Original Article
Glutaminase 2- a valuable predictor for hepatocellular carcinoma patients' survival

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Abstract: Glutaminase2 is a p53 target gene and is known to play an important role in energy metabolism. Glutaminase2 has been reported to be downregulated in human hepatocellular carcinomas. However, the prognostic value of glutaminase2 expression in hepatocellular carcinoma patients is still unclear. Here, we investigated the prognostic value of glutaminase2 expression in liver cancer patients. Glutaminase2 mRNA expression was determined in tumor tissues and non-tumor tissues by real-time PCR. For evaluation of the prognostic value of glutaminase2 expression to each clinicopathologic factor, Kaplan-Meier method and Cox's Proportional Hazard Model (univariate analysis and multivariate analysis all were used) were employed. A simple risk scoredevised by using significant variables obtained from Cox's regression analysis for further predicting the HCC patients' prognosis. We observed reduced glutaminase2 mRNA level in cancerous tissues in comparison to non-cancerous tissues. Glutaminase2 expression was also significantly correlated with hepatitis B surface antigen expression, histological grade and tumor stage. More importantly, Kaplan-Meier analysis showed that patients with high glutaminase2 expression had longer disease-free survival and overall survival compared with those with low expression of glutaminase2. Cox's regression analysis indicated that glutaminase2 expression, histological grade, and tumor stage might be significant prognostic factors for disease-free survival and overall survival. Finally, we found that patients whose total score more than 2 are more likely to die or relapse than patients whose total score less than 2. Glutaminase2 expression in liver tumors is a potential prognostic tool for patients. The risk scoring system is useful in predicting survival of liver cancer patients after tumor resection.

Keywords: GLS2, hepatocellular carcinoma, biomarker, risk scoring system, prognosis

Introduction

Hepatocellular carcinoma (HCC) with a high mortality is one of the most common cancers worldwide, especially in Asia [1]. Based on molecular profiling, several prognostic markers for HCC are also used in clinic [2], but only a few genes have been identified as useful. So it is challenging to evaluate the prognosis of HCC patients.

Energy metabolism has been considered a crucial hallmark of cancer [3, 4]. Increased aerobic glycolysis (also known as the Warburg effect) and glutaminolysis are commonly found in many malignancies [5, 6]. During malignancy development and progression, the glutamine (Gln) pathway provides a variety of essential products to sustain biological function and cell proliferation, such as ATP generation and macromolecules for biosynthesis [6, 7]. Mitochondrial glutaminase is the key enzyme that converts glutamine to glutamate in glutaminolysis [8, 9], and it plays a crucial role in regulating cellular catabolism and maintaining redox balance in cancer cells [9-11]. *Glutaminase 2 (GLS2)* gene islocated in chromosome 12, and the proteins encodedby GLS2 gens are highly expressed in normal adult liver [12]. As a mitochondrial glutaminase, Glutaminase 2 (GLS2) can catalyze the hydrolysis of glutamine to glutamate and it has been identified as a p53 target gene to influence the energy metabolism [13, 14]. By decreasing reactive oxygen species (ROS) levels, GLS2 can regulate antioxidant defense function in cells; moreover, the oxida-

tive stress is known to contribute to genetic instability and cancer initiation and progression, and GLS2 can protect cells from oxidative stress, so cancer development can be inhibited by GLS2 [15, 16]. Interestingly, one study has been provided evidence showing that epigenetic silencing of GLS2 via promoter hypermethylation is common in human liver and colon cancers, and GLS2 appears to be a functional tumor suppressor involved in the liver and colon tumorigenesis. However, no specific associations between clinical outcomes and GLS2 expression have been identified.

We hypothesized that GLS2 could be used as a pathological and prognostic biomarker for HCC patients. Therefore, we investigated the expression of GLS2 in a large set of HCC specimens. The results validated the relevance of GLS2 expression to HCC clinical outcomes.

Material and methods

Specimen cohorts

Seventy-two patients (56 males and 16 females) from Huashan Hospital (Shanghai, China) were included in this study. All the patients underwent radical hepatic resection for HCC between 2008 and 2010. The age of the patients ranged from 16 to 84 years (mean ± standard deviation [SD], 53.67 ± 12.30 years). The criteria for radicality has been published [17]. To identify any tumor recurrence, all the patients after surgery were followed postoperatively for 3-6 weeks using ultrasonography, computed tomography (CT), and angiography if necessary. Serum α-fetoprotein (AFP) levels were measured in the outpatient clinic. None of the patients in this study received any preoperative chemotherapy or embolization therapy. The tumor tissues and the adjacent non-tumor tissues were collected from these patients above as frozen samples. The distance between adjacent non-tumor tissue and tumor tissue boundary was 2 cm, beyond of which was regarded as distant normal tissue. The selected tumor areas had more than 80% of tumor cells as being confirmed by histology examination. Classification of tumor stages using the tumor-node-metastasis (TNM) stage was based on the 7th edition of the AJCC (American Joint Committee on Cancer) cancer staging manual [18].

All patients were given informed consent for obtaining the study specimens. Experiments and procedures were in accordance with the Helsinki Declaration of 1975, and approved by the Human Ethics Committee of Shanghai Fudan University.

Follow-up

Follow-up ended at death or June 1st, 2013, whichever came first. Follow-up imaging was performed every 3-6 months for 2 years and then every 6-12 months. According to the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1) [19], the appearance of one or more new malignant lesions on multiphase computed tomography (CT) scan or magnetic resonance (MR) imaging denotes disease progression. Disease-free survival (DFS) was defined as the time period from the date of surgery operation to the first cancer recurrence (local or distant). Overall survival (OAS) was calculated from the date of cancer resection to death or the last contact.

RNA/DNA extraction and reverse transcription

Total RNA and genomic DNA from human tissue samples were extracted using Trizolreagent (Invitrogen) according to the manufacturer's instructions and their concentrations were quantified by NanoDrop 1000 (Wilmington, DE., USA). A reverse transcription reaction was performed using 1 μg of total RNA with High Capacity cDNA Reverse Transcriptionkit (Applied Biosystems, Foster City, CA, USA).

Quantitative real-time PCR

The mRNA level of Gls2 was determined by real-time PCR using SYBR Green Master Mix Kit and ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde-3- phosphate dehydrogenase (GAPDH) was used as an internal control. The $2^{-\Delta\Delta ct}$ method was used to analyze the relative changes in Gls2 expression from real-time PCR experiments [20]. Real-time PCR was performed in triplicate. Primers used for Gls2 were: Gls2-F 5'-TCCAGCTGT-GTTCTGTGGAG-3' and Gls2-R 5'-GCAAACTGGCCAGAGAA GTC-3' (175 bp product).

Statistical analysis

Kruskal-Wallis test and One-way ANOVA were used to examine the statistical difference
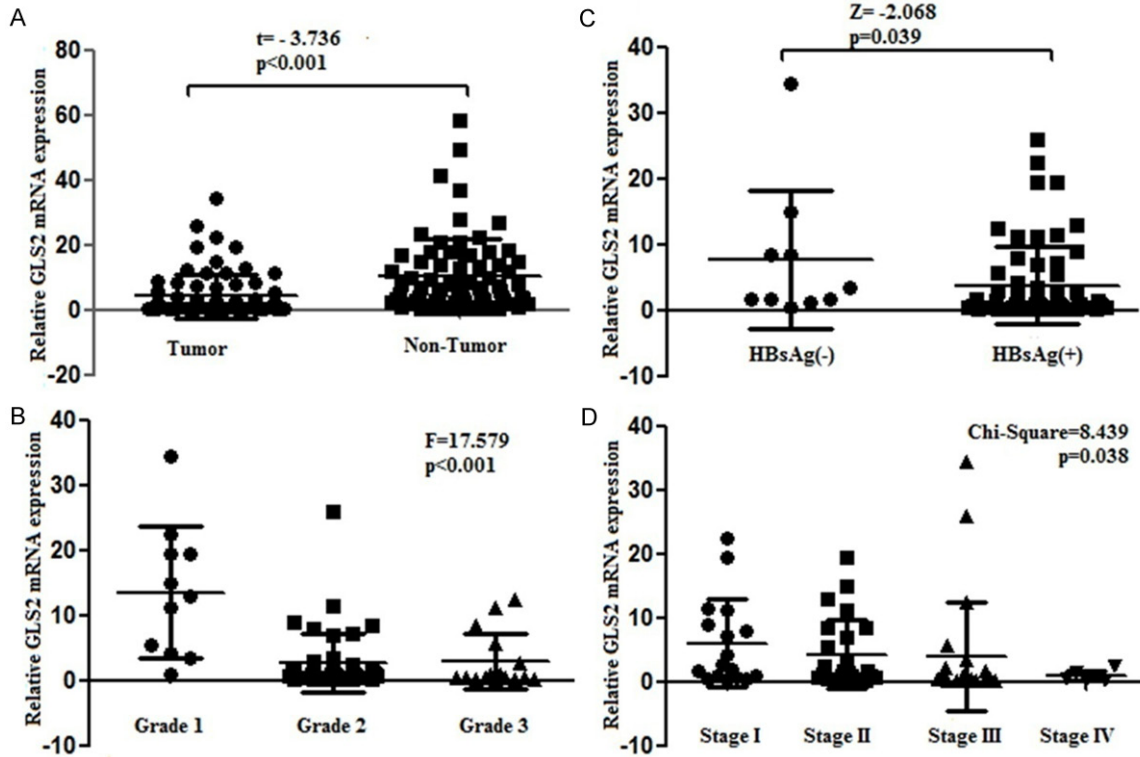
Figure 1. Clinicopathologic features and expression of GLS2. The expression of GLS2 in HCC tissues and adjacent non-tumor tissues was determined by real-time PCR. A. 72 pairs of samples were from liver tissue, including tumor tissue and adjacent non-tumor tissue; *P* value according to the independent-samples t-test. B. The expression of GLS2 mRNA in different histological grade (three-tier grading scheme) of primary HCC tissues; *P* value according to the One-way ANOVA. C. The expression of GLS2 mRNA in HBsAg positive(+) group and HBsAg negative(-) group; *p* value according to the Mann-Whitney U-test. D. The expression of GLS2 mRNA in different TNM stage of primary HCC tissues; *P* value according to the Kruskal-Wallis test.
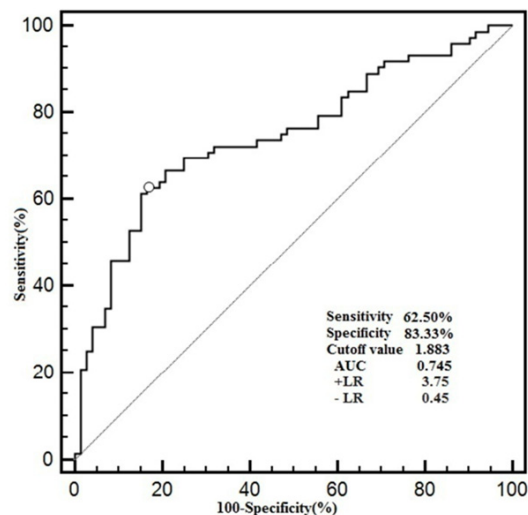

Figure 2. ROC curves of GLS2 expression to indentify the cutoff value of relative GLS2 mRNA level. +LR, positive likelihood ratio, 3,75; -LR, negative likelihood ratio, 0.45; sensitivity = 62.50%; specificity = 83.33%; AUC = 0.745; cutoff value = 1.883.

among three groups or more. The Mann-Whitney U-test or independent-samples t-test were used to compare continuous variables between two groups. The diagnostic performance of GLS2 was assessed by receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC). The optimal cutoff value was determined to maximize the sum of sensitivity and specificity; negative likelihood ratio and positive likelihood ratio were computed for the cutoff GLS2 value.

Survival curves were plotted using the Kaplan-Meier method and the statistical significance between groups was determined using the log-rank test. Independent variables predicting survival were evaluated using a multiple stepwise regression analysis using the Cox model. A simple risk score devised by using significant variables obtained from multiple stepwise Cox's regression analysis with P < 0.05. The discrimi-
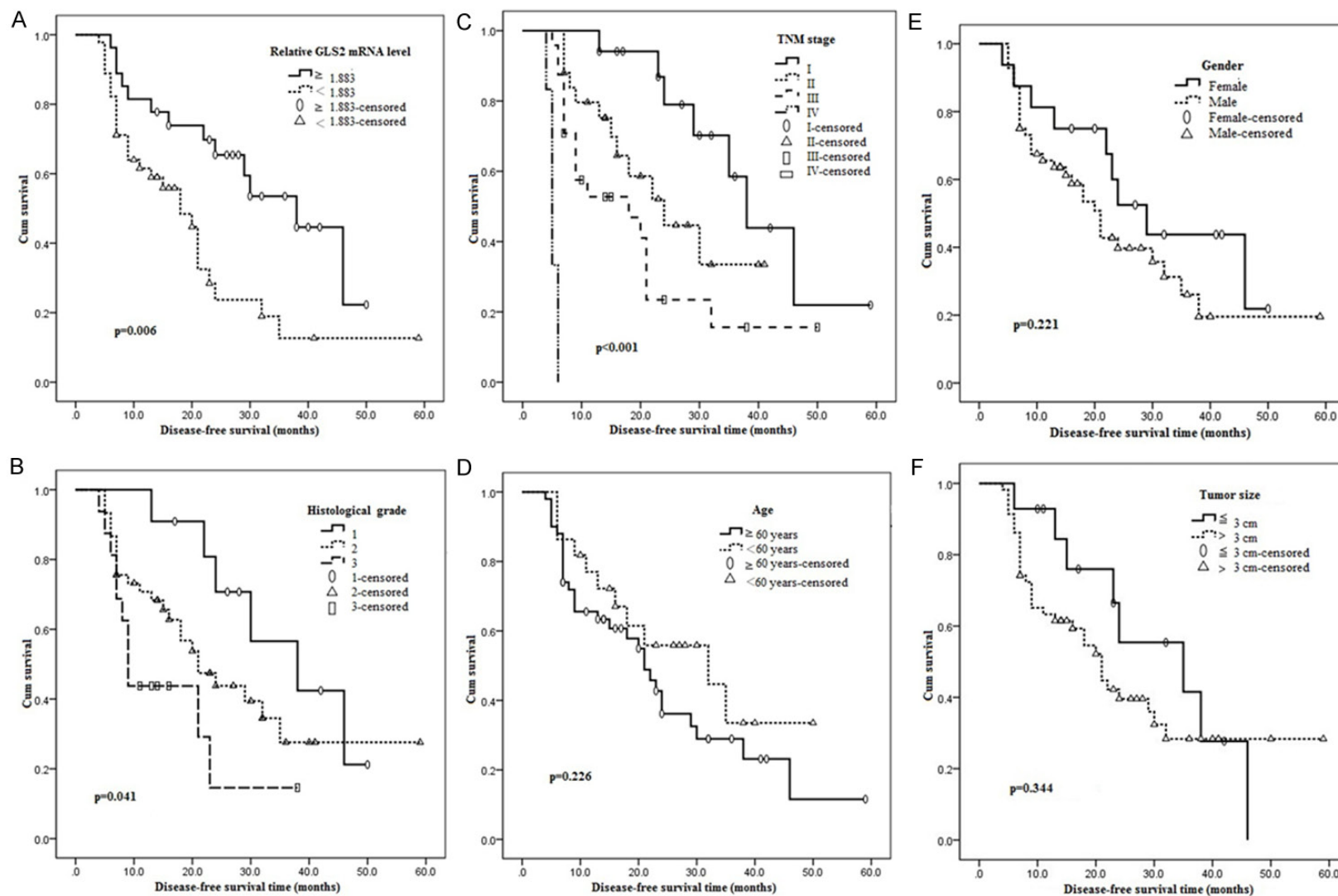
Figure 3. The impact of the clinicopathologic features on patients disease-free survival after radical resection for HCC was evaluated using the Kaplan-Meier method; *p* value according to the log-rank test. (A) Patients with high GLS2 expression tended to have longer DFS compared with those with low GLS2 expression. Histological grade (B) and TNM stage (C) were significantly correlated with DFS. Age (D), gender (E) and tumor size (F) had no relevant with DFS.
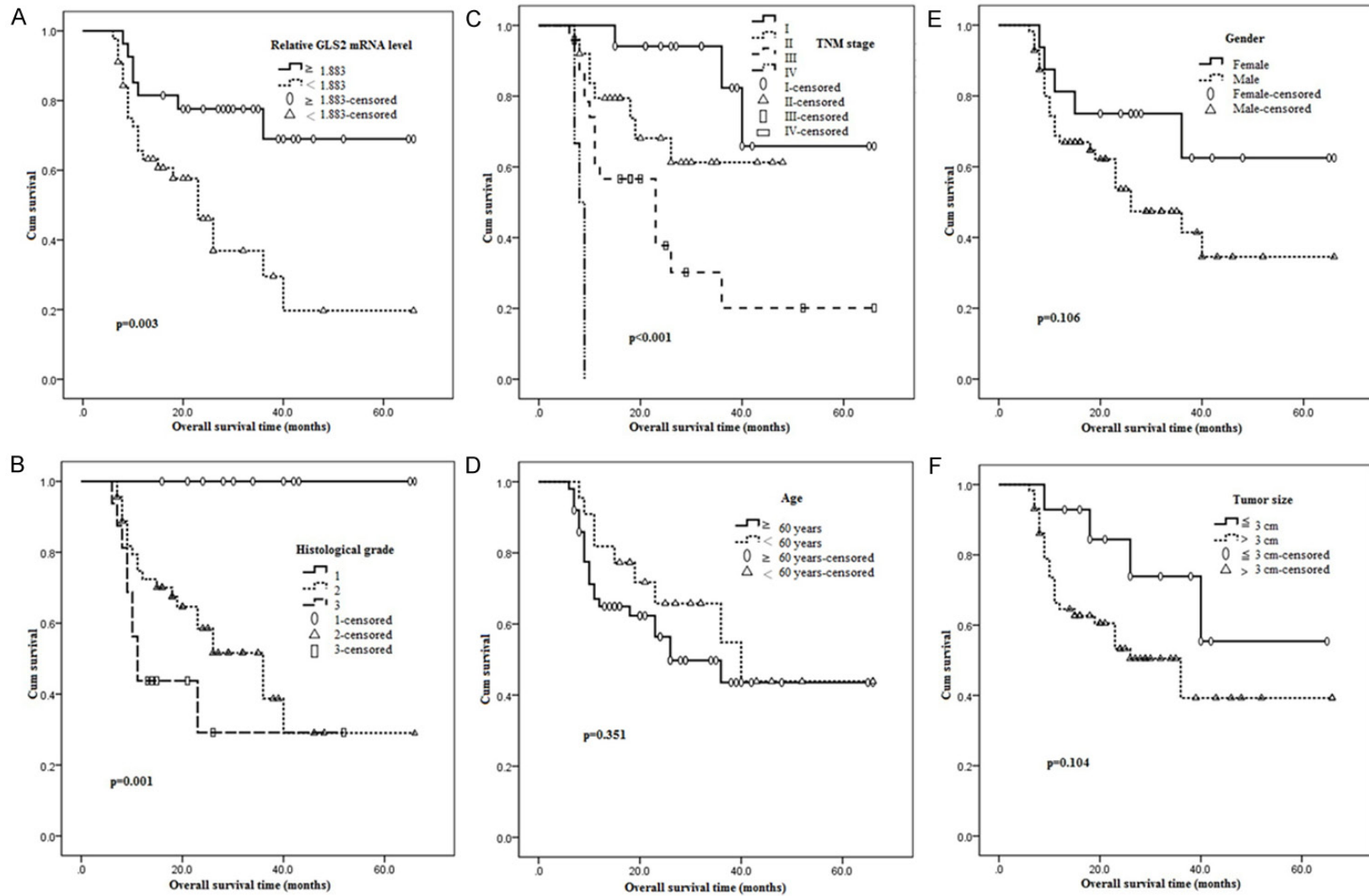
Figure 4. The impact of the clinicopathologic features on patients overall survival after radical resection for HCC was evaluated using the Kaplan-Meier method; *p* value according to the log-rank test. (A) Patients with high GLS2 expression tended to have longer OAS compared with those with low GLS2 expression. Histological grade (B) and TNM stage (C) were significantly correlated with OAS. Age (D), gender (E) and tumor size (F) had no relevant withOAS.

Table 1. Univariate analysis of prognostic factors in patients with HCC as evaluated by disease-free survival and overall survival

Variable	Number	Disease-free survival			Over-all survival		
		RR (95% CI)	β	*P* value	RR (95% CI)	β	*P* value
Gender							
Male	56	1.58 (0.75-3.34)	0.457	0.233	2.13 (0.82-5.57)	0.757	0.122
Female	16	Reference			Reference		
Age							
< 60 years	50	Reference			Reference		
≥ 60 years	22	1.51 (0.76-3.01)	0.415	0.238	1.43 (0.66-3.10)	0.358	0.364
Tumor size							
≤ 3 cm	14	Reference			Reference		
> 3 cm	58	1.44 (0.66-3.13)	0.366	0.355	2.30 (0.80-6.57)	0.831	0.121
Histological grade							0.001
1 or 2	56	Reference			Reference		
3	16	2.12 (1.04-4.29)	0.750	0.038	2.47 (1.15-5.29)	0.902	0.020
Tumor stage							
I or II	42	Reference			Reference		
III or IV	30	3.05 (1.65-5.66)	1.116	< 0.001	4.24 (2.03-8.89)	1.445	< 0.001
Relative Gls2 mRNA level							
< 1.883	45	2.47 (1.26-4.84)	0.903	0.009	3.32 (1.42-7.76)	1.199	0.006
≥ 1.883	27	Reference			Reference		

RR: risk ratio; 95% CI: 95% confidence interval. β: regression coefficient of the Cox proportional hazards model. *P*-value < 0.05 according to univariate Cox proportional hazards model. Histological grade: according to the three-tier grading scheme. Tumor stage: tumor-node-metastasis stage, according to the 7th edition of the AJCC (American Joint Committee on Cancer) cancer staging manual.

nation capabilities of the simple risk score was also presented by ROC curve and AUC. All statistical tests were two-sided, and *P* values less than 0.05 were considered as statistically significant. The statistical analyses were performed using SPSS version 21.0, MedCalc version 11.4 and GraphPad Prism version 5.0.

Result

GLS2 expression was down-regulated in HCC tissues

We obtained 72 HCC patients in this study, the median age of liver cancer patients was 53.67 years old (range of 16 to 84 years old). Then the HCC patients were grouped by tissue type (tumor tissue group and non-tumor tissue group), HBsAg expression (HBsAg positive group and HBsAg negative group), histologic grade (divided into grade 1, 2 and 3 groups) and tumor stage (divided into stage I, II, III and IV groups) respectively. Thus we can further confirm the difference of GLS2 expression in these groups above (**Figure 1**). GLS2 expres-

sion was significantly down-regulated in human primary HCC tissues when compared with adjacent non-tumor tissues (**Figure 1A**). The expression of GLS2 mRNA was also significantly related to histological grade (P < 0.001, **Figure 1B**), the serum HBsAg (P = 0.039, **Figure 1C**), and TNM stage of HCCs (P = 0.038, **Figure 1D**).

ROC curve of GLS2 and determination of optimal cutoff GLS2 expression value

We considered the tumor tissue group and non-tumor tissue group as HCC group and normal group; then ROC curve was plotted by software MedCalc 11.4 to evaluate the diagnostic efficacy of GLS2 for HCC (**Figure 2**). The optimal cutoff GLS2 expression value was 1.883 according to the ROC curve for HCC diagnosis, and corresponding diagnostic indexes are as follows: sensitivity 62.50%, specificity 83.33%, negative likelihood ratio 0.45, positive likelihood ratio 3.75 and AUC 0.745. For convenient to statistical analysis, patients were further categorized into two groups as GLS2 low expres-

Table 2. Multivariate analysis of prognostic factors in patients with HCC as evaluated by disease-free survival

Parameter	β	RR	95% CI	P
Relative Gls2 mRNA level	0.604	1.81	1.03-3.79	0.045
< 1.883				
≥ 1.883 (reference)				
Tumor stage	1.177	3.26	1.49-7.06	0.003
III or IV				
I or II (reference)				
Histological grade	0.638	1.86	1.05-3.92	0.042
3				
1 or 2 (reference)				
Age	0.581	1.63	0.76-3.84	0.069
≥ 60 years				
< 60 years (reference)				
Gender	0.145	1.16	0.51-2.60	0.726
Male				
Female (reference)				
Tumor size	0.126	1.13	0.47-2.74	0.780
> 3 cm				
≤ 3 cm (reference)				

RR: risk ratio; 95% CI: 95% confidence interval. β: regression coefficient of the Cox proportional hazards model. *P*-value < 0.05 according to univariate Cox proportional hazards model. Histological grade: according to the three-tier grading scheme. Tumor stage: tumor-node-metastasis stage, according to the 7th edition of the AJCC (American Joint Committee on Cancer) cancer staging manual.

sion (< 1.883) and GLS2 high expression group (≥ 1.883).

Correlations between disease-free survival, overall survival and clinicopathological factors for HCCs

Subsequently, we used the Kaplan-Meier method to further investigate the impact of these clinical factors above on DFS and OAS. As shown in the **Figures 3A** and **4A**, patients with high GLS2 expression tended to have longer DFS and OAS compared with those with low GLS2 expression. **Figures 3B**, **4B**, and **3C**, **4C** showed that histologic grade and tumor stage were significantly correlated with DFS and OAS. Age (60 years old was taken as cutoff value according to Gokcan's study [21]), gender and tumor size (3 cm was considered as cutoff value according to Milan criteria) showed no relevant with DFS (**Figure 3D-F**) and OAS (**Figure 4D-F**).

HCC patients' DFS and OAS can be affected by GLS2 expression, tumor stage and histological grade according to Univariate analysis and multivariate analysis

Furthermore, the univariate COX's Proportional Hazard Model, in which tumor size, age, gender, histologic grade, tumor stage, and GLS2 expression were respectively included, showed that loss of GLS2 expression was an independent prognostic factor for DFS (RR = 2.47, P = 0.009) and OAS (RR = 3.32, P = 0.006) in hepatic carcinoma patients. The results also showed that high histological grade and later tumor stage were independent unfavorable factors for DFS and OAS (**Table 1**).

A multivariable analysis including the significant prognostic factors in the univariate analysis for DFS and OAS after radical resection for HCC is summarized in **Tables 2** and **3**. The expression of GLS2 was one of the independent risk factors in the multivariable analysis for DFS (P = 0.045, RR = 1.81; **Table 2**; **Figure 5A**) and OAS (P = 0.037, RR = 2.59; **Table 3**; **Figure 5B**). Tumor stage and histological grade were also significant correlated with DFS (**Table 2**) and OAS (**Table 3**), while poorer tumor stage appeared to have more significant impact on DFS (tumor stage III or IV vs. I or II, RR = 3.26, P = 0.003) and OAS (tumor stage III or IV vs. I or II, RR = 3.31, P = 0.008).

A simple risk score for predicting the HCC patients' prognosis

After that, a simple risk score devised by using significant variables in the Cox model P < 0.05. The score was the weighted sum of those variables of which the weights were defined as the quotient (rounded to nearest integer) of corresponding estimated coefficients from a Cox's regression analysis divided by the smallest regression coefficient in the same Cox model (formula: Each score = β_1/β_2; β_1 was the regression coefficient of the significant variable with p < 0.05; β_2 was the smallest one among the regression coefficients of significant variables; each score was rounded to nearest integer, and total score was the sum of each score) (**Tables 4** and **5**). The total score ranged from 0 to 4. HCC patients were divided into two groups by

Table 3. Multivariate analysis of prognostic factors in patients with HCC as evaluated by overall survival

Parameter	β	RR	95% CI	P
Relative Gls2 mRNA level	0.951	2.59	1.06-6.32	0.037
< 1.883				
≥ 1.883 (reference)				
Tumor stage	1.196	3.31	1.36-8.03	0.008
III or IV				
I or II (reference)				
Histological grade	0.737	1.95	1.04-4.16	0.033
3				
1 or 2 (reference)				
Age	0.586	1.80	0.76-4.23	0.180
≥ 60 years				
< 60 years (reference)				
Gender	0.365	1.44	0.52-3.96	0.480
Male				
Female (reference)				
Tumor size	0.370	1.45	0.45-4.64	0.534
> 3 cm				
≤ 3 cm (reference)				

RR: risk ratio; 95% CI: 95% confidence interval. β: regression coefficient of the Cox proportional hazards model. *P*-value < 0.05 according to univariate Cox proportional hazards model. Histological grade: according to the three-tier grading scheme. Tumor stage: tumor-node-metastasis stage, according to the 7th edition of the AJCC (American Joint Committee on Cancer) cancer staging manual.

the endpoint of DFS (recurrence: yes or no) or endpoint of OAS (death: yes or no), and the total score was considered as diagnostic test. Then two ROC curves were performed by software MedCalc11.4 and the AUC were 0.676 and 0.773 (**Figure 6A**, **6B**). The optimal cutoff points of the two ROC curves all were score 2. For clinical and informative application, patients were further categorized into two risk groups as low-risk (score < 2) and high-risk group (score ≥ 2). From the **Figure 6C** and **6D**, we can find that patients whose score more than 2 are more likely to die or relapse than patients whose score less than 2. By applying the cutoff point of 2, the sensitivity and specificity of this cutoff value to predict death of liver cancer patient after surgery were 59.4% and 85.0%, and to predict recurrence of HCC patient after operation were 50.0% and 86.7%.

Discussion

In this study, we found that the GLS2 mRNA expression was significantly decreased in majority of primary HCCs that we examined com-pared with non-tumor liver tissues (**Figure 1A**). This result is consistent with previous reports from other groups [13, 14]. These results clearly demonstrated that the down-regulation of GLS2 is a common event in primary HCCs. Many researches have demonstrated that p53 plays a critical role in prevention of HCC [22, 23]. As a direct p53 target, GLS2 can contribute greatly to the function of p53 in tumor suppression in HCC. The aberrant activation of the PI3K/AKT signaling is frequently observed in HCC, which plays a critical role in liver tumorigenesis [24-26]. One study has been showed that GLS2 is an important negative regulator of the PI3K/AKT signaling in HCC [27], which strongly suggests that the negative regulation of PI3K/AKT signaling contributes greatly to GLS2's role in suppression of HCC. However, it still remains unclear how GLS2 negatively regulates the PI3K/AKT signaling.

As is well known, cell cycle checkpoints are important control mechanisms in maintaining tissue homeostasis, especially the G2/M checkpoint, which blocks the entry into mitosis when DNA is damaged [28]. The G2/M transition can be regulated by p53 protein either through the induction of p21 and stratifin (a protein that normally sequesters cyclin B1-cdc2 complexes in the cytoplasm [29, 30]), or through the induction of apoptosis [31, 32]. Since GLS2 has been considered as a direct p53 target gene [13, 14], one group investigated the impact ofGLS2 on cell cycle progression, and finally found that GLS2 high expression induced G2/M phase cell cycle arrest [33], which indicated that GLS2 played an important role in tumor suppression. It has been also reported that GLS2 acted as tumor suppressor may via reduction of DNA damage and mutations induced by oxidative stress [15]. Furthermore, recent studies have been showed that the promoter region of *GLS2* is hypermethylated in a high percentage of HCCs but not in their matched adjacent non-tumor liver tissues [27]. The hypermethylation of *GLS2* promoter was also observed in different HCC cell lines, and demethylation of *GLS2* promoter by 5-Aza-dC greatly induced GLS2 expression in these cell lines [27, 33]. These results strongly suggest that *GLS2* promoter hypermethylation is an important mechanism
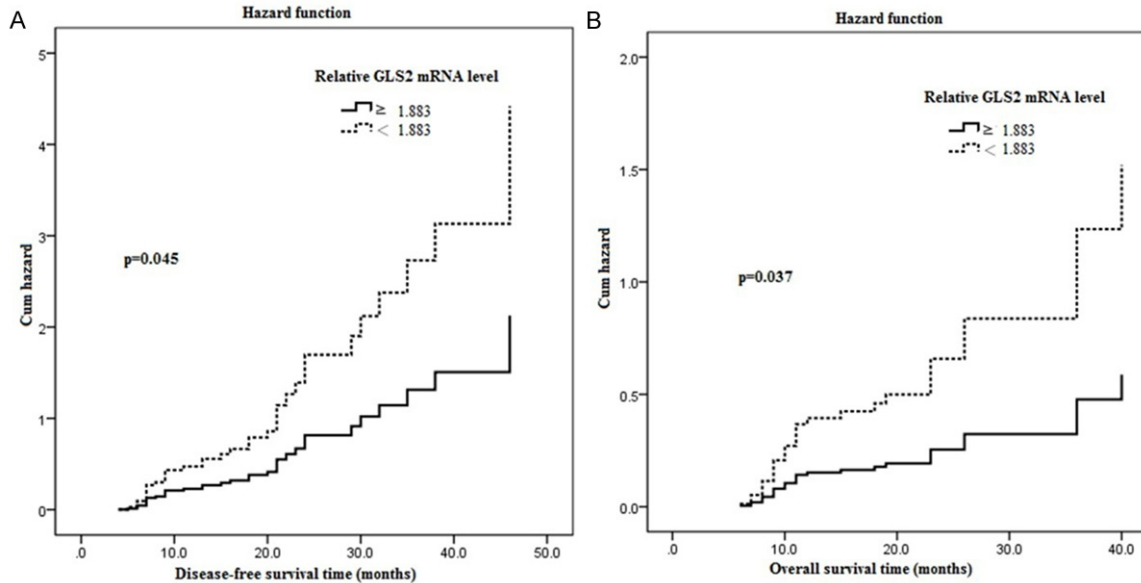
Figure 5. The risk of GLS2 expression for disease-free survival (A) and overall survival (B) with Cox's regression analysis; *P* value < 0.05 according to the multivariate Cox proportional hazards model.

Table 4. Components of the disease-free survival prediction score

Factors	Score (rounded to nearest integer)	Score origin
Relative Gls2 mRNA level		
≥ 1.883	0	
< 1.883	1	0.604/0.604
Tumor stage		
I or II	0	
III or IV	2	1.177/0.604
Histological grade		
1 or 2	0	
3	1	0.638/0.604

Table 5. Components of the overall survival prediction score

Factors	Score (rounded to nearest integer)	Score origin
Relative Gls2 mRNA level		
≥ 1.883	0	
< 1.883	1	0.951/0.737
Tumor stage		
I or II	0	
III or IV	2	1.196/0.737
Histological grade		
1 or 2	0	
3	1	0.737/0.737

contributing to the decreased GLS2 expression in HCC.

We further investigated the correlation between GLS2 expression and clinicopathologic features of liver cancer. GLS2 expression was significantly correlated with tumor stage, HBsAg expression and differentiation in histology (**Figure 1B-D**). Compared with the later tumor stage and poorer histologic grade of HCC patients, we found that early TNM stage and benign differentiation in histology seem to be associated with high expression of GLS2. Then the ROC curve was performed and AUC was 0.745. Other corresponding diagnostic indexes like sensitivity, specificity, and positive likelihood ratio were 62.50%, 83.33% and 3.75. These results implied that GLS2 can be an efficient biomarker for HCC diagnosis. Regrettably, we only measured the GLS2 expression level in tissue and neglected expression level of GLS2 in serum, which hinder the further study of GLS2's diagnostic efficacy. Then 72 HCC patients were divided into GLS2 high expression and GLS2 low expression group according to the cutoff point 1.883. Further survival analysis with Kaplan-Meier method indicated that patients with
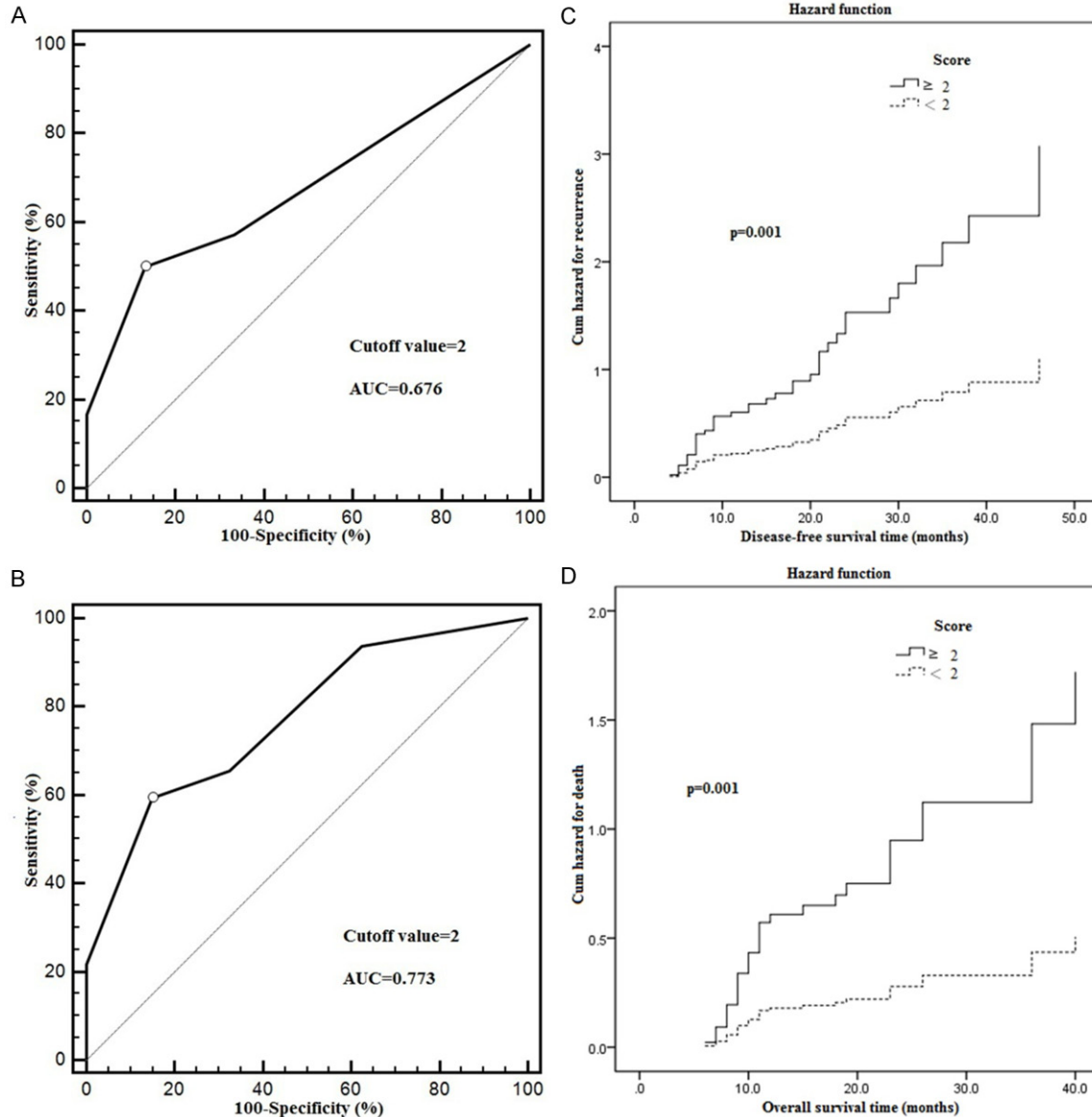
Figure 6. ROC curve with simplified risk score to predict the HCCs prognosis and cumulative risk for HCCs survival. A. ROC curve with simplified risk score to predict the recurrence of HCC patients after surgery; AUC = 0.676, cutoff value = 2, sensitivity = 50.0%, specificity = 86.7%. B. ROC curve with simplified risk score to predict the death of HCC patients after tumor resection; AUC = 0.773, cutoff value = 2, sensitivity = 59.4%, specificity = 85.0%. C. Cumulative risk for HCC relapse after operation. D. Cumulative risk for the death of HCC patients after surgery. *P* value was confirmed with Cox proportional hazards model.

high GLS2 expression have longer DFS and OAS compared to the others with low expression of GLS2 (**Figures 3A**, **4A**). What's more, histologic grade and tumor stage were also significantly correlated with DFS (**Figure 3B**, **3C**) and OAS (**Figure 4B**, **4C**) according to the Kaplan-Meier analysis. These results were consistent with the study according to univariate Cox regression analysis (**Table 1**).

Traditionally, tumor size, histologic grade and tumor stage are still the most important prognostic indicators. However, we found some patients with a relatively early TNM stage have shorter DFS and OAS in our follow-up process, which is also inconsistent with our previous study (the results of Kaplan-Meier analysis). So Cox regression analysis was applied to determine significant prognostic factor for DFS and

OAS. The result shows that GLS2 expression, histologic grade and tumor stage are the significant prognostic factors. Moreover, we found that the hazard ratio (HR or RR) of GLS2 expression for DFS and OAS are respectively 1.81 (P = 0.045, **Table 2**) and 2.59 (P = 0.037, **Table 3**), indicating that the group with lower GLS2 expression may have about 1.81 times risk of liver cancer relapse and 2.59 times risk of death. In order to dig deeper to research the impact of GLS2 expression, histological grade and tumor stage on DFS and OAS. Therefore, we developed a simple score composed of the three variables to predict the risk of HCC relapse and death after tumor resection. Patients with a prediction score of < 2 and ≥ 2 had distinctly different risk of HCC relapse and death. Notably, patients with score less than 2 are low risk of HCC recurrence and death (**Figure 6C**, **6D**). Identification of patients risk could initiate an individualized surveillance program for HCC patients after tumor resection.

Apart from the reports that GLS2 can inhibit the occurrence or development of malignant tumors through various mechanisms, our results showed the expression of GLS2 in liver non-tumor tissue is significant higher than that in liver malignant tumor, and the advanced extent of hepatic cancer is correlated with lower expression of GLS2. More importantly, we found that the patients with higher GLS2 expression have better cumulative survival. These results together indicate that GLS2 acts as a tumor suppressor in the development of hepatic carcinoma and could well be considered as a novel biomarker for prognosis in liver cancer. The scoring system including GLS2 from this study can provide some evidence to predict the recurrence and death of HCC.

In conclusion, this study generated valuable evidence that the high expression of GLS2 in HCC leads to a better prognosis in terms of both DFS and OAS after radical resection. GLS2 can be a useful predictor of survival in hepatocellular carcinoma patients. What's more, the scoring system including GLS2 acts as predictive model firstly used in our study to predict HCC patients' survival and this predictive model can be a potential prognostic tool for liver cancer patients.

Disclosure of conflict of interest

None.

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