity, respectively. (B) Correlation heatmap of *GINS* subunit expression and their top 20 correlated genes. ***, P<0.001. FPKM, fragments per kilobase million; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS3*, Go-Ichi-Nii-San3; *GINS4*, Go-Ichi-Nii-San4; *GINS*, Go-Ichi-Nii-San; ccRCC, clear cell renal cell carcinoma.

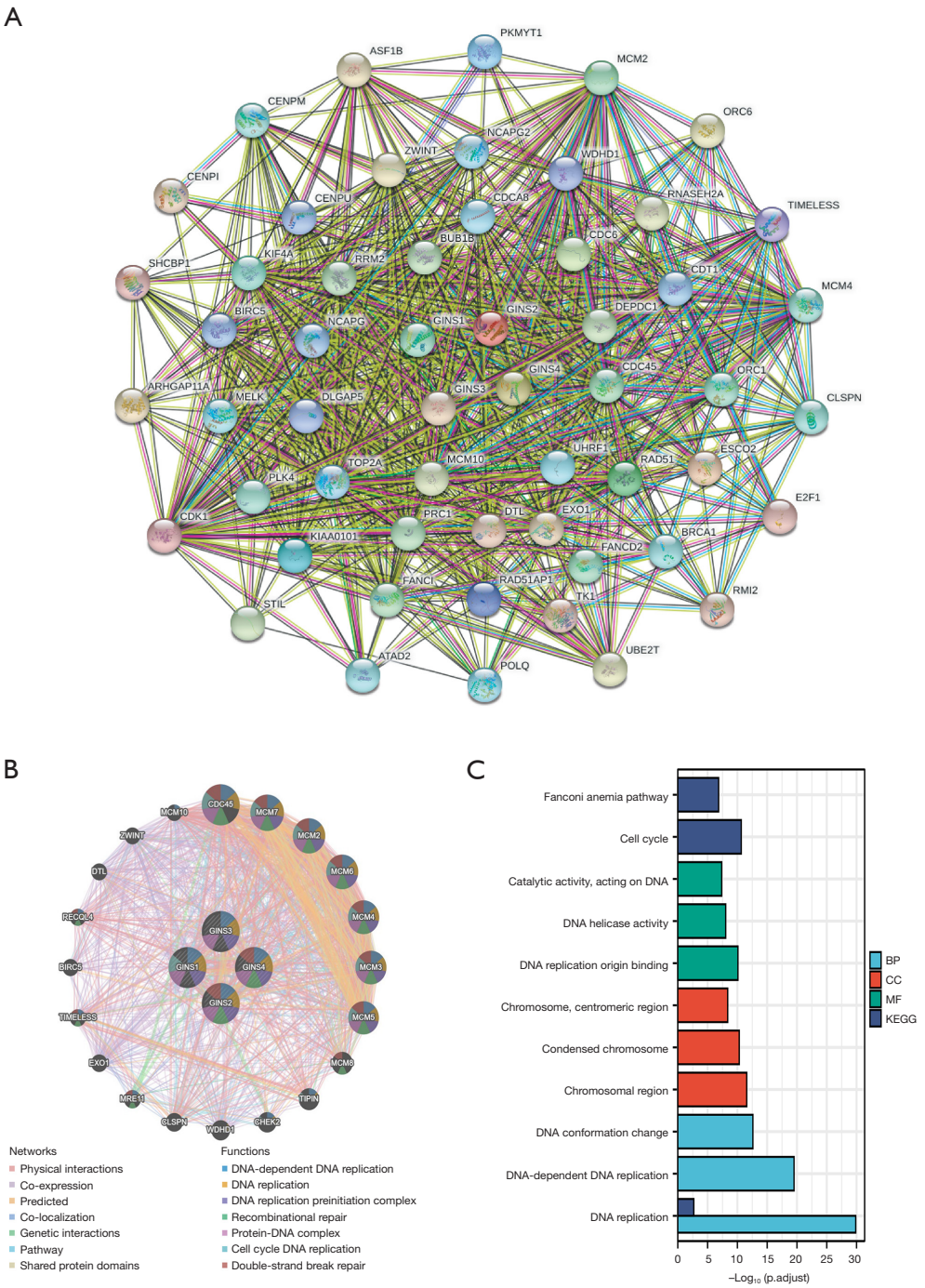

Figure 12 *GINS* subunit-related gene analysis. (A) Protein-protein interaction network of the differentially expressed *GINS* subunits. (B) GeneMANIA was used to identify the predicted genes that were correlated with the *GINS* subunits; 20 of the predicted genes were located in the outer circle while *GINS1/2/3/4* were located in the inner circle. The color of each line illustrates the different types of relationships. The color inside the gene dots indicates the functions in which these genes are involved. (C) The GO and KEGG pathway terms of the 55 genes with the strongest interaction with the *GINS* subunits. BP, biological progress; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopaedia of Genes and Genomes; *GINS*, Go-Ichi-Nii-San; *GINS1*, Go-Ichi-Nii-San1; *GINS2*, Go-Ichi-Nii-San2; *GINS3*, Go-Ichi-Nii-San3; *GINS4*, Go-Ichi-Nii-San4; GO, Gene Ontology.

synovial sarcoma (38,39). Silencing *MALAT1* can reduce GINS1 expression by interfering with the transcription process, inhibiting the proliferation of non-small cell lung cancer (40). This is consistent with the differential expression, correlation of clinicopathological parameters, and clinical prognosis analysis results of *GINS1* in this study. The expression of *GINS1* was significantly higher in the tumor samples of the ccRCC patients than the normal tissues. In terms of tumor grade and stage, both stage IV and grade IV showed the highest expression levels. A high expression level also affected the OS of the ccRCC patients.

GINS2 is a newly discovered gene located on chromosome 16q24. It encodes proteins with a relative molecular weight of 21,000 Da and a mRNA length of 1,196 bp. Its structure is a replicative helicase, it is capable of opening double strands before replication, and it thus plays an important role in initiating DNA replication (41). The overexpression of *GINS*2 affects the occurrence and progression of various tumors. It has been reported that *GINS2* is upregulated or downregulated in cholangiocarcinoma (42), lung cancer (43), breast cancer (44), and cervical cancer (10), and influences the early development and metastasis of these tumors. Methyltransferase can promote the methylation of CpG island gene loci, leading to the development of pancancer. Previous studies have shown that *GINS2* is closely related to methyltransferase in pancancer, including a variety of RCCs, such as kidney chromophobe (KICH) and kidney papillary cell carcinoma (KIRP). Therefore, it can also inhibit the overexpression of *GINS2* and thus inhibit tumor development (16). In this study, we found that *GINS2* was highly expressed in the tumor tissues of ccRCC patients, and was expressed in the highest grade and stage of tumors, indicating that it may be significantly correlated with tumorigenesis and malignant progression in ccRCC patients. In terms of its diagnostic value, *GINS2* had an AUC of 0.932. Based on these results, we believe that its differential expression could serve as an important biomarker in the diagnosis of ccRCC patients.

Like *GINS1* and *GINS2*, *GINS3* also contributes to cancer progression in humans. Research has shown that the overexpression of *GINS3* reduced the survival of breast cancer patients (1), and in human colorectal cancer, the knockdown of the *GINS3* gene inhibited the proliferation of cancer cells. In addition, Bu *et al.* found that *GINS3* is a potential biomarker for pancreatic cancer (15). The *GINS3* protein is an important component of the Cdc45-MCM-GINS (CMG) helicase, and its role is to separate DNA double strands to ensure DNA affinity to DNA polymerases during replication (45). The *GINS3* gene profoundly affects the vitality of human cells (46). CMG helicase components include *CDC45* (47), *MCM5* (48), *MCM3*, *MCM*7 (49), *GINS2* (50), *MCM4*, and *GINS1* (45). Similarly, in the co-expression and correlation analyses in this study, the analysis of the 20 genes with the highest co-expression and correlation with *GINS3* also included some of the above genes. In the PPI network analysis, *GINS3*, as a core gene, was also established to have multiple association lines with these genes. Thus, we demonstrated that *GINS3* protein, as a member of the *GINS* complex, is critical in DNA replication and thus further affects tumor progression.

GINS4, also known as *Sld5*, plays a role in the initiation and elongation of DNA replication (51). *GINS4* regulates early embryogenesis in mice and plays an important role in cell cycle progression and the maintenance of genomic stability in drosophila, suggesting that it has an effect on tumorigenesis (8,52). Study has shown that *GINS4* activates *Rac1* and *CDC42 in vivo* and *in vitro*, thereby activating their downstream signaling pathways (53). These pathways affect cell proliferation and apoptosis, and promote the migration and invasion of gastric cancer cells. *GINS4* could also induce ferroptosis, a kind of programmed cell death, in EMT-specific LUAD. In short, *GINS4* has great potential to provide therapeutic targets for tumors (54). In addition, *GINS4* has been shown to be associated not only with immune cell infiltration in ESCC, but also with immune cell infiltration in many other cancers. In this study, we used the TIMER tool to analyze the relationship between *GINS4* and the level of immune invasion of ccRCC, and found that *GINS4* was negatively correlated with the purity of ccRCC patients and positively correlated with infiltrating B cells (r=0.228, P=7.96e−07), $CD4^+$ T cells (r=0.105, P=0.03), $CD8^+$ T cells (r=0.276, P=1.81e−09), macrophages (r=0.34, P=1.34e−13), neutrophils (r=0.361, P=1.45e−15), and dendritic cells (r=0.318, P=3.74e−12). Therefore, we believe that *GINS4* could be used as an immune-related prognostic gene in ccRCC patients.

This study is the first to explore the differential expression of *GINS* subunits in the tumor tissues of ccRCC patients in a relatively comprehensive way, and its correlation with clinicopathological parameters, diagnostic and prognostic value, immune infiltration level and other relevant aspects; however, there are still some limitations. First, most of our research was conducted through online databases, resulting in some heterogeneity in the research results. Second, the conclusions of this

study were obtained through a bioinformatics analysis. Therefore, further clinical trial validation is still needed to obtain more accurate results, and *GINS* subunits will be used as new biomarkers to predict transitional diagnosis and prognosis of ccRCC, providing better guidance for the clinical treatment of ccRCC patients. In the future, scientists will need to focus on developing more precise and individualized treatments, with immunotherapy, targeted therapy, and combination therapy options being the main trends. Among them, we believe that targeted therapy has a more prominent advantage in its transformation to clinical application. In addition, researchers need to further explore unique biomarkers to prevent drug resistance in patients with RCC and develop personalized treatment plans.

Conclusions

We used bioinformatics methods to systematically analyze the differential expression, prognosis, and immunological correlation of *GINS* subunits in ccRCC. Our findings suggest that *GINS1* may serve as a novel biomarker or potential therapeutic target for patients with ccRCC.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-24-95/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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