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Original contribution

PIGU overexpression adds value to TNM staging in the prognostic stratification of patients with hepatocellular carcinoma☆,☆☆

Jianzhong Cao MD[1], Penghui Wang MD[1], Jiemin Chen MD[1], Xiaodong He MD*

Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China

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Summary Phosphatidylinositol glycan anchor biosynthesis class U (PIGU), which is a critical subunit of the glycosylphosphatidylinositol transamidase (GPI-T) complex, has been reported to be an oncogene in bladder cancer. However, the expression and prognostic significance of PIGU in hepatocellular carcinoma (HCC) remain unclear. In this study, we conducted bioinformatics, quantitative real-time polymerase chain reaction, and immunohistochemistry analysis to investigate the expression profile of GPI-T subunits in HCC tissues, finding that PIGU was the most significantly overexpressed GPI-T subunit in HCC tissues at both the RNA and protein levels. Using Kaplan-Meier analysis and Cox proportional hazards regression models, we then comprehensively explored the prognostic impact of overexpressed PIGU in HCC patients in 2 independent HCC cohorts, and the results showed that overexpressed PIGU was an independent predictor for poor survival in HCC patients. Furthermore, based on the constructed nomogram, we proposed a risk score combining PIGU expression with the standard TNM staging system and provided a more powerful tool for the prognostic stratification of HCC patients. We also investigated the potential functional role of PIGU in HCC by performing bioinformatic analysis, indicating that PIGU might be involved in cell cycle–related biological processes in HCC. In conclusion, our findings suggest that PIGU overexpression provides independent and complementary prognostic information in HCC patients and that incorporation of this information with the traditional TNM staging system can improve prognostic stratification.

1. Introduction

Hepatocellular carcinoma (HCC) is a major public health problem, with more than 700 000 new cases and deaths occurring yearly worldwide, and China alone accounts for approximately 50% [1]. Because of a low rate of early diagnosis and lack of treatment options in the advanced stage, the prognosis of HCC remains poor [2]. HCC tends to be heterogeneous, both clinically and biologically, with different outcomes and treatment responses even in patients with the same TNM stage [3]. Therefore, identification of molecular biomarkers that

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* Corresponding author at: Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No.1 Shuaifuyuan, Beijing 100730, China.
E-mail address: hexdpumch@sina.com (X. He).

[1] J. Cao, P. Wang, and J. Chen contributed equally to this work.

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accurately predict clinical outcomes could be of substantial aid in better patient stratification and may even lead to novel targeted therapies in specific subsets of patients.

Glycosylphosphatidylinositol (GPI) membrane anchoring of proteins is a widespread phenomenon that specifically tethers proteins to lipid bilayers [4]. This posttranslational glycolipid modification is introduced into proteins via GPI transamidase (GPI-T), which contains 5 known subunits, namely, phosphatidylinositol glycan anchor biosynthesis class K, phosphatidylinositol glycan anchor biosynthesis class T, glycosylphosphatidylinositol anchor attachment 1 (GPAA1), phosphatidylinositol glycan anchor biosynthesis class S, and phosphatidylinositol glycan anchor biosynthesis class U (PIGU) [5]. Overexpression of one or more GPI-T subunits has been reported in various tumors, such as breast cancer, ovarian cancer, and uterine cancer, indicating that GPI-T itself may be tumorigenic [6,7]. PIGU, a hydrophobic protein that is essential for GPI-T activity, has been reported to be the first oncogenic GPI-T subunit [8]. PIGU is overexpressed in bladder cancer, breast cancer, and lymphoma. In bladder cancer, PIGU exerts its oncogenic role via increasing GPI-T activity and anchoring substrate proteins such as urokinase plasminogen activator surface receptor (UPAR) [9]. Genome-wide association studies analysis showed that PIGU polymorphism is associated with an increased susceptibility to cutaneous melanoma [10]. Moreover, researchers discovered that the presence of a reciprocal balanced translocation event between asparagine-linked glycosylation protein 5 homolog (ALG5) and PIGU can give rise to 2 novel gene fusions (ALG5-PIGU and PIGU-ALG5), implicating PIGU as a potential oncogene in prostate cancer [11]. Nevertheless, the expression profile, clinical importance, and biological role of PIGU in HCC are unclear and need further exploration.

In the present study, we investigated the expression profile of GPI-T in HCC tissues and provided the first evidence that PIGU is the most significantly overexpressed GPI-T subunit in HCC tissues at both the RNA and protein levels. Using Kaplan-Meier analysis, subgroup analysis, and Cox proportional hazards regression models, we then comprehensively explored the prognostic impact of overexpressed PIGU in HCC patients in 2 independent HCC cohorts. Furthermore, based on the constructed nomogram, we proposed a risk score combining PIGU expression with the standard TNM staging system and provided a more powerful tool for prognostic stratification of HCC patients. We also investigated the potential functional role of PIGU in HCC by performing bioinformatic analysis, indicating that PIGU may be involved in cell cycle–related biological processes.

2. Materials and methods

2.1. Patients and tissue specimens

Forty paired fresh-frozen HCCs and adjacent nontumor tissue samples used in quantitative real-time polymerase chain reaction (qRT-PCR) were randomly collected from HCC patients who had underwent hepatectomy at Peking Union Medical College Hospital (PUMCH; Beijing, China) between 2010 and 2014. All samples were collected immediately after resection of the tumors and then stored in liquid nitrogen.

One hundred sixty-six HCC, 31 paratumor, and 36 normal liver tissue samples were used in tissue microarray (TMA) analysis. HCC tissue samples were consecutively chosen from 166 HCC patients who had underwent hepatectomy at PUMCH between January 2010 and December 2011. Normal liver tissues were collected from patients with hepatolithiasis who were treated in the same hospital. Complete clinicopathological and follow-up data are available for the 166 HCC samples.

All diagnoses were confirmed pathologically. The study protocol was approved by the ethics committee of Peking Union Medical College Hospital. Informed consent was obtained from each patient.

2.2. TMA construction and immunohistochemistry

TMAs were constructed in the present study. In brief, hematoxylin and eosin sections were assessed, and an appropriate area of tumor was marked on the corresponding paraffin block. While avoiding necrotic tissue, the representative 1.5-mm-diameter tissue cores were removed by a hollow needle and reembedded into a recipient paraffin block at a defined position. Two TMA blocks were then built in PUMCH.

TMA slides were pretreated at 65°C for 2 hours, followed by deparaffinization. Antigen retrieval was performed using citrate buffer (pH 6) at a temperature of 97°C for 20 minutes. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide for 10 minutes at room temperature. Nonspecific binding of the antibody was blocked by incubating with 5% normal goat serum in phosphate-buffered saline containing 0.1% Tween 20 for 1 hour at room temperature. The slides were then incubated with primary antibodies against PIGU (1:500; Abcam; ab192255, Cambridge, MA, USA) overnight at 4°C. After washing, slides were incubated for 2 hours at room temperature with the secondary antibody conjugated to horseradish peroxidase (1:100; Dako, Glostrup, Denmark). Horseradish peroxidase activity was detected using the Liquid DAB+ Substrate Chromogen System (Dako). Finally, sections were counterstained with hematoxylin and photographed.

PIGU expression was evaluated using the "hybrid scoring system" (H-score) criteria based on both the percentage of positively stained cells and on the intensity of staining. In brief, the H-score was calculated as the sum of the product of the staining intensity in tumor cells (0, no staining; 1, weak staining; 2, moderate staining; 3, intense staining) and the extent of cells showing that staining intensity (0-100%). Therefore, the possible H-score ranged from 0 to 300, and the scores for each core reported by 2 independent readers were averaged.

2.3. RNA isolation, reverse transcription, and qRT-PCR

Total RNA was extracted from tissues using TRIzol (Invitrogen, Carlsbad, CA) and reverse transcribed using the GoScript Reverse Transcription System (Promega Corporation, Madison, WI) according to the manufacturer's instruction. Then qRT-PCR was performed using LightCycler 480 real-time PCR (Roche, Basel, Switzerland), and the data were normalized to GAPDH messenger RNA (mRNA) expression. The primers for qRT-PCR were available in supplementary data (Supplementary Table 1).

2.4. TCGA database and bioinformatic analysis

The Cancer Genome Atlas (TCGA) database stores genomic and clinical data for a number of cancers, including HCC, and the data have been made public for appropriate analysis according to the publication guidelines [12]. The gene expression (FPKM) and clinical data of the HCC patients from the TCGA liver cancer (LIHC) data set were obtained by using UCSC Xena Browser (https://xenabrowser.net/). In the TCGA LIHC cohort, RNAseq data were available in 371 HCC tissues and 50 adjacent nontumor tissues, and 365 of the 371 HCC cases contain detailed clinical and follow-up data. Another RNAseq data set comprising 50 HCC samples (GSE65485) was downloaded from GEO data sets [13].

Gene set enrichment analysis (GSEA) was performed to identify the pathways that are correlated with PIGU expression in the TCGA HCC data set [14]. Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis was conducted to predict the function of PIGU using DAVID Bioinformatics Tool [15,16].

2.5. Statistical analysis

Student *t* test was applied for the comparison of the 2 groups. The correlation coefficients were determined using the Pearson correlation test. Survival curves were determined using the Kaplan-Meier method and compared using the log-rank test. Hazard ratios (HRs) of death associated with PIGU expression and other predictor variables were estimated by the univariate Cox proportional hazards regression model, and a multivariate Cox model was constructed to estimate the adjusted HR for PIGU expression. Based on the results of the multivariable analysis, the nomogram was formulated by R software with the survival and rms package. Calibration of the nomogram for 5-year overall survival (OS) was performed by comparing the predicted survival with the observed survival. The model performance for predicting outcome was also evaluated by calculating the concordance index (*C* index). The prognostic performance of the nomogram-based risk score was then evaluated by survival receiver operating characteristic (ROC) analysis. *P* values less than .05 were considered statistically significant. All statistical analyses were conducted using the SPSS statistical software package (version 22.0; SPSS, Chicago, IL), GraphPad Prism 7 (GraphPad Software, La Jolla, CA), and R software (V 3.3.3; R Foundation for Statistical Computing, Vienna, Austria).

3. Results

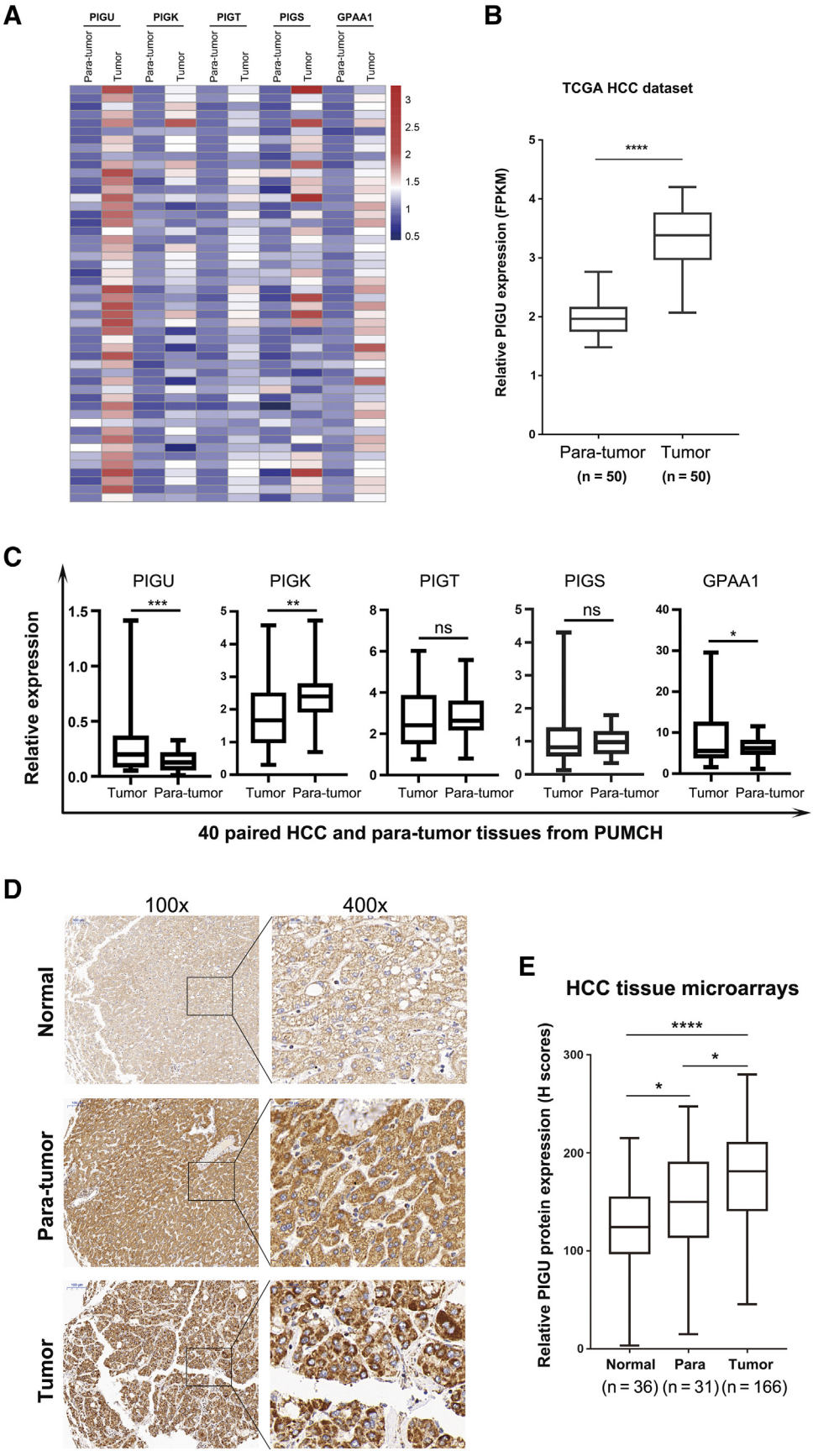
3.1. PIGU is the most significantly overexpressed GPI-T subunit in HCC

First, we searched the TCGA database to identify the expression profile of the GPI-T complex genes in HCC. We found that almost all 5 GPI-T subunits tended to be up-regulated in HCC tissues compared with paratumor tissues, and PIGU was the most significant overexpressed subunit (Fig. 1A and B). To further examine the expression pattern of the GPI-T subunits, we performed qRT-PCR in clinical HCC samples. The results confirmed that PIGU was consistently overexpressed in HCC tissues (Fig. 1C). We then used immunohistochemistry (IHC) analysis in HCC TMAs to investigate PIGU expression at the protein level, and we found that PIGU protein expression was also increased in HCC tissues compared with paratumor or normal liver tissues (Fig. 1D and E). Collectively, these data demonstrated that the GPI-T subunit PIGU was significantly overexpressed in HCC tissues at both the RNA and protein levels.

3.2. Overexpressed PIGU correlates with poor survival in HCC

To investigate the prognostic value of PIGU expression in HCC patients, we performed a Kaplan-Meier analysis in the TCGA HCC data set (TCGA cohort), and the result showed that HCC patients with high PIGU expression at the mRNA level had significantly poorer survival compared with those

Fig. 1 PIGU is the most significantly overexpressed GPI-T subunit in HCC. A, Heatmap of GPI-T subunits in 50 paired HCC and paratumor tissues from the TCGA database. The average FPKM values of the genes in paratumor tissues were normalized to 1. B, Expression level of PIGU in 50 paired HCC and paratumor tissues from the TCGA database (****$P < .0001$). C, qRT-PCR analysis of the GPI-T subunits expression in 40 paired HCC and paratumor tissues from PUMCH (****$P < .0001$ and **$P < .01$; not significant [ns], $P > .05$). D, Representative photographs of PIGU expression in TMA sections of normal liver, paratumor, and HCC tissues. Original magnifications ×100 (left) and ×400 (right). E, Comparison of the H-scores of PIGU between normal liver (n = 36), paratumor (n = 31), and HCC (n = 166) tissues (****$P < .0001$ and *$P < .05$).


A
PIGU
PIGK
PIGT
PIGS
GPAA1
Para-tumor
Tumor
3
2.5
2
1.5
1
0.5
B
TCGA HCC dataset
Relative PIGU expression (FPKM)
****
Para-tumor
(n = 50)
Tumor
(n = 50)
C
PIGU
PIGK
PIGT
PIGS
GPAA1
Relative expression
***
**
ns
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*
Tumor
Para-tumor
40 paired HCC and para-tumor tissues from PUMCH
D
100x
400x
Normal
Para-tumor
Tumor
E
HCC tissue microarrays
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(n = 166)

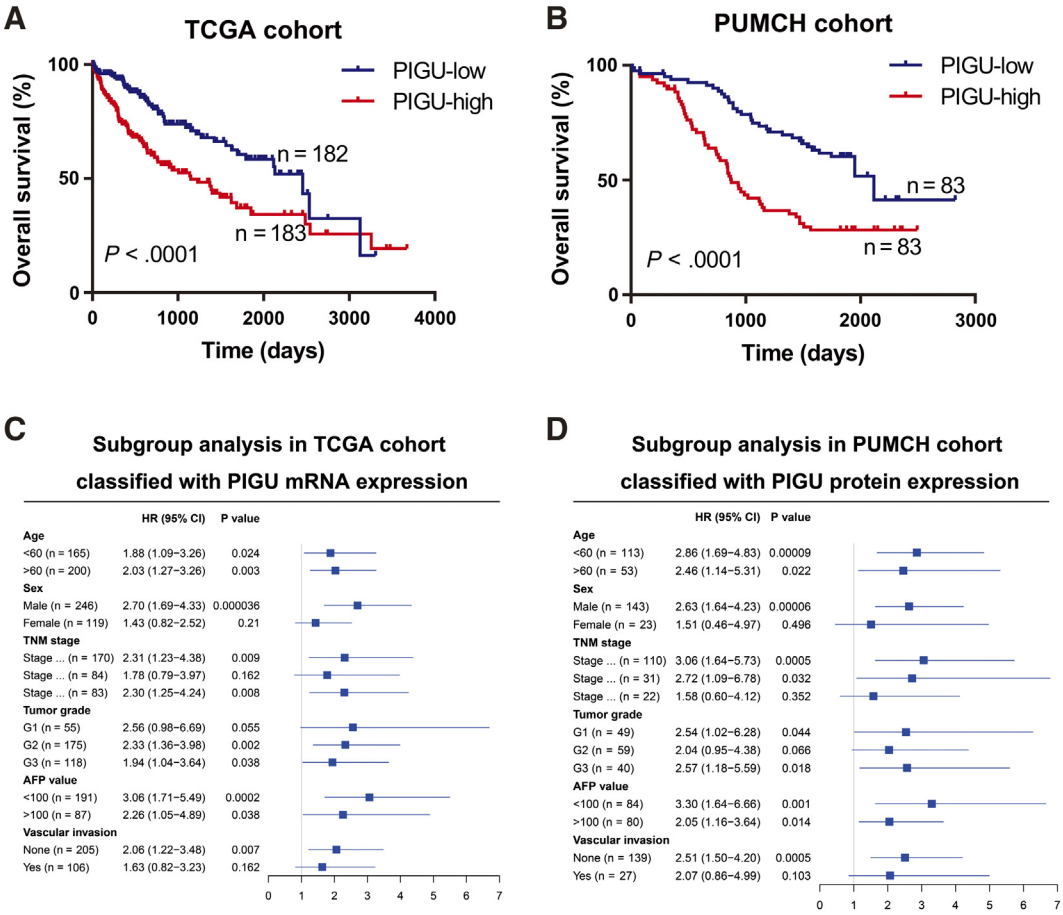

Fig. 2 Overexpressed PIGU correlates with poor survival in HCC. A and B, Kaplan-Meier curves for HCC patients in the TCGA or PUMCH cohorts by PIGU, mRNA, or protein expression. C and D, Prognostic impact of PIGU overexpression in the subgroups of HCC patients in the TCGA or PUMCH cohorts. The lines indicate the range from the lower to the upper bound of the 95% confidence interval of the corresponding HR; the box indicates the corresponding HR.

with low PIGU expression (median survival time, 1149 and 2456 days, respectively; Fig. 2A). To further confirm the above finding, we performed IHC analysis in HCC TMAs (PUMCH cohort) to evaluate PIGU protein expression, and the Kaplan-Meier analysis demonstrated that high PIGU protein expression indeed identified a subgroup of HCC patients with poor survival (median survival time, 934 and 2117 days, respectively, for PIGU-high and PIGU-low protein expression groups; Fig. 2B).

When stratified by the clinicopathological variables, PIGU overexpression showed a significant prognostic impact in most subgroups in the TCGA cohort, except showing a tendency in subgroup female (HR, 1.43; P = .21), TNM stage II (HR, 1.78; P = .162), grade 1 (HR, 2.56; P = .055), and vascular invasion (HR, 1.63; P = .162; Fig. 2C). In the PUMCH cohort, high PIGU expression at the protein level also showed a significant prognostic impact in most subsets of the HCC patients, except within subgroup female (HR, 1.51; P = .496), TNM stage III (HR, 1.58; P = .352), grade 2 (HR, 2.04; P = .066), and vascular invasion (HR, 2.07; P = .103; Fig. 2D).

3.3. PIGU overexpression is an independent prognostic predictor for OS in HCC

We then sought to explore whether overexpressed PIGU predicted poor OS independently using a multivariate Cox proportional hazard model. In the TCGA cohort, the result showed that PIGU mRNA overexpression was a predictor of poorer OS in HCC patients independent of TNM stage (Table 1). In the PUMCH cohort, the multivariate Cox proportional hazards analysis demonstrated that PIGU protein overexpression remained an independent predictor of shorter OS time in HCC patients after adjusting for TNM stage, tumor grade, and AFP value (Table 2). Taken together, these data confirmed that PIGU overexpression, at both the mRNA and protein levels, served as an independent predictor of poor survival in HCC patients after adjusting for potential confounding factors.

3.4. A risk score integrating PIGU expression with TNM staging better predicts OS in HCC

Considering that PIGU expression and TNM stage were the most significant and independent prognostic factors in both HCC cohorts, we investigated whether adding the PIGU expression level to the widely used TNM staging system would improve the prediction of prognosis in HCC patients. We constructed a nomogram that incorporated PIGU expression and TNM stage in the TCGA cohort (Fig. 3A and Supplementary Table 2). The calibration plots presented an acceptable agreement in both the primary TCGA cohort and the PUMCH validation cohort between the nomogram prediction and actual observation for 5-year OS (Fig. 3B and C). Moreover, the Harrell *C* index for the nomogram established to predict OS was much higher than that of the TNM staging system in both the TCGA (0.68 versus 0.61) and PUMCH (0.71 versus 0.65) cohorts.

To investigate the performance of the established nomogram in stratifying the risk of patients, we then added up the scores assigned by the nomogram and obtained a risk score for each patient. According to the risk scores, we grouped the HCC patients into 3 subgroups, namely, low-risk (risk score 0), medium-risk (risk scores 4-9), and high-risk (risk scores 10-18) groups. Kaplan-Meier analysis showed that each risk group represented a distinct prognosis (Fig. 4A and B). Furthermore, the survival ROC analysis demonstrated that the risk score showed better performance than the TNM stage in predicting 5-year survival of HCC patients in both the TCGA cohort (area under curve, 0.72 versus 0.65) and the PUMCH cohort (area under curve, 0.788 versus 0.705; Fig. 4C and D). Altogether, these results indicated that the established nomogram was an accurate risk prediction tool, and the nomogram-based risk score tended to predict OS better than the TNM stage in HCC patients.

3.5. PIGU may be involved in cell cycle regulation in HCC, as revealed by bioinformatic analysis

To preliminarily explore the biological role of PIGU in HCC, we performed GSEA analysis using TCGA HCC RNA-seq data, and the results showed that multiple cell cycle–related biological processes, such as DNA repair, MYC targets, E2F targets, and G2M checkpoint, were significantly enriched among the PIGU-associated genes in HCC (Fig. 5A and B). Moreover, we conducted the KEGG pathway enrichment analysis for PIGU coexpressed genes (Pearson correlation coefficient ≥ 0.35), and we found that the cell cycle pathway was also significantly enriched (Supplementary Table 3). Hierarchical clustering analysis revealed that these enriched cell cycle pathway genes separated PIGU-high HCC from PIGU-low HCC in the 2 independent HCC cohorts, indicating that these cell cycle genes may represent a functional module and be involved in the PIGU-regulated program (Fig. 5C and D).

Table 1 Cox proportional hazard regression analysis for OS in TCGA cohort

Variables	n [a]	Univariate analysis		Multivariate analysis	
		HR (95%CI)	*P*	HR (95%CI)	*P*
Age (y)					
<60	165	Reference			
≥60	200	1.21 (0.85-1.72)	.28		NA
Sex					
Male	246	Reference			
Female	119	1.23 (0.86-1.75)	.26		NA
TNM stage					
Stages I-II	254	Reference		Reference	
Stages III-IV	87	2.45 (1.69-3.55)	.000002	2.33 (1.60-3.38)	.00001
Tumor grade					
G1-2	230	Reference			
G3-4	130	1.12 (0.78-1.60)	.54		NA
AFP value					
<100	191	Reference			
≥100	87	1.15 (0.73-1.80)	.55		NA
PIGU expression					
Low	182	Reference		Reference	
High	183	2.04 (1.43-2.92)	.00009	1.99 (1.36-2.92)	.0004

Abbreviations: CI, confidential interval; NA, not adopted; AFP, α-fetoprotein.

[a] Age, sex, and PIGU expression data are available in all cases (365 in total); TNM stage (341 cases), tumor grade (360 cases), and AFP value (278 cases) data are also available for analysis.

Table 2 Cox proportional hazard regression analysis for OS in PUMCH cohort

Variables	n [a]	Univariate analysis		Multivariate analysis	
		HR (95%CI)	*P*	HR (95%CI)	*P*
Age (y)					
<60	113	Reference			
≥60	53	1.12 (0.72-1.75)	.62		NA
Sex					
Male	143	Reference			
Female	23	0.92 (0.50-1.70)	.80		NA
TNM stage					
Stages I-II	141	Reference			
Stages III-IV	25	3.27 (1.99-5.39)	.000003	3.17 (1.85-5.41)	.00003
Tumor grade					
G1-2	108	Reference			
G3-4	54	1.87 (1.21-2.89)	.005	2.20 (1.39-3.48)	.0008
AFP value					
<100	84	Reference			
≥100	80	1.73 (1.12-2.67)	.014	1.13 (0.71-1.80)	.599
PIGU expression					
Low	83	Reference			
High	83	2.52 (1.62-3.90)	.00004	2.95 (1.86-4.69)	.000005

Abbreviations: CI, confidential interval; NA, not adopted; AFP, α-fetoprotein.

[a] Age, sex, TNM stage, and PIGU expression data are available in all cases (166 in total); tumor grade (162 cases) and AFP value (164 cases) data are also available for analysis.

4. Discussion

Existing studies have shown that the expression and prognostic significance of PIGU in HCC remain unknown. This study represents the first investigation of dysregulated PIGU expression and its prognostic impact on HCC patients. Our results indicated that PIGU was the most significantly overexpressed GPI-T complex gene in HCC tissues and that HCC patients with high PIGU expression had a poorer survival. Moreover, our study revealed that the nomogram-based

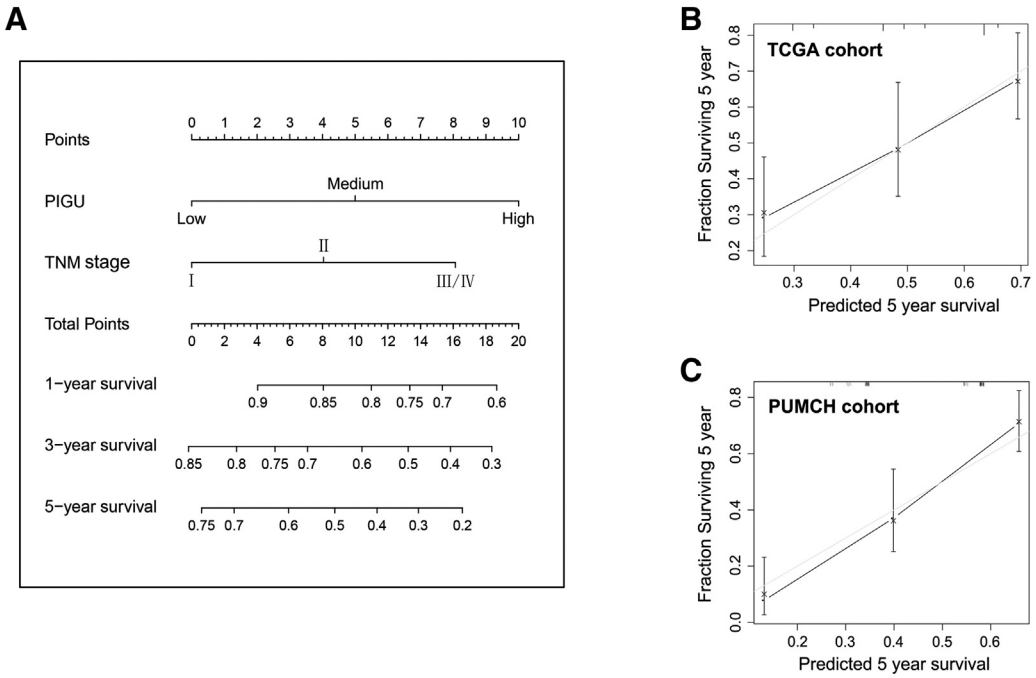

Fig. 3 Construction of a prognostic nomogram integrating PIGU expression with TNM staging in HCC patients. A, Prognostic nomogram for HCC patients in the TCGA cohort. B and C, The calibration curves for predicting the 5-year survival of HCC patients in the TCGA and PUMCH cohorts. Nomogram-predicted OS is plotted on the *x*-axis, and actual OS is plotted on the *y*-axis.

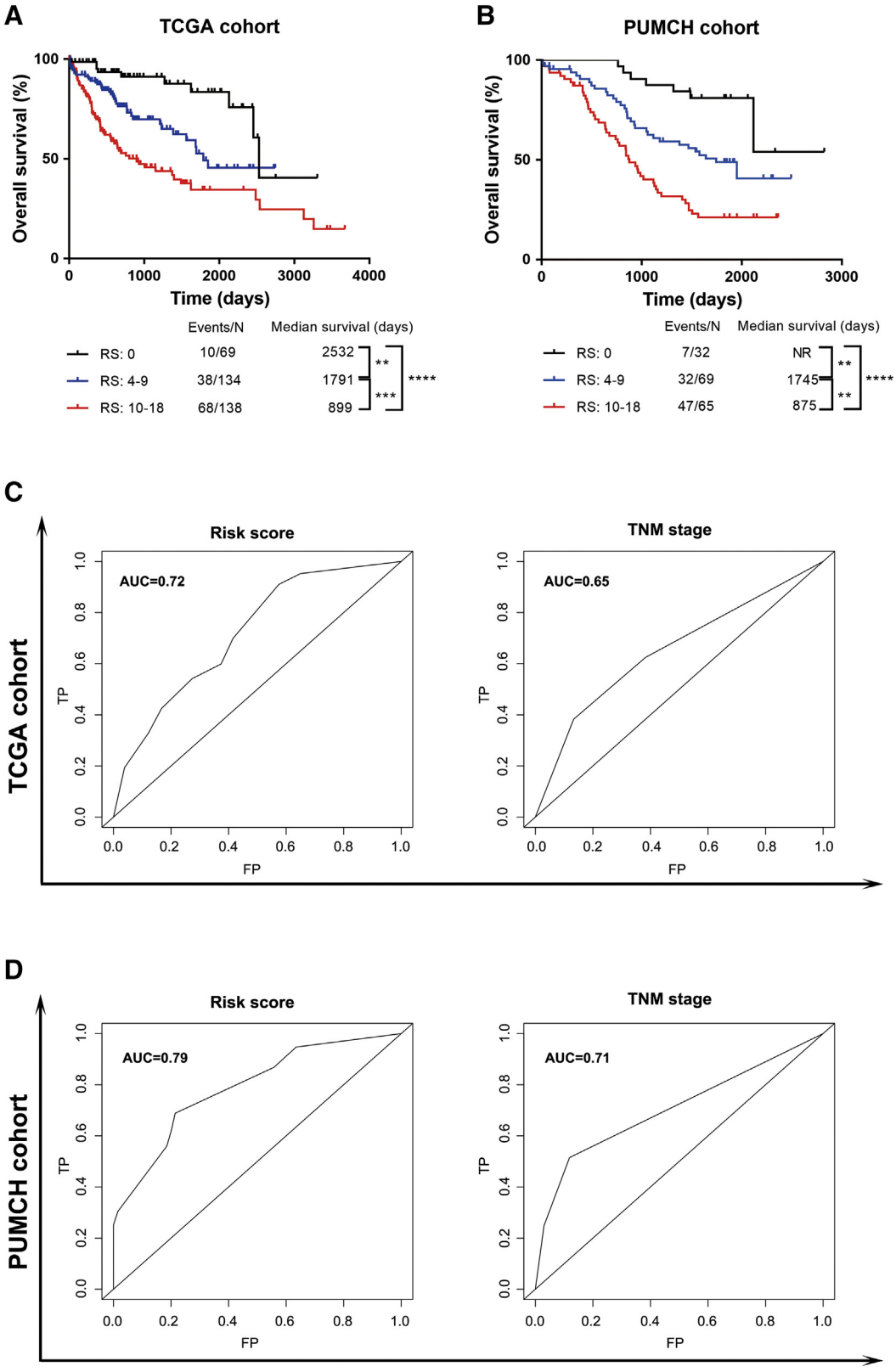

Fig. 4 The nomogram-based risk score shows superior performance in the prognostic stratification of HCC patients. A and B, Kaplan-Meier curves of HCC patients in the TCGA or PUMCH cohorts stratified by the risk scores (not reached [NR]; ****$P < .0001$, ***$P < .001$, and **$P < .01$). C and D, Survival ROC analysis in terms of the 5-year OS for HCC patients in the TCGA and PUMCH cohorts. AUC indicates area under curve; FP, false positive; TP, true positive.

risk score, which incorporated PIGU expression with the standard TNM staging system, served as a more powerful tool for the prognostic stratification of HCC patients. Preliminary bioinformatic analysis indicated that PIGU might be involved in cell cycle regulation in HCC.

As reported by a range of studies, GPI-anchored proteins are fundamentally involved in various cancers [7]. In 2004, the discovery of PIGU as an oncogene in human bladder cancer provided the first evidence that GPI-T might be tumorigenic [9]. After that, many studies were carried out to investigate the relationship between GPI-T and cancer. By investigating the expression of the GPI-T subunits in 19 different human cancers, researchers demonstrated a more frequent expression of GPI-T subunits in cancers than in normal tissue, especially in breast, ovarian, and uterus cancer [6]. In breast cancer, PIG-T and GPAA1 were significantly overexpressed, contributing to tumorigenesis and invasion in human breast cancer cells [17]. An increased expression level and elevated copy number for GPAA1 were reported in head and neck squamous carcinoma and HCC [18,19]. All the studies mentioned above demonstrated the deregulated expression and functional contribution of the GPI-T subunits in different cancers, indicating potential implications in diagnosis, prognosis, and therapeutic intervention. Therefore, we hypothesized that the GPI-T subunits might also be dysregulated in HCC, and a specific subunit could serve as a molecular marker for prognostic prediction in HCC patients. In our study, by integrating the RNAseq data from the TCGA HCC data set and the qRT-

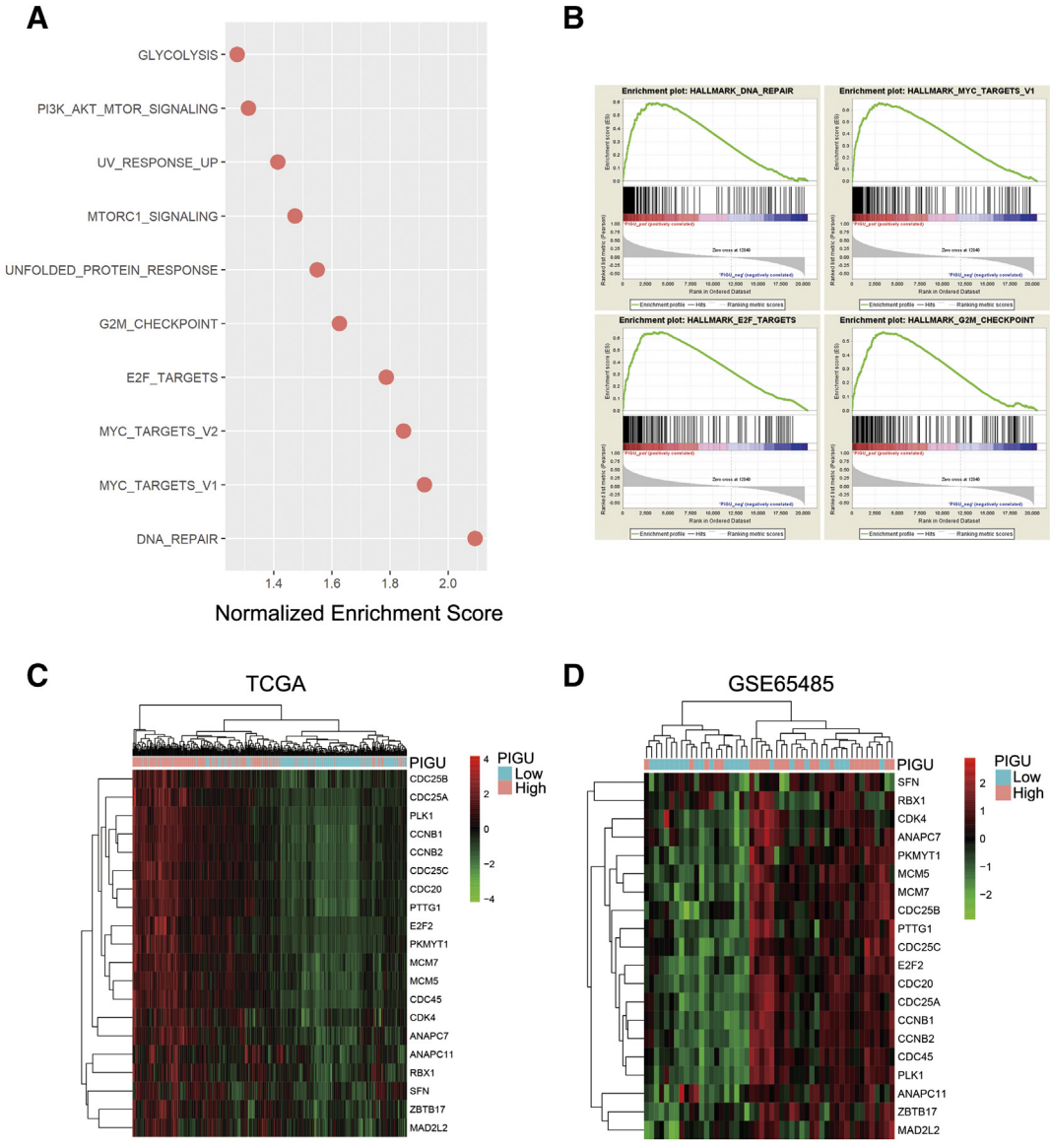

Fig. 5 PIGU may be involved in cell cycle regulation in HCC. A, GSEA of the PIGU-related pathways in HCC. B, Association between the top enrichment gene sets and PIGU expression in HCC by GSEA analysis. C and D, Heatmaps of the enriched cell cycle genes in 2 independent HCC cohorts and their association with PIGU expression.

PCR data from fresh-frozen HCC tissues, we demonstrated that PIGU was the most significantly overexpressed GPI-T subunit in HCC tissues. The expression of GPAA1 was also increased in HCC, which was consistent with a previous study [19]. We then performed IHC analysis to detect PIGU expression at the protein level in HCC TMAs, validating PIGU overexpression at the protein level in HCC. Kaplan-Meier curve and univariate and multivariate Cox proportional hazards regression analysis were then performed in 2 independent HCC cohorts, showing that overexpressed PIGU was an independent predictor for poor survival in HCC patients. Moreover, we constructed a nomogram and then proposed a risk score that combined PIGU expression with the standard TNM staging system. The Kaplan-Meier analysis showed that the nomogram-based risk score could stratify HCC patients into subgroups with distinct prognoses, and the survival ROC analysis demonstrated that the risk score had better performance in predicting 5-year survival of HCC patients than did the standard TNM staging system. All these results provide strong support for the notion that a specific GPI-T subunit (PIGU) not only is overexpressed in HCC but also serves as a potent molecular marker for prognostic prediction in HCC patients.

PIGU is the fifth subunit of the GPI-T complex, which is important for the biological function of many membrane-associated proteins [8]. PIGU was found to be oncogenic in bladder cancer, giving the first hint for the involvement of GPI-T in cancer [9]. Another study also found the presence of a reciprocal balanced translocation event between ALG5 and PIGU in prostate cancer [11]. In bladder cancer, PIGU promoted UPAR expression and STAT-3 phosphorylation, indicating that the GPI-anchoring function of PIGU had an important role in its oncogenic ability [9]. However, the impact of PIGU in HCC and its mechanism have not yet been reported. In this study, we performed preliminary bioinformatic analysis to investigate the potential functional role of PIGU in HCC, and we found that PIGU might be involved in several cell cycle–related processes. Whether the mechanisms of PIGU in terms of HCC progression depend on the GPI-anchoring pathway requires further investigation.

In conclusion, our results indicate that high PIGU expression is predictive of a poor prognosis in HCC and that the nomogram-based risk score incorporating PIGU expression with the standard TNM staging system tends to be a more powerful tool for prognostic stratification of HCC patients. Preliminary bioinformatic analysis indicated that PIGU may be involved in cell cycle regulation in HCC. Additional studies are required to clarify the molecular mechanisms through which PIGU promotes HCC development.

Author contributions

X. He and J. Cao designed the research. J. Cao, P. Wang, and J. Chen performed the research. J. Cao analyzed the data. X. He and J. Cao wrote the manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.08.013.

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