Original Article

A novel prognostic biomarker: *GINS3* is correlated with methylation and immune escape in liver hepatocellular carcinoma

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Background: Liver cancer remains one of the tricky malignancies nowadays. GINS complex subunit 3 (*GINS3*), part of the *GINS* tetrameric complex, is significantly upregulated in many cancers, including liver hepatocellular carcinoma (LIHC). With the development of liver cancer treatment, immune and molecular targeted therapy gradually becomes a promising treatment. However, the key target for liver cancer is still indistinct. Herein, the underneath mechanism of *GINS3* was investigated to verify its role as a biomarker in LIHC.

Methods: Genomic expression, genetic alteration, and methylation analyses were obtained from The Cancer Genome Atlas (TCGA), Clinical Proteomic Tumor Analysis Consortium (CPTAC), The University of Alabama at Birmingham CANcer (UALCN), and Human Protein Atlas (HPA), cBioPortal, and MethSurv databases. Subsequently, the diagnostic and prognostic role of *GINS3* in LIHC were analyzed based on data from receiver operating characteristic (ROC), Kaplan-Meier plotter (KM-plotter), and univariate and multivariate cox regression analyses. The functional analyses were conducted with GeneMANIA and STRING databases, gene-gene, and protein-protein interaction (PPI) networks, Gene Ontology (GO) term, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Tumor Immune Estimation Resource (TIMER), Tumor-Immune System Interaction Database (TISIDB), and Gene Expression Profiling Interactive Analysis (GEPIA) were utilized to explore the internal connection with the immune escape.

Results: Through the analyses of genomic expression, *GINS3* was significantly upregulated in LIHC and positively correlated with higher T classification. ROC analysis indicated *GINS3* as a potential biomarker in the diagnosis of LIHC. KM-plotter, univariate and multivariate cox regression analyses both associated *GINS3* with poor prognosis in LIHC patients. *GINS3* genetic alteration, gene-gene interaction, PPI networks, and enrichment analysis further revealed that *GINS3* played a pivotal role in the progression of LIHC. Furthermore, hypermethylation of *GINS3* at different cytosine-guanine (CpG) sites was correlated with better or worse overall survival (OS) in LIHC and *GINS3* was also closely correlated with m6A modification. Moreover, results supported that *GINS3* could influence the tumor microenvironment and relate to the immune checkpoints.

Conclusions: Taken together, comprehensive analyses from this study supported *GINS3* as a novel targeted biomarker in LIHC.

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Keywords: GINS complex subunit 3 (*GINS3*); liver hepatocellular carcinoma (LIHC); biomarker; methylation; immune escape

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Introduction

Nowadays, liver cancer, the sixth most common cancer, has become the most frequent fatal malignancy (1,2). Liver cancer usually develops with the risk factor of hepatitis virus, alcohol, obesity, smoking, and diabetes (2,3). In addition, the patient's odds are dismayingly low unless liver cancer is diagnosed early (4). Despite the booming development in medical technology, there is still a startling lack of treatments for liver cancer (4,5). Therefore, researchers have devoted themselves to investigating the potential biomarkers for further effective treatments (6,7). To be perspective, combing immune checkpoint inhibitors and gene-targeted drugs has recently been unveiled as an auspicious cancer treatment (8). Hence, searching for underlying targeted genes and understanding the molecular mechanisms are urgently needed in liver hepatocellular carcinoma (LIHC).

The *GINS* tetrameric complex integrates with MCM and CDC45 turning into the productive CDC45-MCM-GINS (CMG) helicases required for genome duplication (9). GINS complex subunit 3 (*GINS3*) (*Psf3*), part of the *GINS* tetrameric complex, is also required for helicase activity which is involved in regulating DNA replication (10-13). In the meantime, variants of *GINS3* display impaired growth, S phase progression defects, and decreased *Psf3* protein stability (10). Moreover, dysregulation of *GINS3* expression has been reported in correlation with poor prognosis as a biomarker in many cancers (14,15). However, the exact role of *GINS3* in LIHC is still obscure.

In human cancer, tumor-associated immune cells can be divided into two types: tumor-antagonizing [effector T cells, natural killer (NK) cells dendritic cells (DCs), M1-polarized macrophages and N1-polarized neutrophils] and tumor-promoting immune cells [regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs)] (16). Moreover, the tumor microenvironment (TME) has already been substantiated to interact with cancer cells and then plays a determinative role in tumor survival and progression (17,18). Furthermore, the TME of liver cancer can affect tumor progression, invasion, metastasis, and recurrence (19). It is well known that immune escape is a key cause of tumor progression, and liver fibrosis has been proven crucial in promoting tumor immune escape (20,21). Meanwhile, changes in DNA methylation status were common in tumors. DNA hypermethylation might affect the DNA structure and then repress gene transcription which would silence the related gene (22). Strikingly, the m6A methylation might play dual biological functions in human cancer (23) and regulate TME by affecting immune-inflamed, immune-excluded, and immune-desert respectively (24). Consequently, studying the internal function of LIHC TME and methylation would contribute to a better comprehension of the molecular mechanism and the development of immunotherapy.

Hence, considering the regulatory roles of *GINS3* in DNA replication, in this study, multiple online public databases were utilized to evaluate the expression and protein levels of *GINS3* in LIHC. Further, the diagnostic and predictive roles of *GINS3* were also analyzed by those resources. Eventually, based on our findings, *GINS3* was concluded to be an auspicious prognostic biomarker and is associated with methylation and immune escape in

Highlight box

Key findings

- This study's comprehensive analyses supported *GINS3* as a novel targeted biomarker in liver hepatocellular carcinoma (LIHC).

What is known and what is new?

- Liver cancer has become the most frequent fatal malignancy. Despite the booming development in medical technology, there is still a startling lack of treatments for liver cancer. And several researchers have certified *GINS3* as a reliable biomarker for diagnosis and prognosis in many cancers;
- *GINS3* was significantly upregulated and correlated with poor prognosis methylation, and immune escape in LIHC.

What is the implication, and what should change now?

- *GINS3* could be a promising targeted biomarker for more advanced and effective treatments in LIHC.

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LIHC. With the findings of this study, hopefully, advanced immunotherapies and targeted therapies in LIHC will be developed in the near future. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2565/rc).

Methods

The Cancer Genome Atlas (TCGA) datasets

The data on *GINS3* mRNA expression were obtained from TCGA datasets (25) (http://cancergenome.nih.gov/), which were further used to analyze the differential expression of *GINS3* in 33 types of human cancer, in 50 LIHC tissues with their paired adjacent normal liver tissues, and in 374 LIHC tissues with 50 normal liver tissues. Clinical characteristics of LIHC patients and associated genes data were also downloaded. Moreover, the correlation between the expression level of *GINS3* and the expression of m6A-related genes in LIHC samples and the differences in expression in m6A-related genes between the high and low *GINS3* expression groups were also analyzed. M6A-related genes include *METTL3*, *YTHDC1*, *YTHDC2*, *METTL14*, *RBM15*, *RBM15B*, *IGF2BP1*, *IGF2BP2*, *IGF2BP3*, *VIRMA*, *WTAP*, *YTHDF1*, *YTHDF2*, *YTHDF3*, *ZC3H13*, *HNRNPA2B1*, *HNRNPC*, *RBMX*, *FTO* and *ALKBH5* (26). In addition, the expression correlation of *GINS3* with immune checkpoints including PD-1, PD-L1, PD-L2, LAG3, CTLA4, and TIM3 in LIHC was also evaluated by data from TCGA. P value <0.05 was considered statistically significant.

Protein expression analysis

The Clinical Proteomic Tumor Analysis Consortium (CPTAC) database (https://proteomics.cancer.gov/programs/cptac) is a central repository for the public dissemination of proteomic sequence datasets which can comprehensively characterize cancer types and support clinically-relevant research projects that elucidate biological mechanisms of response in clinical trials (27). UALCAN (http://ualcan.path.uab.edu/) is a user-friendly and interactive web resource for analyzing cancer data, which can provide protein expression analysis options using data from the CPTAC dataset. In this study, protein expression of *GINS3* in LIHC was analyzed by UALCAN using CPTAC datasets.

The Human Protein Atlas (HPA) database (https://www.proteinatlas.org) is an open-access resource with the aim to map all the human proteins in cells, tissues, and organs. Here, the immunohistochemical (IHC) staining results of *GINS3* in normal liver tissue and LIHC tissue were collected from HPA.

Survival and prognostic analysis

Kaplan-Meier plotter (KM-plotter) (http://kmplot.com), a potent public online database, is capable to assess the effect of all gene expressions (mRNA, miRNA, protein) on survival in over 30,000 samples from 21 cancer types, including liver cancer (n=364). Here, the overall survival (OS), progression-free survival (PFS), disease-specific survival (DSS), and relapse-free survival (RFS) of *GINS3* in liver cancer were analyzed with associated patient samples separated into two groups by median expression. The hazard ratio (HR) with a 95% confidence interval (CI) and log-rank P value were also contained.

Gene-gene interaction and protein-protein interaction (PPI) networks

GeneMANIA (http://www.genemania.org) is a flexible user-friendly website for generating hypotheses about gene function, analyzing gene lists, and prioritizing genes for functional assays (28). STRING (https://string-preview.org/) is an online database for PPI network functional enrichment analysis. In this study, gene-gene and PPI networks of *GINS3* were conducted by GeneMANIA and STRING respectively.

Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The top 300 genes most positively associated with *GINS3* from the TCGA database were implemented in functional enrichment, containing biological processes (BPs), cellular components (CCs), molecular functions (MFs), and the KEGG pathway. Functional enrichment analyses of co-expression genes were performed by the EnrichGO and EnrichKEGG function in the R package "ClusterProfiler" and visualized by the package "ggplot2", with the enrichment value set to P<0.05.

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cBioPortal database analysis

The cBioPortal database for Cancer Genomics (https://www.cbioportal.org/) provides an open-access web resource for the interactive exploration of multidimensional cancer genomics data sets. Here the cBioPortal was employed to identify different types of *GINS3* genetic alterations in different cancers and then focused on the alteration in LIHC. KM plots for survival outcomes including OS, DSS, PFS, and RFS of *GINS3* alterations were contained and the log-rank test was performed.

Methylation analysis

MethSurv (https://biit.cs.ut.ee/methsurv/), a web portal providing univariable and multivariable survival analysis based on DNA methylation biomarkers using TCGA data, was implemented to investigate the DNA methylation sites of *GINS3* and evaluate the prognostic values of corresponding cytosine-guanine (CpG) methylation. The HR with 95% CI of the OS was computed and P<0.05 was considered statically significant.

Tumor Immune Estimation Resource (TIMER) analysis

TIMER (http://timer.cistrome.org/) is a comprehensive online resource for the systematical analysis of immune infiltrates across diverse cancer types by inputting the gene expression profile data of tumor samples. In this study, TIMER was utilized to elucidate the correlation between *GINS3* expression in LIHC and six tumor-infiltrating immune cells (B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and DCs). Moreover, we assessed how *GINS3* expression correlated with the expression of particular immune checkpoint genes including *PDCD1* (PD-1), *CD274* (PD-L1), *PDCD1LG2* (PD-L2), *LAG3*, *CTLA4*, and *HAVCR2* (TIM3).

Tumor-Immune System Interaction Database (TISIDB) analysis

TISIDB (http://cis.hku.hk/TISIDB/) is an online web for tumor and immune system interaction, which integrates multiple heterogeneous data types (29). Here, TISIDB analysis was conducted to identify the expression of *GINS3* and 28 types of tumor-infiltrating lymphocytes (TILs) across human cancers. The study was performed in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

The statistical analysis calculated by the online database in this study was mentioned above. ROC curve was performed to identify the cutoff value of *GINS3* using the R software package "pROC". Univariate and multivariate logistic regression analyses were employed to the correlation between *GINS3* expression and clinical characteristics. The heat maps of the correlations between *GINS3* and the top 50 positively or negatively associated genes were generated by the R software package "pheatmap" with Spearman's correlation. P value <0.05 or log-rank P value <0.05 was considered statistically significant.

Results

Analysis of upregulated GINS3 mRNA and protein expression in LIHC

In this study, the TCGA database was utilized for the first time to evaluate the mRNA expression pattern of *GINS3* across 33 different cancer types relative to normal tissues. As shown in *Figure 1A*, compared with normal tissues, *GINS3* was significantly upregulated in 26 cancer types including LIHC. This indicated that the mRNA expression of *GINS3* was aberrant across different cancer types.

Next, further analyses of the *GINS3* expression data from the TCGA and HPA database were conducted to evaluate the mRNA and protein expression of *GINS3* in LIHC. As shown in *Figure 1B*,*1C*, paired data analysis revealed that the mRNA expression of *GINS3* was significantly higher than that in the adjacent normal tissues (n=50) (1.380±0.642 *vs.* 0.501±0.218, P<0.001), which was also confirmed in unpaired data analysis in 374 LIHC tissues compared with 50 adjacent normal tissues [1.270 (0.928–1.851) *vs.* 0.455 (0.349–0.601), Mann-Whitney U test, P<0.001]. *GINS3* was also assessed at a protein level through the National Cancer Institute's CPTAC dataset. The result showed that the total protein expression of *GINS3* was significantly higher in LIHC tumor tissues compared to normal tissues (*Figure 1D*, P<0.001).

Furthermore, immunohistochemical staining by the HPA database demonstrated that normal liver tissues had negative or medium *GINS3* IHC staining, while LIHC tumor tissues had medium or strong staining (*Figure 1E*).

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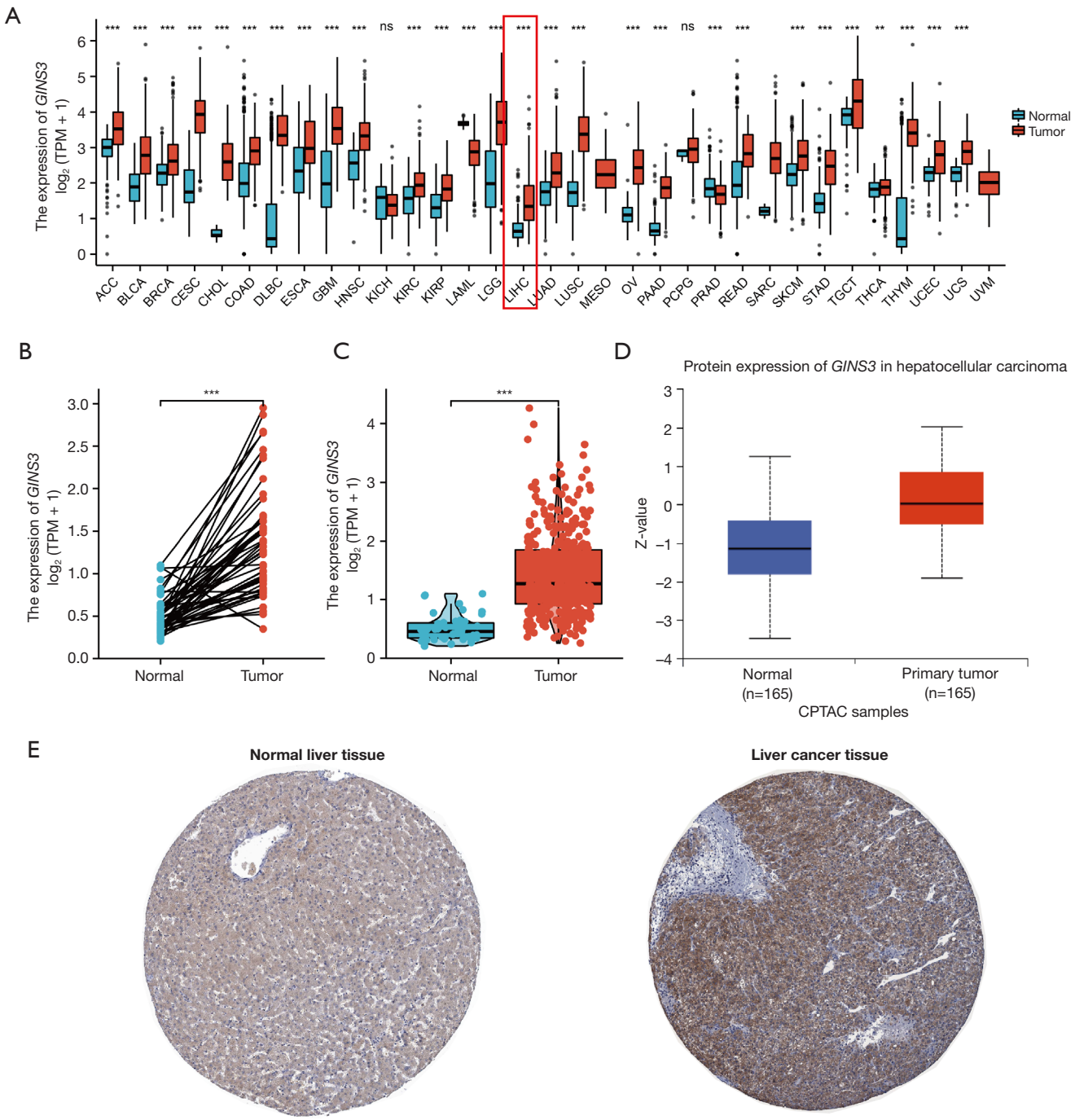

Figure 1 The expression analysis of *GINS3*. (A) Expression profile of *GINS3* in 33 different cancer types by TCGA database. (B) *GINS3* expression in 50 LIHC and matched-adjacent normal paired samples. (C) *GINS3* expression in 374 LIHC and 50 normal unpaired samples. (D) The protein expression levels of *GINS3* are based on CPTAC (https://proteomics.cancer.gov/programs/cptac). (E) The protein levels of *GINS3* are stained by IHC based on HPA (https://www.proteinatlas.org); the source of the images was as follows: normal liver tissue available at https://www.proteinatlas.org/ENSG00000181938-GINS3/tissue/liver#img; liver cancer tissue available at https://www.proteinatlas.org/ENSG00000181938-GINS3/pathology/liver+cancer#img. **, P<0.01; ***, P<0.001. ns, no significance; TPM, transcripts per million; TCGA, The Cancer Genome Atlas; LIHC, liver hepatocellular carcinoma; CPTAC, Clinical Proteomic Tumor Analysis Consortium; IHC, immunohistochemical; HPA, Human Protein Atlas.

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Table 1 Clinical characteristics of the patients (TCGA)

Characteristic	Total (n=374)	Low expression of *GINS3* (n=187)	High expression of *GINS3* (n=187)	P
T stage, n (%)				0.003
T1	183	107 (28.8)	76 (20.5)	
T2	95	43 (11.6)	52 (14.0)	
T3	80	30 (8.1)	50 (13.5)	
T4	13	3 (0.8)	10 (2.7)	
N stage, n (%)				0.623
N0	254	124 (48.1)	130 (50.4)	
N1	4	1 (0.4)	3 (1.2)	
M stage, n (%)				1.000
M0	268	129 (47.7)	139 (51.1)	
M1	4	2 (0.7)	2 (0.7)	
Age (years), median (IQR)	373	62.0 (52.0, 69.5)	60.5 (51.0, 68.0)	0.133

TCGA, The Cancer Genome Atlas; IQR, interquartile range.

Relationship between GINS3 expression and clinical features of LIHC patients

To evaluate the relation between the mRNA expression of *GINS3* and clinical features of LIHC samples, Chi-square test, Fisher test and Mann-Whitney U-test were conducted. The statistics showed that the expression level of *GINS3* was significantly upregulated in patients with higher T classification (P=0.003) (*Table 1*). However, no statistically significant association was found between the expression levels of *GINS3* and other clinical features including N classification and M classification (*Table 1*). The above results were consistent with the actual clinical practice that liver cancer was usually found at an advanced stage due to multiple factors.

Diagnostic and prognostic value of GINS3 in LIHC

To estimate the value of *GINS3* in distinguishing LIHC samples from normal samples, ROC and Kaplan-Meier (KM) curve analyses were performed. Results showed *GINS3* had an area under the curve (AUC) value of 0.939 (95% CI: 0.909–0.969) (*Figure 2A*). Those results suggested *GINS3* as a potential biomarker for distinguishing LIHC tissue from normal tissue.

Next, in order to clarify how *GINS3* expression related to prognosis in LIHC, cancer cases were divided into high and low expression groups based on the expression level of *GINS3*, and then those data were analyzed by the KM-plotter. As shown in *Figure 2B-2E*, compared to low expression of *GINS3*, higher expression was significantly connected to OS, DSS, PFS, and RFS (all P<0.001).

To better evaluate the prognostic value of *GINS3* expression, further exploration was performed to unveil the correlation between the mRNA expression of *GINS3* and prognosis. Results (*Figure 2F*) demonstrated that higher expression of *GINS3* was a hazard factor to all T stages, cancer grades, American Joint Committee on Cancer (AJCC) stage, and no microvascular invasion in LIHC. Interestingly, higher expression of *GINS3* was obviously a risk factor for people regardless of gender and race. However, *GINS3* was a protective factor for LIHC patients with alcohol consumption and hepatitis virus.

Moreover, to further estimate the prognostic potential of *GINS3* in LIHC, univariate and multivariate cox regression analyses were performed (*Table 2*). In the univariate cox regression analysis, T3 & T4, M1, stage III & IV, and high *GINS3* expression were all independent risk factors for OS (all P<0.1); in the multivariate cox regression analysis, only high *GINS3* expression was the independent risk factor for OS (P=0.052). In conclusion, those results suggested that high *GINS3* expression was associated with poor clinical prognosis.

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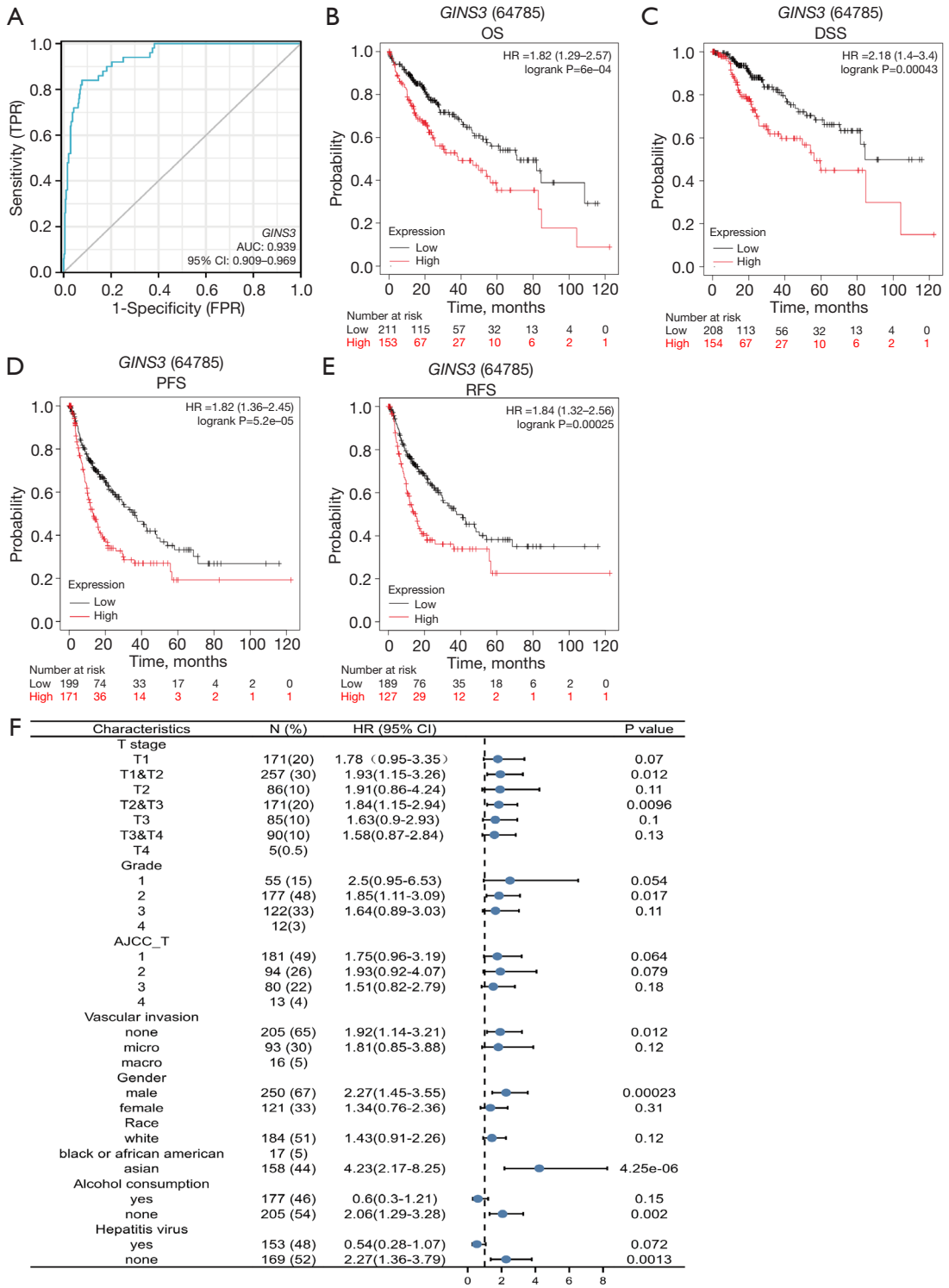

Figure 2 Diagnostic and prognostic value of *GINS3*. (A) ROC curve of *GINS3* in LIHC patients. (B-E) Kaplan-Meier analysis of OS, DSS, PFS, and RFS. (F) Forest plot of the correlation between *GINS3* and clinicopathological parameters in LIHC patients. ROC, receiver operating characteristic; LIHC, liver hepatocellular carcinoma; FPR, false positive rate; TPR, true positive rate; AUC, area under the curve; OS, overall survival; DSS, disease-specific survival; PFS, progression-free survival; RFS, relapse-free survival; HR, hazard ratio; CI, confidence interval; AJCC, American Joint Committee on Cancer.

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Table 2 Univariate and multivariate analysis of the correlation of *GINS3* expression with OS among LIHC patients

Characteristics	Total (n=373)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
T stage	370				
T1	183	Reference	–	Reference	–
T2	94	1.428 (0.901–2.264)	0.129	1.517 (0.841–2.736)	0.166
T3 & T4	93	2.949 (1.982–4.386)	<0.001	2.242 (0.296–16.996)	0.435
N stage	258				
N0	254	Reference	–	–	–
N1	4	2.029 (0.497–8.281)	0.324	–	–
M stage	272				
M0	268	Reference	–	Reference	–
M1	4	4.077 (1.281–12.973)	0.017	1.882 (0.575–6.158)	0.296
Pathologic stage	349				
I & II	259	Reference	–	Reference	–
III & IV	90	2.504 (1.727–3.631)	<0.001	1.317 (0.178–9.733)	0.787
Gender	373				
Female	121	Reference	–	–	–
Male	252	0.793 (0.557–1.130)	0.200	–	–
Age, years	373				
≤60	177	Reference	–	–	–
>60	196	1.205 (0.850–1.708)	0.295	–	–
Residual tumor	344				
R0	326	Reference	–	–	–
R1 & R2	18	1.604 (0.812–3.169)	0.174	–	–
GINS3	373				
Low	187	Reference	–	Reference	–
High	186	1.633 (1.153–2.313)	0.006	1.569 (0.996–2.471)	0.052

OS, overall survival; LIHC, liver hepatocellular carcinoma; CI, confidence interval.

Enrichment analysis of GINS3-interacting genes and proteins in LIHC

To find out the essential biological pathways and molecular mechanisms through the BP, enrichment analyses were conducted by GeneMANIA and STRING databases. As shown in *Figure 3A*, the 20 most frequently altered genes which closely related to *GINS3* include *GINS2*, *GINS4*, *GINS1*, and *MCM2*. Meanwhile, the PPI network of *GINS3* through STRING analysis showed 11 nodes including GINS4, CDC45, CDT1, and ORC3 (*Figure 3B*).

Next, the co-expression genes of *GINS3* were investigated to learn about the sequence of gene interaction by utilizing the TCGA database. Results found the top 50 genes that were positively and negatively correlated with *GINS3* in LIHC (*Figure 3C,3D*). Meanwhile, to investigate *GINS3*-related pathways and biological functions, the KEGG and GO enrichment analyses were performed by using the top 300 genes that are positively related to

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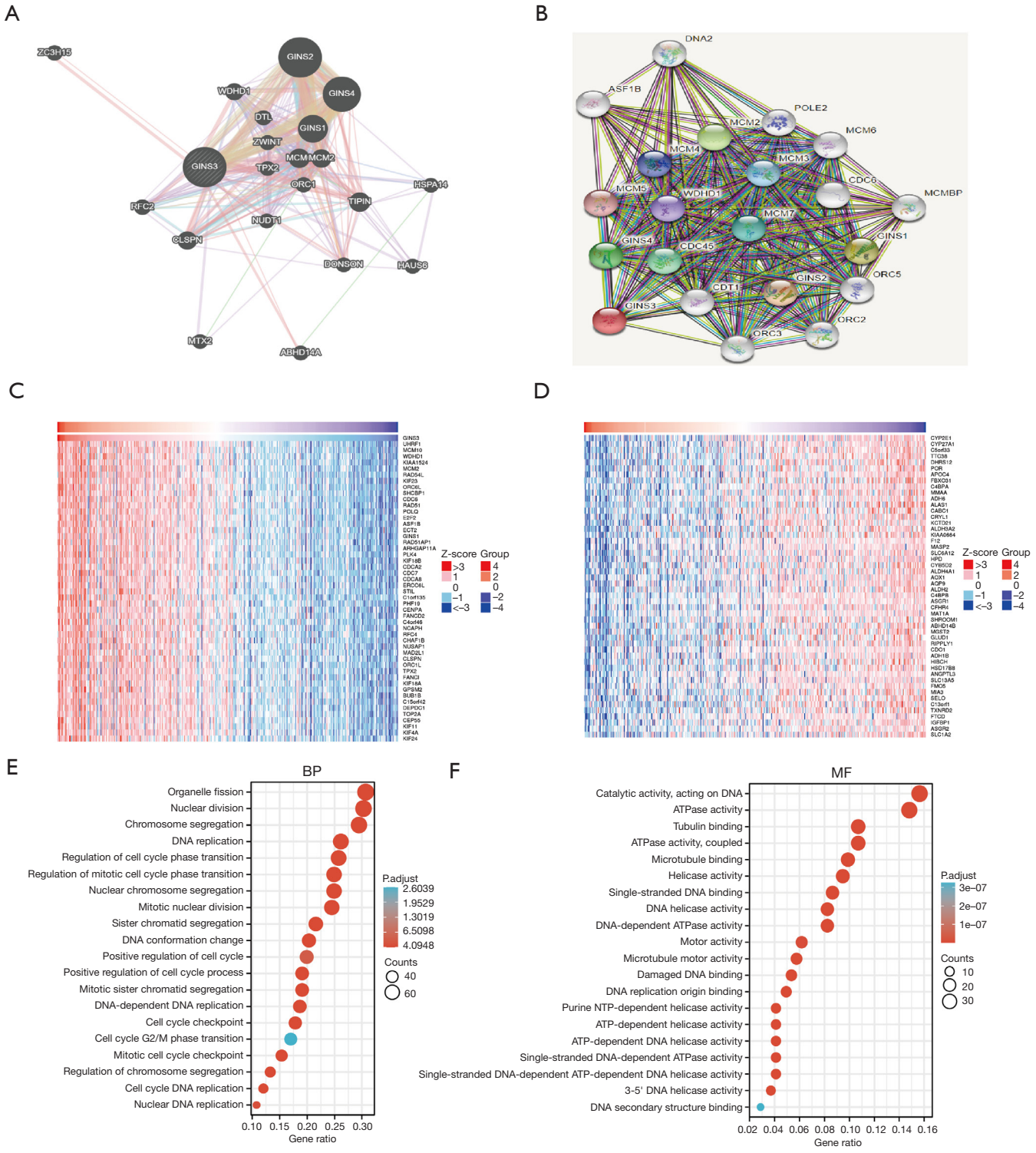

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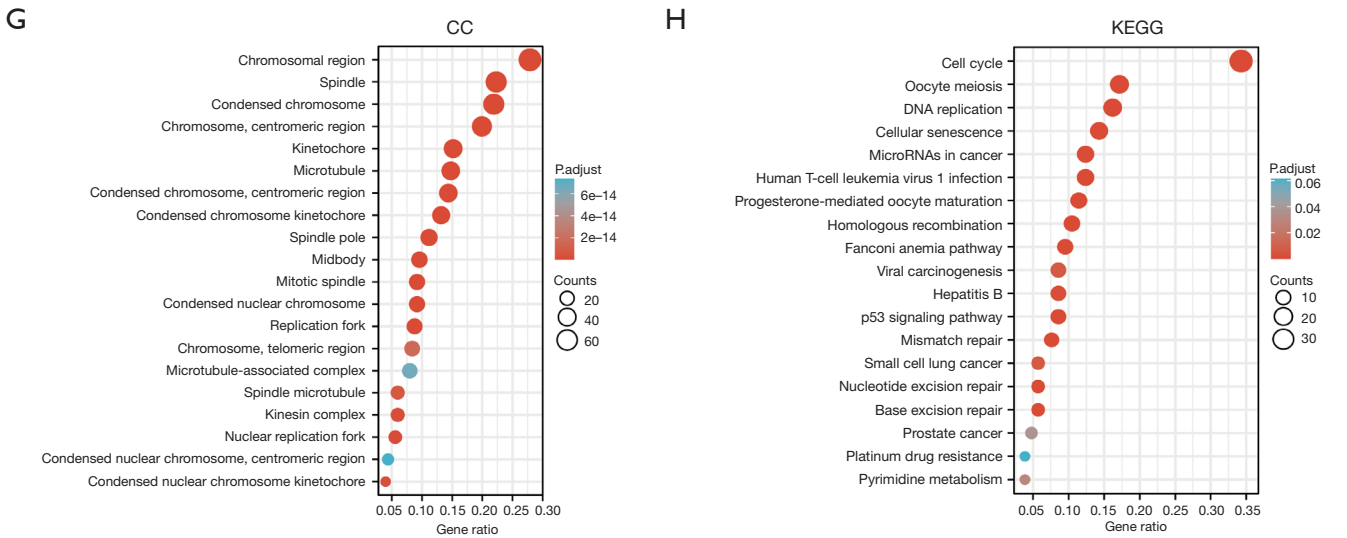

Figure 3 Enrichment analysis of *GINS3* functional networks in LIHC. (A) The gene-gene interaction network of *GINS3* by GeneMANIA. (B) The PPI network of *GINS3* by STRING. (C) Heatmap shows the top 50 genes positively correlated with *GINS3* in LIHC. (D) Heatmap shows the top 50 genes negatively correlated with *GINS3* in LIHC. (E-G) Top 20 enrichment terms in BP, MF, and CC categories in LIHC. (H) Top 19 KEGG enrichment pathways in LIHC. ATP, adenosine triphosphate; NTP, nucleotide triphosphate; LIHC, liver hepatocellular carcinoma; PPI, protein-protein interaction; BP, biological process; MF, molecular function; CC, cellular component; KEGG, Kyoto Encyclopedia of Genes and Genomes.

GINS3. The top 20 significant terms of BP, MF, and CC enrichment analyses are demonstrated in *Figure 3E-3G*. In terms of BP, *GINS3* was enriched in organelle fission, nuclear division, chromosome segregation, and DNA replication. In terms of CC, *GINS3* was enriched in the chromosomal region, spindle, and condensed chromosome. In terms of MF, *GINS3* was enriched in catalytic activity, acting on DNA. Moreover, the top 19 KEGG pathways for *GINS3* and its-correlated genes are presented in *Figure 3H*. Results showed that *GINS3* was closely connected with many DNA replications and cell cycle-related pathways, including cell cycle, oocyte meiosis, DNA replication, and cellular senescence.

Genetic alteration of GINS3 in LIHC

In general, human cancers develop due to the intrinsic factor—the accumulation of genetic alterations. Accordingly, the *GINS3* genetic alterations were investigated in human cancer samples and then focused on LIHC through the cBioPortal database. The analysis showed that different types of *GINS3* genetic alteration existed in different cancers at different frequencies (*Figure 4A*). Besides, the main *GINS3* genetic alteration in LIHC was the deep deletion and the percentage was 0.2% (*Figure 4A,4B*). Meanwhile, for clarity, two pie charts of the mutation types are shown in *Figure 4C*. Missense substitutions occurred in approximately 38.29% of the samples, synonymous substitution occurred in approximately 7.43% of the samples, frameshift insertion occurred in approximately 1.14% of the samples, and other types occurred in approximately 13.71% of the samples (*Figure 4C*). The substitution mutations mainly occurred at C>T (25.64%) and G>A (24.36%). Moreover, a systematic study was performed to ascertain the correlation between *GINS3* genetic alterations and the clinical survival prognosis of LIHC patients. Patients with genetic alteration of *GINS3* in LIHC showed a better prognosis in DSS (P=0.0706) and OS (P=0.0400), but not DFS (P=0.828) and PFS (P=0.364), compared to patients without *GINS3* genetic alterations (*Figure 4D*).

Methylation of GINS3 and its correlation with m6A methylation regulators in LIHC

To investigate the methylation of *GINS3* in LIHC, the DNA methylation sites of *GINS3* were analyzed and the prognostic values of corresponding cytosine-guanine (CpG) methylation was evaluated through the MethSurv.

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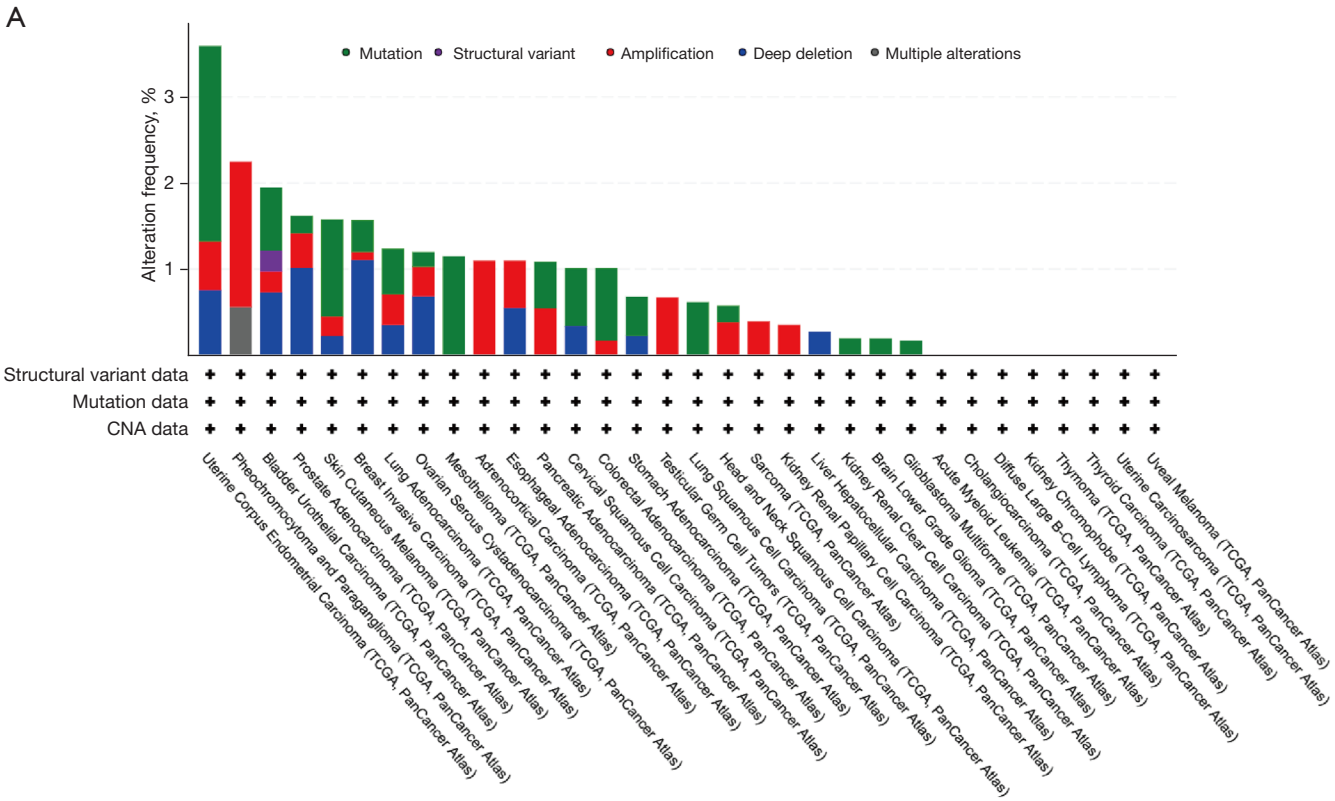

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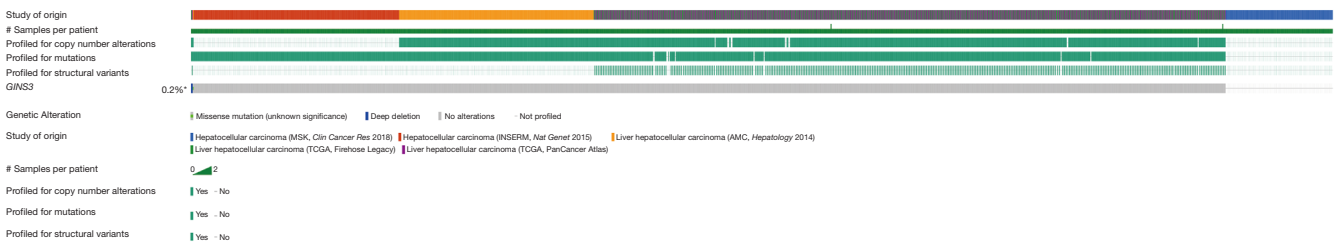

C

Colour	Mutation type	Number of samples (%)
	Nonsense substitution	0 (0.00%)
	Missense substitution	67 (38.29%)
	Synonymous substitution	13 (7.43%)
	Inframe insertion	0 (0.00%)
	Frameshift insertion	2 (1.14%)
	Inframe deletion	0 (0.00%)
	Frameshift deletion	0 (0.00%)
	Complex mutation	0 (0.00%)
	Other	24 (13.71%)
	Total unique samples	175

Colour	Mutation type	Number of samples (%)
	A>C	2 (2.56%)
	A>G	7 (8.97%)
	A>T	2 (2.56%)
	C>A	7 (8.97%)
	C>T	20 (25.64%)
	C>G	3 (3.85%)
	G>A	19 (24.36%)
	G>C	1 (1.28%)
	G>T	7 (8.97%)
	T>A	3 (3.85%)
	T>C	5 (6.41%)
	T>G	3 (3.85%)
	Total unique samples	78

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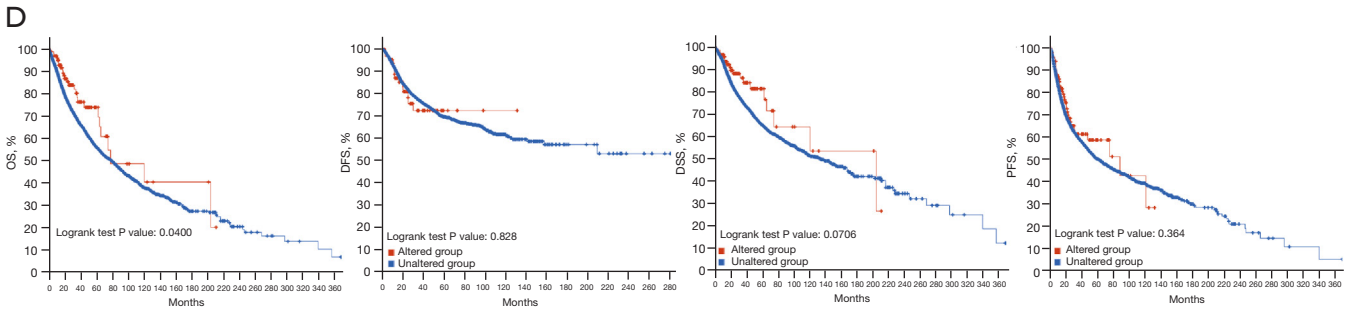

Figure 4 *GINS3* genetic alteration in LIHC. (A) Genetic alteration of *GINS3* in different cancers. (B) OncoPrint visual summary of *GINS3* genetic alteration. (C) Pie charts of the *GINS3* mutation types. (D) Kaplan-Meier plots comparing OS, DFS, DSS, and PFS in patients with/without *GINS3* genetic alterations. CNA, copy-number alterations; TCGA, The Cancer Genome Atlas; MSK, Memorial Sloan Kettering Cancer Center; AMC, Acta Medica Colombiana; LIHC, liver hepatocellular carcinoma; OS, overall survival; DSS, disease-specific survival; PFS, progression-free survival; RFS, relapse-free survival.

Totally, 12 methylation CpG sites of *GINS3* were observed and cg01783195 had the highest DNA methylation (*Figure 5A*). And 6 methylation CpG sites were related to the prognosis of LIHC patients. Hypermethylation of *GINS3* at cg03919836 and cg05732130 sites was related to a worse OS in LIHC, while hypermethylation of *GINS3* at cg02655227, cg05425326, cg09431182, and cg26635363 sites was related to a better OS (*Table 3*).

Furthermore, the TCGA-LIHC data was analyzed to determine the correlation between *GINS3* expression and 20 m6A-related genes in LIHC, which were demonstrated as a heat map and scatter plots (*Figure 5B*,*5C*). Results showed that *GINS3* expression was positively correlated with 12 m6A-related genes in LIHC, including *METTL3* (r=0.506, P<0.001), *YTHDC1* (r=0.522, P<0.001), *RBM15* (r=0.478, P<0.001), *RBM15B* (r=0.548, P<0.001), *IGF2BP2* (r=0.341, P<0.001), *IGF2BP3* (r=0.506, P<0.001), *WTAP* (r=0.545, P<0.001), *YTHDF1* (r=0.600, P<0.001), *YTHDF2* (r=0.496, P<0.001), *HNRNPA2B1* (r=0.616, P<0.001), *HNRNPC* (r=0.586, P<0.001), and *RBMX* (r=0.628, P<0.001). Moreover, 424 LIHC patient samples were divided into two groups by median expression of *GINS3* to explore the relationship of 20 m6A-related genes with *GINS1* expression. Higher expression of *METTL3*, *YTHDC1*, *RBM15*, *RBM15B*, *IGF2BP1*, *IGF2BP3*, *VIRMA*, *WTAP*, *YTHDF1*, *YTHDF2*, *YTHDF3*, *HNRNPA2B1*, HNRNPC, *RBMX*, and *ALKBH5* were observed in the high *GINS3* expression group compared with the low *GINS3* expression group (*Figure 5D*). Those results indicated that *GINS3* was closely correlated with m6A modification which was involved in the tumor progression including LIHC.

Assessment of the correlation between GINS3 expression and immune cell infiltration in LIHC

To access the level of immune cell infiltration in the LIHC microenvironment, the correlation between *GINS3* expression and six types of tumor-infiltrating immune cells was analyzed by the TIMER database. Results demonstrated that *GINS3* expression was related to tumor purity (cor =0.1, P=6.43e−02), B cell (cor =0.399, P=1.31e−14), $CD8^+$ T cell (cor =0.388, P=1.06e−18), $CD4^+$ T cell (cor =0.342, P=6.88e−11), macrophage (cor =0.494, P=2.37e−22), neutrophil (cor =0.435, P=2.41e−17), DC (cor =0.467, P=7.61e−20) (*Figure 6A*). Moreover, further study of the correlation between *GINS3* expression and 28 types of TILs across human cancers was conducted in the TISIDB database (*Figure 6B*). The above results suggested that *GINS3* somehow could affect the immune infiltration and then make a progress in LIHC progression.

Relationship between GINS3 expression and immune checkpoints in LIHC

To further evaluate the correlation between *GINS3* expression and immune escape, the investigation was performed for the immunotherapy checkpoint genes including *PDCD1* (PD-1), *CD274* (PD-L1), *PDCD1LG2* (PD-L2), *LAG3*, *CTLA4*, and HAVCR2 (TIM3). The heatmap suggested that the above immune checkpoint genes were positively correlated with *GINS3* expression (*Figure 7A*). In more detail, scatter plots showed the close association of individual immune checkpoint genes with

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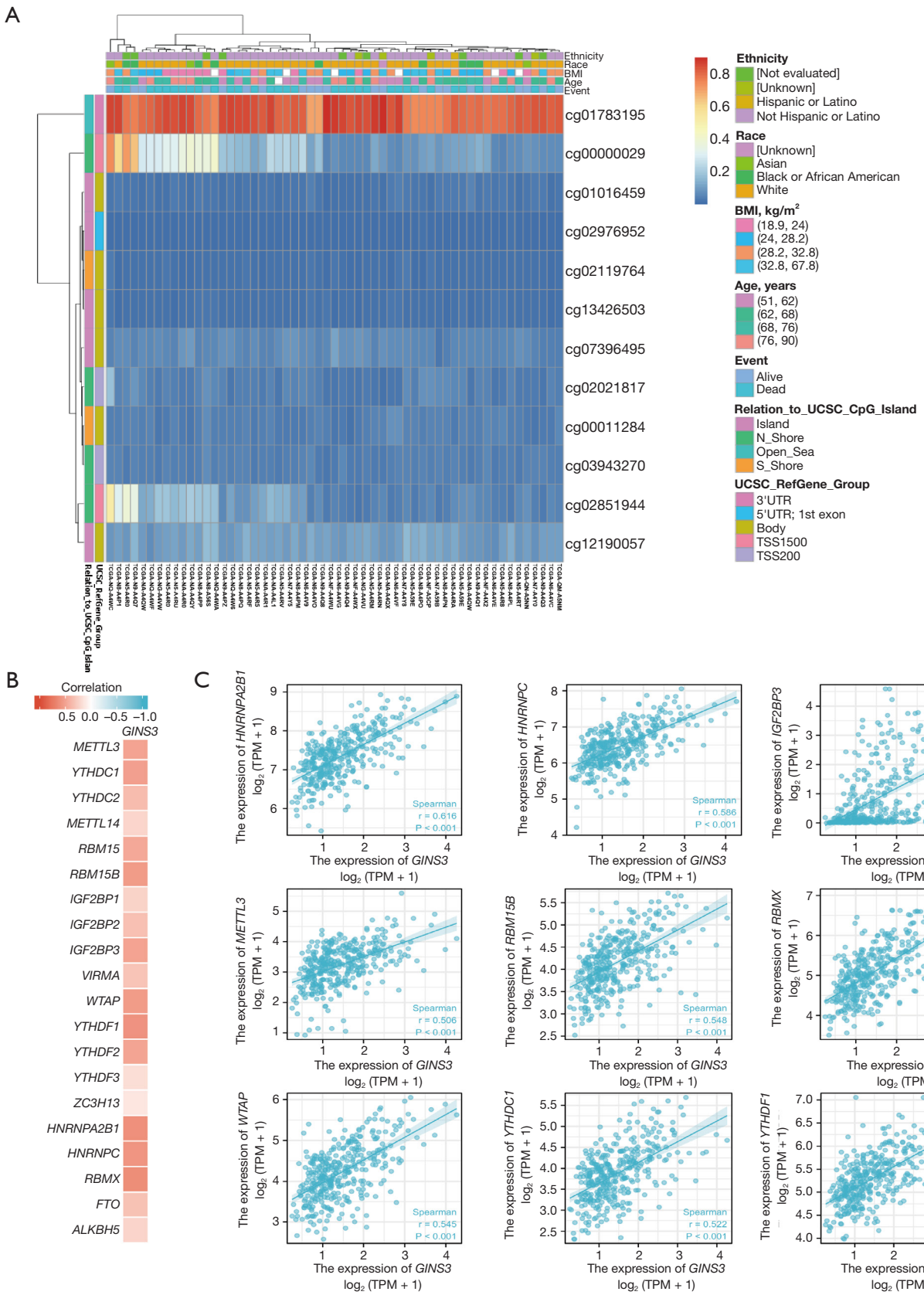

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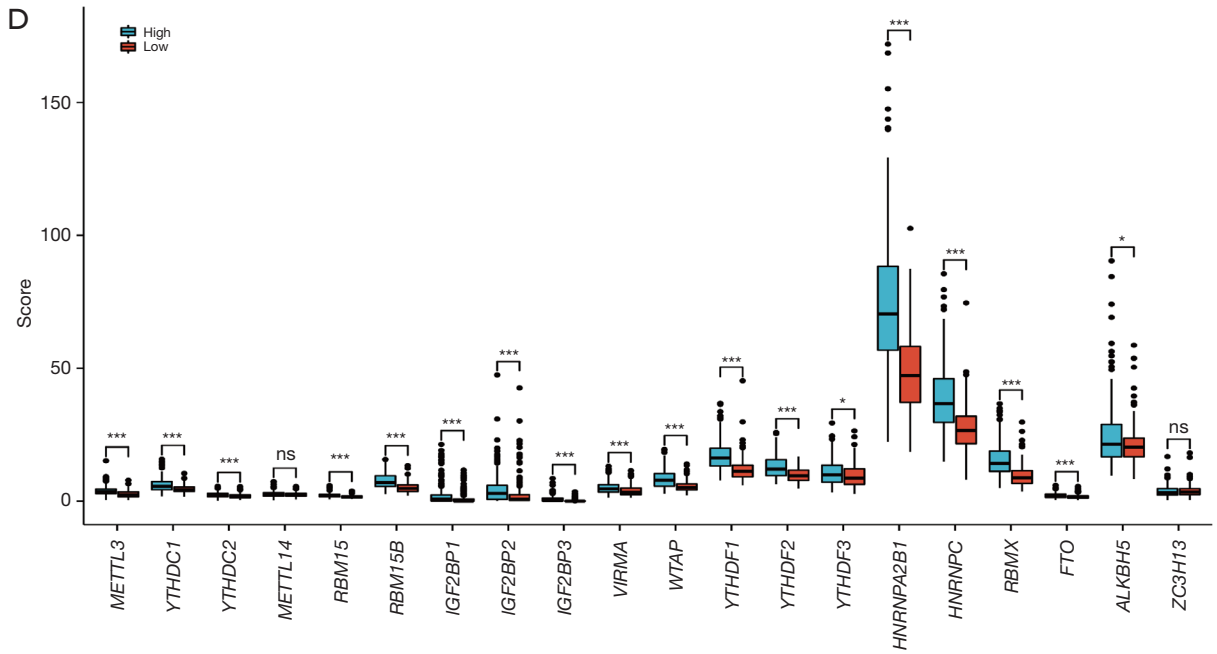

Figure 5 Methylation analysis of *GINS3* in LIHC. (A) Visualization of the CpG methylation sites of *GINS3* in LIHC. (B) The correlation between *GINS3* expression and m6A-related genes in LIHC based on TGCA datasets. (C) The scatter plots of the correlation between *GINS3* and night m6A-related genes with the highest correlation coefficient. (D) The differential expression of m6A-related genes in the high and low *GINS3* expression groups in LIHC. *, P<0.05; ***, P<0.001. UCSC, University of California, Santa Cruz; BMI, body mass index; CpG, cytosine-guanine; UTR, untranslated region; LIHC, liver hepatocellular carcinoma; ns, no significance; TGCA, The Cancer Genome Atlas; TPM, transcripts per million.

Table 3 Effect of hypermethylation level on prognosis in LIHC

Name	HR (95% CI)	P value
5'UTR;1stExon-Island-cg00885732	0.734 (0.478, 1.128)	0.16
TSS200-Island-cg02655227*	0.645 (0.44, 0.947)	0.025
5'UTR;1stExon-Island-cg03919836*	1.607 (1.031, 2.504)	0.036
TSS200-Island-cg04246829	0.823 (0.562, 1.204)	0.32
TSS1500-N_Shore-cg04840669	1.369 (0.879, 2.131)	0.16
3'UTR-Open_Sea-cg05425326*	0.672 (0.46, 0.981)	0.039
TSS200-Island-cg05732130*	1.948 (1.38, 2.749)	0.0001
TSS200-Island-cg09431182*	0.573 (0.396, 0.829)	0.0031
Body-Island-cg15518345	1.067 (0.758, 1.503)	0.71
Body-Island-cg26635363*	0.424 (0.282, 0.637)	<0.01
TSS200-Island-cg26851141	1.340 (0.947, 1.895)	0.099

*, methylation CpG sites related to prognosis (P<0.05). LIHC, liver hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; UTR, untranslated region; CpG, cytosine-guanine.

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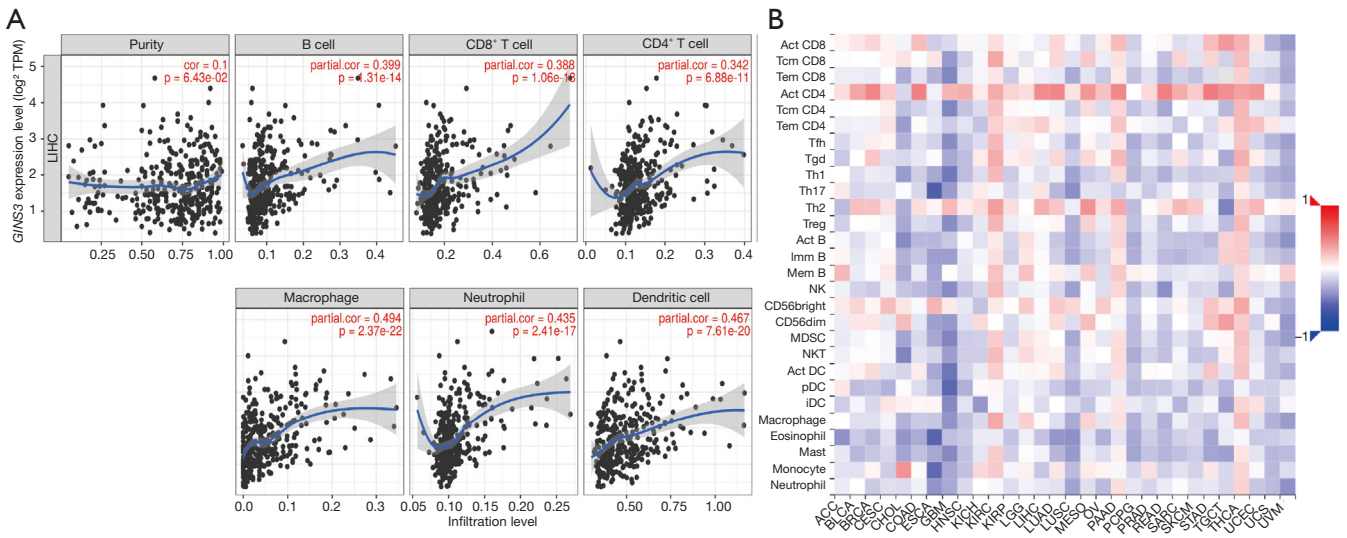

Figure 6 Correlation of *GINS3* and immune cell infiltration in LIHC. (A) The correlation between *GINS3* expression and tumor-infiltrating immune cells from the TIMER database. (B) The correlation between *GINS3* expression and 28 types of TILs across human cancers from the TISIDB database. Act, activated; Tcm, central memory T cell; Tem, effector memory T cell; Tfh, T follicular helper cell; Tgd, gamma delta T cell; Treg, regulatory T cells; Imm, immature; Mem, memory; NK, natural killer; MDSC, myeloid derived suppressor cell; NKT, natural killer T; DC, dendritic cell; pDC, plasmacytoid dendritic cell; iDC, immature dendritic cell; LIHC, liver hepatocellular carcinoma; TIMER, Tumor Immune Estimation Resource; TILs, tumor-infiltrating lymphocytes; TISIDB, Tumor-Immune System Interaction Database; TPM, transcripts per million.

GINS3 expression (*Figure 7B*). The correlation indicated the important effect of immune escape in *GINS3*-mediated carcinogenesis of LIHC and then hopefully advanced immunotherapy could be employed in LIHC treatment.

Discussion

For developing the potential therapeutic strategies, it is pivotal to have a comprehensive study of the molecular mechanisms in LIHC. Therefore, in this study, great efforts were made to elucidate that *GINS3* was correlated with prognosis, methylation, and immune escape in LIHC.

As a part of the *GINS* subcomplex, *GINS3* is required for helicase activity and is, therefore, involved in DNA replication (9). Also, research has proved that DNA replication drives tumor development and is considered a hallmark of cancer (30,31). Therefore, recently, researchers have focused on the potential correlation between the *GINS* subunits and cancer (32-34). However, the expression and role of *GINS3* in LIHC have not been clarified yet. Here, elucidation of the internal connection between *GINS3* and LIHC was made by an integrated analysis of public datasets. Results demonstrated that *GINS3* was significantly upregulated in many cancers based on pan-cancer analysis and then further analyses proved that *GINS3* is conspicuously upregulated in LIHC. Additionally, it was exhibited that the expression level of *GINS3* was significantly upregulated in patients with higher T classification. Those results suggested *GINS3* could be a sensitive index for LIHC diagnosis.

Several researchers have certified *GINS3* as a reliable biomarker for diagnosis and prognosis in many cancers. In malignant colon cancer, a study showed that overexpression of *GINS3* is closely connected with higher-grade tumors and might be a potential biomarker (35). Besides, it has been proved that high *GINS3* expression was a poor prognostic marker for pulmonary adenocarcinoma (36). Meanwhile, *GINS3* also acted as a factor significantly affecting the prognosis of colorectal cancer patients (37). On the other hand, it was hypothesized that survival time might be prolonged by suppressing the expression of *GINS3*. Therefore, great efforts were made to investigate the diagnostic and prognostic value of *GINS3* in LIHC for more treatment possibilities. The ROC curve analysis proved *GINS3* had a good diagnostic value in LIHC. Meanwhile, the KM analyses showed that high *GINS3*

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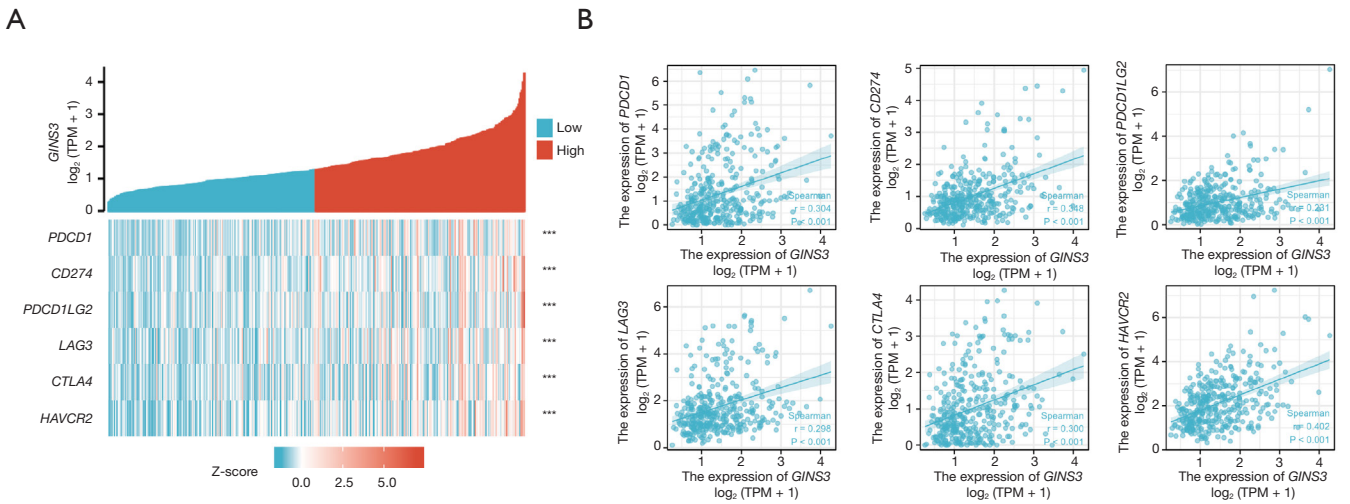

Figure 7 Correlation of *GINS3* and immune checkpoints in LIHC. (A) Heatmap of immune checkpoints including *PDCD1* (PD-1), *CD274* (PD-L1), *PDCD1LG2* (PD-L2), *LAG3*, *CTLA4*, and *HAVCR2* (TIM3). (B) Scatter plots of the correlation between *GINS3* expression and immune checkpoints in LIHC. ***, P<0.001. LIHC, liver hepatocellular carcinoma; TPM, transcripts per million.

expression was significantly correlated with poor OS, PFS, DSS, and RFS. Also, the forest plot and univariate and multivariate cox regression analyses both indicated high *GINS3* expression as a risk factor in LIHC. According to strong evidence, *GINS3* was confirmed to be a promising biomarker in the diagnosis and prognosis of LIHC.

Previous study has proved that the *GINS* gene is essential for the viability of human cells (38). *GINS1*, another component of the *GINS* complex, is an essential component of the RAS/RAF/MAPK signaling pathway which is related to various cancers (39,40). In melanoma, the RAS/RAF/MAPK signaling pathway, regulated by microRNA-340, could suppress the tumorigenic phenotype (41). However, especially in LIHC, the essential biological pathways and the underlying mechanism of *GINS3* have not been clarified yet. In this study, co-expression analyses were conducted and found that *GINS3* was significantly correlated with *GINS4*, *GINS1*, *GINS2*, and *MCM2*. Meanwhile, the KEGG and GO enrichment analyses indicated that *GINS3* was enriched in the chromosomal region and highly involved in cell cycle progression by affecting organelle fission, DNA replication, catalytic activity, and ATPase activity. The above results suggested that *GINS3* might be an essential prerequisite for the progression of LIHC and therefore, could be a promising therapeutic target of LIHC.

Genetic alteration is known to be associated with DNA replication, DNA damage repair, cancer, and senescence. And the accumulation of genetic alterations has been perceived as a driving factor in cancer progression (42). Based on this, the exact pattern of *GINS3* genetic alteration was probed into. Analyses showed that *GINS3* genetic alteration which existed in multiple cancers was only 0.2% in LIHC. The alteration was mainly deep deletion and missense substitutions that occurred at C>T and G>A. Moreover, the results also showed a better prognosis in LIHC patients with genetic alteration of *GINS3*. In conclusion, genetic alteration of *GINS3* played a certain role in LIHC progression.

Changes in DNA methylation status were common in all forms of cancer. Generally, DNA hypermethylation might affect the DNA structure and then repress gene transcription which would silence the related gene (22). However, the CpG island was the methylated site to regulate the expression of downstream genes (22). Therefore, the analyses were conducted to learn that there were 12 methylation CpG sites of *GINS3* and some were associated with prognosis in LIHC. Meanwhile, m6A methylation, the most important and abundant form of internal modifications in eukaryotic cells, has been proven to be pivotal in tumor proliferation, migration, and invasion (26,43). By regulating oncogenes or suppressor genes, the m6A methylation could interfere with the immune infiltration phenotype and the progression of cancer (23,44). Moreover, the m6A methylation also could regulate the expression level of m6A and the activity of m6A methyltransferases, therefore affecting the role of

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m6A in cancer (45,46). The m6A methylation had been certified targeting the IL-7/STAT5/SOCS pathways to control T cell homeostasis which regulates TME in turn (47). Interestingly, dysregulated m6A methylation was proved to associate with hepatocellular (48,49). A paper from Qi *et al.* demonstrated a close association between the high expression of m6A-related genes and poor OS in LIHC (50). Also, the key m6A-related genes *METTL3* and *METTL14* were reported to be active components of the m6A methyltransferase complex and correlated with tumor proliferation, differentiation, tumorigenesis, invasion, and metastasis (51,52). *METLL3* had been proven to possess oncogenic functions in liver cancer, therefore promoting the growth of liver cancer (48). Research revealed that, in LIHC, upregulation of *METTL14* by lipopolysaccharide (LPS) promotes the m6A methylation of MIR155HG, which modulates the expression of programmed death-ligand 1 (PD-L1) by miR-223/STAT1 axis (53). Consequently, further analyses were performed to unveil the exact relation between *GINS3* expression and m6A modification in LIHC. A series of m6A-related genes were positively correlated with *GINS3* expression, including *METTL3*, *YTHDC1*, *RBM15*, *RBM15B*, *IGF2BP2*, *IGF2BP3*, *WTAP*, *YTHDF1*, *YTHDF2*, *HNRNPA2B1*, *HNRNPC*, and *RBMX*. Based on those results, it was speculated that *GINS3* was closely associated with m6A modification and therefore affected the proliferation, migration, and metastasis of LIHC.

TME comprising innate and adaptive immune cells was proved to play a determinative role in tumor survival and progression (17,18). And it is well known that immune escape is a key cause of tumor progression. Therefore, in liver cancer, lots of research had been conducted and found that β-Catenin activation and PD-L1 promoted immune escape (54,55). Furthermore, the activation of STAT3 and NF-κB signaling pathways can induce PD-L1 expression directly and indirectly (55). Meanwhile, liver fibrosis is highly promoted by activated hepatic stellate cells (HSCs) which have immunomodulatory activity by expressing proteins such as PD-L1 and stimulate the expansion of immunosuppressive cells such as Tregs and MDSC (20,21,56). Since the liver is the most frequent organ affected by metastasis after lymph nodes, multiple efforts have been made to unveil that tumor immunity is a fundamental part of metastasis in LIHC (57-60). In addition, the liver itself has an immunosuppressive polarization, however, the metastatic microenvironment underwent remarkable spatial reprogramming of immunosuppressive cells such as $MRC1^{+}CCL18^{+}$ M2-like macrophages (57,58). Strikingly, liver metastasis can alter the immune microenvironment of the liver by inducing systemic loss of antigen-specific T cells and recruiting and polarizing monocyte-derived macrophages (59,60). Thus, we further assessed the association between *GINS3* expression and tumor immune microenvironment in LIHC. By the TIMER database, we found that *GINS3* expression was related to tumor purity, $CD8^{+}$ T cell, $CD4^{+}$ T cell, macrophage, neutrophil, and DC. Combing the above results and *GINS3* expression in 28 types of TILs, it was assumed that *GINS3* could somehow alter the LIHC immune infiltration and then affect LIHC progression.

Recently, more and more studies have extended the scope of immunotherapy by unveiling immune escape as a novel target for cancer treatment (61,62). As for the treatment of liver cancer patients, nonspecific immune stimulation, adaptive cell transfer, immune-checkpoint inhibitors, and DC-based vaccination have been developed as promising immunotherapy (16). Most recently, in LIHC, the combination of atezolizumab (immune-checkpoint inhibitor) and bevacizumab (anti-angiogenic agent) is the first treatment regimen that has been shown to improve OS relative to sorafenib (63). As immune checkpoints are indispensable in immune escape, the correlation between *GINS3* expression and immune checkpoints was investigated in this study. The heatmap and scatter plots showed a strong connection to the immune checkpoints, containing *PDCD1*, *CD274*, *PDCD1LG2*, *LAG3*, *CTLA4*, and *HAVCR*. In conclusion, findings from the mechanism-based hypothesis shed light on *GINS3* as a promising immunotherapeutic target.

Conclusions

In this study, the comprehensive analyses could prove that *GINS3* was significantly upregulated and correlated with poor prognosis methylation, and immune escape in LIHC. Based on the findings of the study, higher *GINS3* expression could be a novel targeted biomarker for more advanced and effective treatments in LIHC.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2565/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2565/coif) and report that this study was supported by grants from the Medical Scientific Research Foundation of Guangdong Province (No. A202109), the Fundamental Research Funds for the Central Universities (No. 21622312), the Basic and Applied Basic Research Project of Guangzhou Basic Research Program (No. 2023A04J01917), the Flagship Specialty Construction Project-General Surgery of the First Affiliated Hospital of the Jinan University (No. 711003), and the Special Foundation for Scientific Research Development of the Affiliated Shunde Hospital of Jinan University (No. 202101004). The authors have no other 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 performed in accordance with the Declaration of Helsinki (as revised in 2013).

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References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Villanueva A. Hepatocellular Carcinoma. N Engl J Med 2019;380:1450-62.
3. Marengo A, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. Annu Rev Med 2016;67:103-17.
4. Gravitz L. Liver cancer. Nature 2014;516:S1.
5. Anwanwan D, Singh SK, Singh S, et al. Challenges in liver cancer and possible treatment approaches. Biochim Biophys Acta Rev Cancer 2020;1873:188314.
6. Luan M, Si H. Novel hypoxia features with appealing implications in discriminating the prognosis, immune escape and drug responses of 947 hepatocellular carcinoma patients. Transl Cancer Res 2022;11:2097-121.
7. Bai E, Dong M, Lin X, et al. Expressional and functional characteristics of checkpoint kinase 1 as a prognostic biomarker in hepatocellular carcinoma. Transl Cancer Res 2022;11:4272-88.
8. Colli LM, Machiela MJ, Zhang H, et al. Landscape of Combination Immunotherapy and Targeted Therapy to Improve Cancer Management. Cancer Res 2017;77:3666-71.
9. Sedlackova H, Rask MB, Gupta R, et al. Equilibrium between nascent and parental MCM proteins protects replicating genomes. Nature 2020;587:297-302.
10. McQuaid ME, Ahmed K, Tran S, et al. Hypomorphic GINS3 variants alter DNA replication and cause Meier-Gorlin syndrome. JCI Insight 2022;7:e155648.
11. Wu L, Liu Y, Kong D. Mechanism of chromosomal DNA replication initiation and replication fork stabilization in eukaryotes. Sci China Life Sci 2014;57:482-7.
12. Jenkyn-Bedford M, Jones ML, Baris Y, et al. A conserved mechanism for regulating replisome disassembly in eukaryotes. Nature 2021;600:743-7.
13. Labib K, Gambus A. A key role for the GINS complex at DNA replication forks. Trends Cell Biol 2007;17:271-8.
14. Tauchi S, Sakai Y, Fujimoto S, et al. Psf3 is a prognostic biomarker in lung adenocarcinoma: a larger trial using tissue microarrays of 864 consecutive resections. Eur J Cardiothorac Surg 2016;50:758-64.
15. Tane S, Sakai Y, Hokka D, et al. Significant role of Psf3

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expression in non-small-cell lung cancer. Cancer Sci 2015;106:1625-34.
16. Lei X, Lei Y, Li JK, et al. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. Cancer Lett 2020;470:126-33.
17. Hinshaw DC, Shevde LA. The Tumor Microenvironment Innately Modulates Cancer Progression. Cancer Res 2019;79:4557-66.
18. Xia L, Oyang L, Lin J, et al. The cancer metabolic reprogramming and immune response. Mol Cancer 2021;20:28.
19. Fu Y, Liu S, Zeng S, et al. From bench to bed: the tumor immune microenvironment and current immunotherapeutic strategies for hepatocellular carcinoma. J Exp Clin Cancer Res 2019;38:396.
20. Vinay DS, Ryan EP, Pawelec G, et al. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. Semin Cancer Biol 2015;35 Suppl:S185-98.
21. Ke MY, Xu T, Fang Y, et al. Liver fibrosis promotes immune escape in hepatocellular carcinoma via GOLM1-mediated PD-L1 upregulation. Cancer Lett 2021;513:14-25.
22. Klutstein M, Nejman D, Greenfield R, et al. DNA Methylation in Cancer and Aging. Cancer Res 2016;76:3446-50.
23. Chen XY, Zhang J, Zhu JS. The role of m(6)A RNA methylation in human cancer. Mol Cancer 2019;18:103.
24. Chong W, Shang L, Liu J, et al. m(6)A regulator-based methylation modification patterns characterized by distinct tumor microenvironment immune profiles in colon cancer. Theranostics 2021;11:2201-17.
25. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015;19:A68-77.
26. Li Y, Xiao J, Bai J, et al. Molecular characterization and clinical relevance of m(6)A regulators across 33 cancer types. Mol Cancer 2019;18:137.
27. Edwards NJ, Oberti M, Thangudu RR, et al. The CPTAC Data Portal: A Resource for Cancer Proteomics Research. J Proteome Res 2015;14:2707-13.
28. Franz M, Rodriguez H, Lopes C, et al. GeneMANIA update 2018. Nucleic Acids Res 2018;46:W60-4.
29. Ru B, Wong CN, Tong Y, et al. TISIDB: an integrated repository portal for tumor-immune system interactions. Bioinformatics 2019;35:4200-2.
30. Macheret M, Halazonetis TD. DNA replication stress as a hallmark of cancer. Annu Rev Pathol 2015;10:425-48.
31. Gaillard H, García-Muse T, Aguilera A. Replication stress and cancer. Nat Rev Cancer 2015;15:276-89.
32. Zhu Z, Yu Z, Rong Z, et al. The novel GINS4 axis promotes gastric cancer growth and progression by activating Rac1 and CDC42. Theranostics 2019;9:8294-311.
33. Rong Z, Luo Z, Zhang J, et al. GINS complex subunit 4, a prognostic biomarker and reversely mediated by Krüppel-like factor 4, promotes the growth of colorectal cancer. Cancer Sci 2020;111:1203-17.
34. Zhan L, Yang J, Liu Y, et al. MicroRNA miR-502-5p inhibits ovarian cancer genesis by downregulation of GINS complex subunit 2. Bioengineered 2021;12:3336-47.
35. Nagahama Y, Ueno M, Haraguchi N, et al. PSF3 marks malignant colon cancer and has a role in cancer cell proliferation. Biochem Biophys Res Commun 2010;392:150-4.
36. Hokka D, Maniwa Y, Tane S, et al. Psf3 is a prognostic biomarker in lung adenocarcinoma. Lung Cancer 2013;79:77-82.
37. Sun X, Sui W, Huang M, et al. Partner of Sld five 3: a potential prognostic biomarker for colorectal cancer. Diagn Pathol 2014;9:217.
38. Luo H, Lin Y, Liu T, et al. DEG 15, an update of the Database of Essential Genes that includes built-in analysis tools. Nucleic Acids Res 2021;49:D677-86.
39. Li S, Wu L, Zhang H, et al. GINS1 Induced Sorafenib Resistance by Promoting Cancer Stem Properties in Human Hepatocellular Cancer Cells. Front Cell Dev Biol 2021;9:711894.
40. Dimri M, Satyanarayana A. Molecular Signaling Pathways and Therapeutic Targets in Hepatocellular Carcinoma. Cancers (Basel) 2020;12:491.
41. Poenitzsch Strong AM, Setaluri V, Spiegelman VS. MicroRNA-340 as a modulator of RAS-RAF-MAPK signaling in melanoma. Arch Biochem Biophys 2014;563:118-24.
42. Garnis C, Buys TP, Lam WL. Genetic alteration and gene expression modulation during cancer progression. Mol Cancer 2004;3:9.
43. Zhang B, Wu Q, Li B, et al. m6A regulator-mediated methylation modification patterns and tumor microenvironment infiltration characterization in gastric cancer. Mol Cancer 2020;19:53.
44. Du J, Ji H, Ma S, et al. m6A regulator-mediated methylation modification patterns and characteristics of immunity and stemness in low-grade glioma. Brief Bioinform 2021;22:bbab013.
45. Jiang X, Liu B, Nie Z, et al. The role of m6A modification

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in the biological functions and diseases. Signal Transduct Target Ther 2021;6:74.
46. Liu J, Harada BT, He C. Regulation of Gene Expression by N(6)-methyladenosine in Cancer. Trends Cell Biol 2019;29:487-99.
47. Li HB, Tong J, Zhu S, et al. m(6)A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathways. Nature 2017;548:338-42.
48. Chen M, Wong CM. The emerging roles of N6-methyladenosine (m6A) deregulation in liver carcinogenesis. Mol Cancer 2020;19:44.
49. Yang Y, Cai J, Yang X, et al. Dysregulated m6A modification promotes lipogenesis and development of non-alcoholic fatty liver disease and hepatocellular carcinoma. Mol Ther 2022;30:2342-53.
50. Qi LW, Jia JH, Jiang CH, et al. Contributions and Prognostic Values of N6-Methyladenosine RNA Methylation Regulators in Hepatocellular Carcinoma. Front Genet 2021;11:614566.
51. Lin S, Choe J, Du P, et al. The m(6)A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells. Mol Cell 2016;62:335-45.
52. Fu Y, Dominissini D, Rechavi G, et al. Gene expression regulation mediated through reversible m^{6}A RNA methylation. Nat Rev Genet 2014;15:293-306.
53. Peng L, Pan B, Zhang X, et al. Lipopolysaccharide facilitates immune escape of hepatocellular carcinoma cells via m6A modification of lncRNA MIR155HG to upregulate PD-L1 expression. Cell Biol Toxicol 2022;38:1159-73.
54. Ruiz de Galarreta M, Bresnahan E, Molina-Sánchez P, et al. β-Catenin Activation Promotes Immune Escape and Resistance to Anti-PD-1 Therapy in Hepatocellular Carcinoma. Cancer Discov 2019;9:1124-41.
55. Wen Q, Han T, Wang Z, et al. Role and mechanism of programmed death-ligand 1 in hypoxia-induced liver cancer immune escape. Oncol Lett 2020;19:2595-601.
56. Li T, Liu J, Wang Y, et al. Liver fibrosis promotes immunity escape but limits the size of liver tumor in a rat orthotopic transplantation model. Sci Rep 2021;11:22846.
57. Wu Y, Yang S, Ma J, et al. Spatiotemporal Immune Landscape of Colorectal Cancer Liver Metastasis at Single-Cell Level. Cancer Discov 2022;12:134-53.
58. Li X, Ramadori P, Pfister D, et al. The immunological and metabolic landscape in primary and metastatic liver cancer. Nat Rev Cancer 2021;21:541-57.
59. Yu J, Green MD, Li S, et al. Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. Nat Med 2021;27:152-64.
60. Brodt P. Role of the Microenvironment in Liver Metastasis: From Pre- to Prometastatic Niches. Clin Cancer Res 2016;22:5971-82.
61. Reisländer T, Groelly FJ, Tarsounas M. DNA Damage and Cancer Immunotherapy: A STING in the Tale. Mol Cell 2020;80:21-8.
62. Topalian SL, Taube JM, Anders RA, et al. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer 2016;16:275-87.
63. Llovet JM, Castet F, Heikenwalder M, et al. Immunotherapies for hepatocellular carcinoma. Nat Rev Clin Oncol 2022;19:151-72.

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