

## Original Article

# Fructose-1,6-bisphosphatase 1 can be a potential predictive marker for the prognosis of hepatocellular carcinoma patients

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**Abstract:** Fructose-1,6-bisphosphatase-1 (FBP1), a gluconeogenesis regulatory enzyme, catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate and inorganic phosphate. FBP1 has been reported to be downregulated in human hepatocellular carcinomas. However, the prognostic value of FBP1 expression in hepatocellular carcinoma patients is still unclear. Here, we investigated the prognostic value of FBP1 expression in liver cancer patients. FBP1 mRNA expression was determined in tumor tissues and non-tumor tissues by real-time PCR. For evaluation of the prognostic value of FBP1 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 score devised by using significant variables obtained from Cox's regression analysis for further predicting the HCC patients' prognosis. We observed reduced FBP1 mRNA level in cancerous tissues in comparison to non-cancerous tissues. FBP1 expression was also significantly correlated with age, histological grade and tumor stage. More importantly, Kaplan-Meier analysis showed that patients with high FBP1 expression had longer disease-free survival and overall survival compared with those with low expression of FBP1. Cox's regression analysis indicated that FBP1 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  $>1$  and  $>2$  are more likely to relapse and die than patients whose total score  $\leq 1$  and  $\leq 2$ . FBP1 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:** Fructose-1,6-bisphosphatase-1 (FBP1), hepatocellular carcinoma, biomarker, risk scoring system, prognosis

## Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, especially in Asia, with a high mortality [1]. It is challenging to evaluate the prognosis of HCC patient. 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.

Metabolic deregulation has been considered a crucial hallmark of cancer [3, 4]. Cancer cells consume excess nutrients and energy as compared with their nonmalignant counterparts due to altered metabolism [5, 6]. Enhanced glucose metabolism accompanied by fermentation (aerobic glycolysis), commonly known as the Warburg effect, is exhibited almost universally by cancer cells [7, 8]. Thus much attention

has focused on regulation of the catabolic pathway of glucose. Fructose-1,6-bisphosphatase-1 (FBP1), which catalyzes the splitting of fructose-1,6-bisphosphate (F-1,6-BP) into fructose 6-phosphate and inorganic phosphate, is a rate-limiting enzyme in gluconeogenesis [9].

It is now widely accepted that constitutively elevated levels of cellular oxidative stress and dependence on mitogenic and anti-apoptotic reactive oxygen species (ROS) signaling in cancer cells are involved in the carcinogenesis [10]. Paradoxically, apart from being involved in proliferative, antiapoptotic, metastatic, and angiogenic signaling, ROS may also exert cytotoxic and proapoptotic functions that would limit tumorigenicity and malignant progression [11, 12]. FBP1 is a multifunctional protein that is, in addition to its function in gluconeogenesis,

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involved in ROS production in chronologically aged cells [13]. Our previous study [14] demonstrated that the growth inhibitory effect of FBP1 as a liver tumor suppressor may also be mediated through enhancing the production of intracellular ROS; compared to normal tissue, FBP1 expression is significantly reduced in liver tumor tissues. Furthermore, studies have been provided evidence showing that epigenetic silencing of FBP1 via promoter hypermethylation is common in human liver, colon and gastric cancers [14, 15]. FBP1 acted as a liver tumor suppressor and loss of FBP1 expression due to promoter DNA methylation has been observed in our previous study [14]; however, no specific associations between clinical outcomes and FBP1 expression have been identified.

We hypothesized that FBP1 could be used as a pathological and prognostic biomarker for HCC patients. Therefore, we investigated the expression of FBP1 in a large set of HCC specimens. The results validated the relevance of FBP1 expression to HCC clinical outcomes.

### Patients 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  $\pm$  standard deviation [SD], 53.67 $\pm$ 12.30 years). Our criteria for radicality have been published [16]. 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 cancer stages using the tumor-node-metastasis (TNM) stage according to the 7th edition of the AJCC (American Joint Committee on Cancer) cancer staging manual [17].

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) [18], 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 Trizol reagent (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  $\mu$ g of total RNA with High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA).

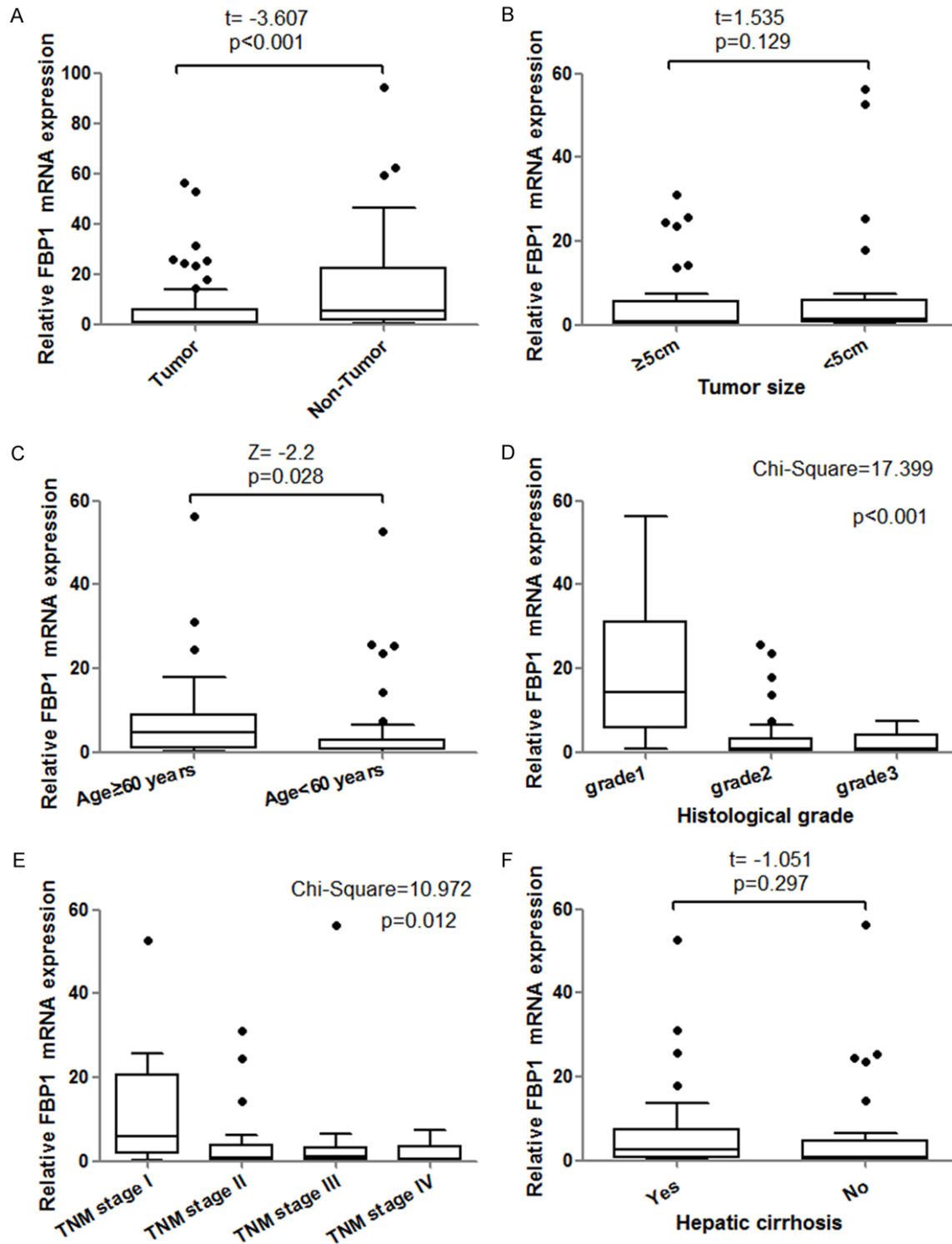
#### *Quantitative real-time PCR*

The mRNA level of FBP1 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 FBP1 expression from real-time PCR experiments [17]. Real-time PCR was performed in triplicate. Primers used for FBP1 were: FBP1-F 5'-ATCCCCTTGATGGATCTTCC-3' and FBP1-R 5'-TCCAGCATGAAGCAGTTGAC-3' (208 bp product).

#### *Statistic analysis*

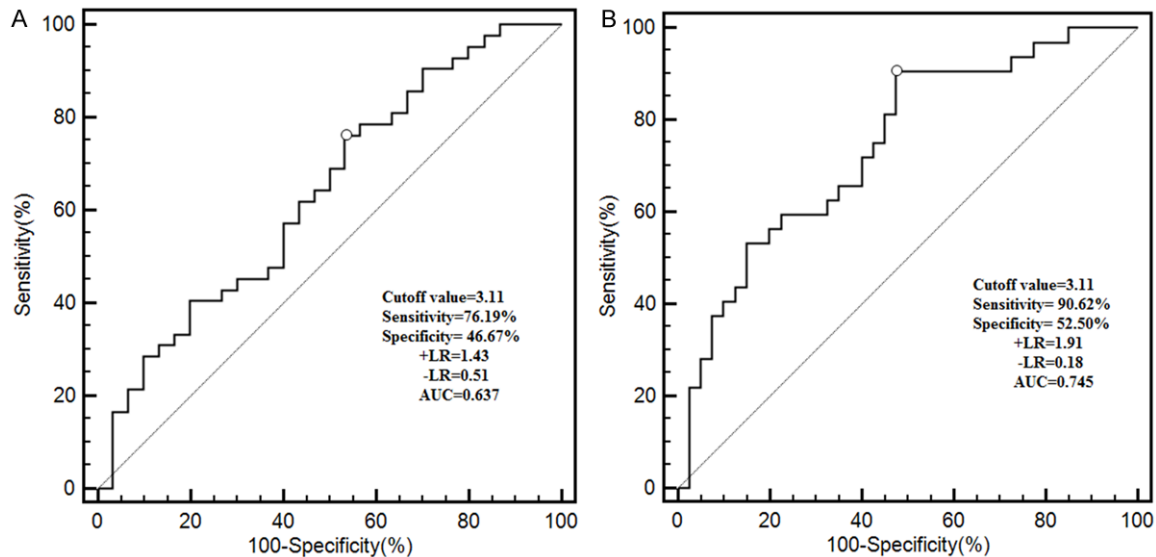
Spearman's rank correlations and Kendall rank correlation were used to investigate the relationship between two variables. Kruskal-Wallis test were used to examine the statistical difference among three groups or more. The Mann-

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**Figure 1.** Clinicopathologic features and expression of FBP1. The expression of FBP1 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 FBP1 mRNA in tumor size  $\geq 5$  cm and  $< 5$  cm groups;  $p$  value according to the independent-samples t-test. C. The expression of FBP1 mRNA in age  $\geq 60$  years and  $< 60$  years groups;  $p$  value according to the Mann-Whitney U-test. D. The expression of FBP1 mRNA in different histological grade (three-tier grading scheme) of primary HCC tissues;  $p$  value according to the Kruskal-Wallis test. E. The expression of FBP1 mRNA in different TNM stage of primary HCC tissues;  $p$  value according to the Kruskal-Wallis test. F. The expression of FBP1 mRNA in hepatic cirrhosis and non hepatic cirrhosis groups;  $p$  value according to the independent-samples t-test.

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**Figure 2.** ROC curves of FBP1 expression to identify the cutoff value of relative FBP1 mRNA level. A. ROC curve of FBP1 expression for disease-free survival. B. ROC curve of FBP1 expression for overall survival. +LR, positive likelihood ratio; -LR, negative likelihood ratio; AUC, area under the ROC curve.

Whitney U-test or independent-samples t-test were used to compare continuous variables between two groups. The diagnostic performance of FBP1 was assessed by receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC). 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 discrimination capabilities of the simple risk score was also presented by ROC curve and AUC. The optimal cutoff value was determined to maximize the sum of sensitivity and specificity. 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.

### Results

#### *Correlation of FBP1 expression and clinicopathologic features*

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). The

bivariate correlation analysis showed that FBP1 expression was related with tissue type (tumor tissue or non-tumor tissue), tumor size, histological grade, age, tumor stage, hepatic cirrhosis (yes or not), disease-free survival time and overall survival time. However, the differences of patient's gender, hepatitis B surface antigen (HBsAg) expression, hepatitis B e antigen (HBeAg) expression,  $\alpha$ -fetoprotein (AFP) levels, intrahepatic metastasis, and lesion location did not appear to have any correlation with FBP1 expression (Supplementary Table 1).

Then the HCC patients were grouped by tissue type (tumor tissue group vs. non-tumor tissue group), tumor size ( $\geq 5$  cm vs.  $< 5$  cm, 5 cm was considered as cutoff value according to Hwang's study [20]), age ( $\geq 60$  years vs.  $< 60$  years, 60 years old was taken as cutoff value according to Gokcan's study [19]), histological grade (divided into grade 1, 2 and 3 groups), tumor stage (divided into stage I, II, III and IV groups) and hepatic cirrhosis (yes vs. not) respectively. Thus we can further confirm the difference of FBP1 expression in these groups above (Figure 1). FBP1 expression was significantly downregulated in human primary HCC tissues when compared with adjacent non-tumor tissues (Figure 1A). The expression of FBP1 mRNA was also significantly related to age ( $P = 0.028$ , Figure 1C), histological grade ( $P < 0.001$ , Figure 1D), and TNM stage of HCCs ( $P = 0.012$ , Figure 1E); nevertheless, it was revealed that tumor

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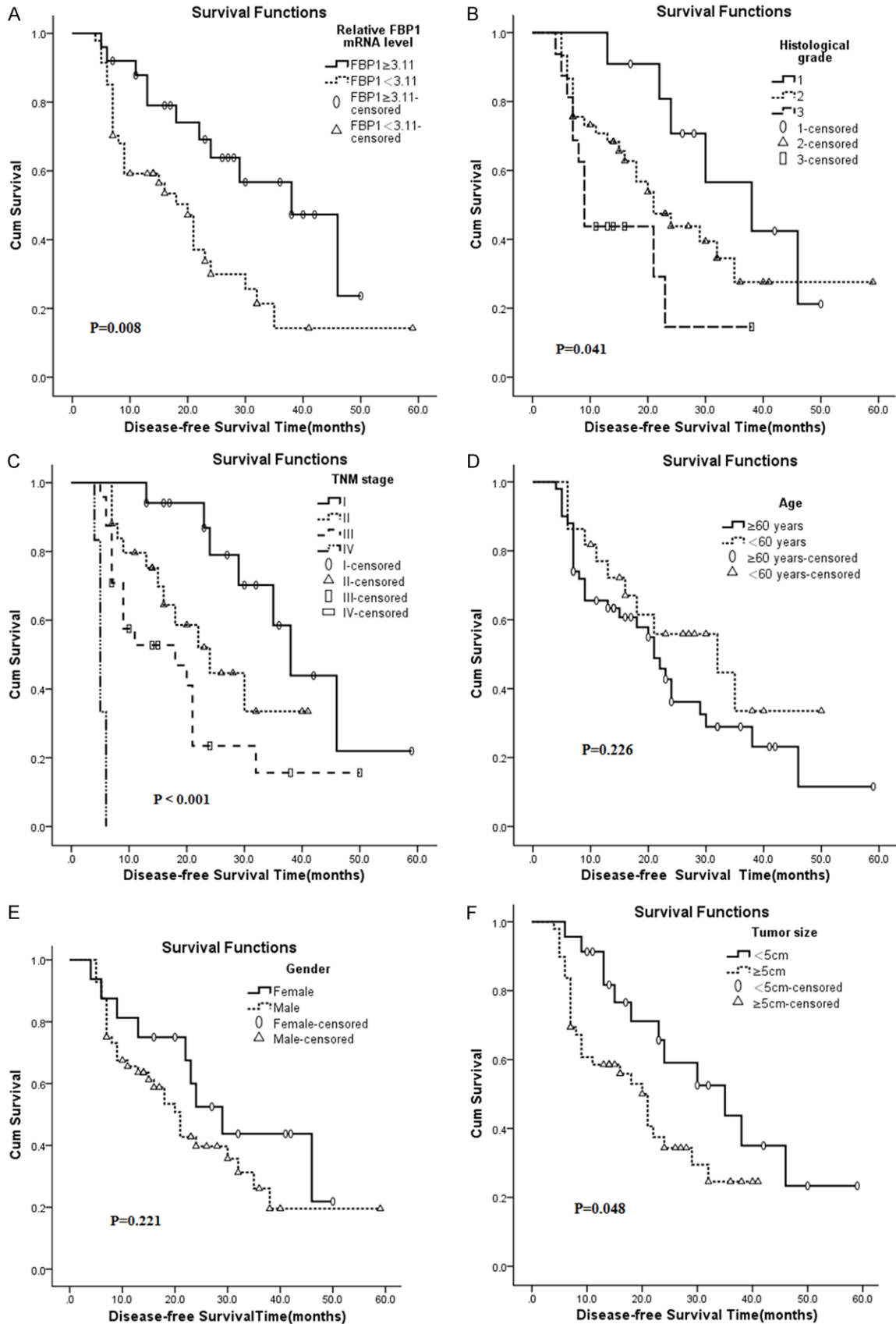
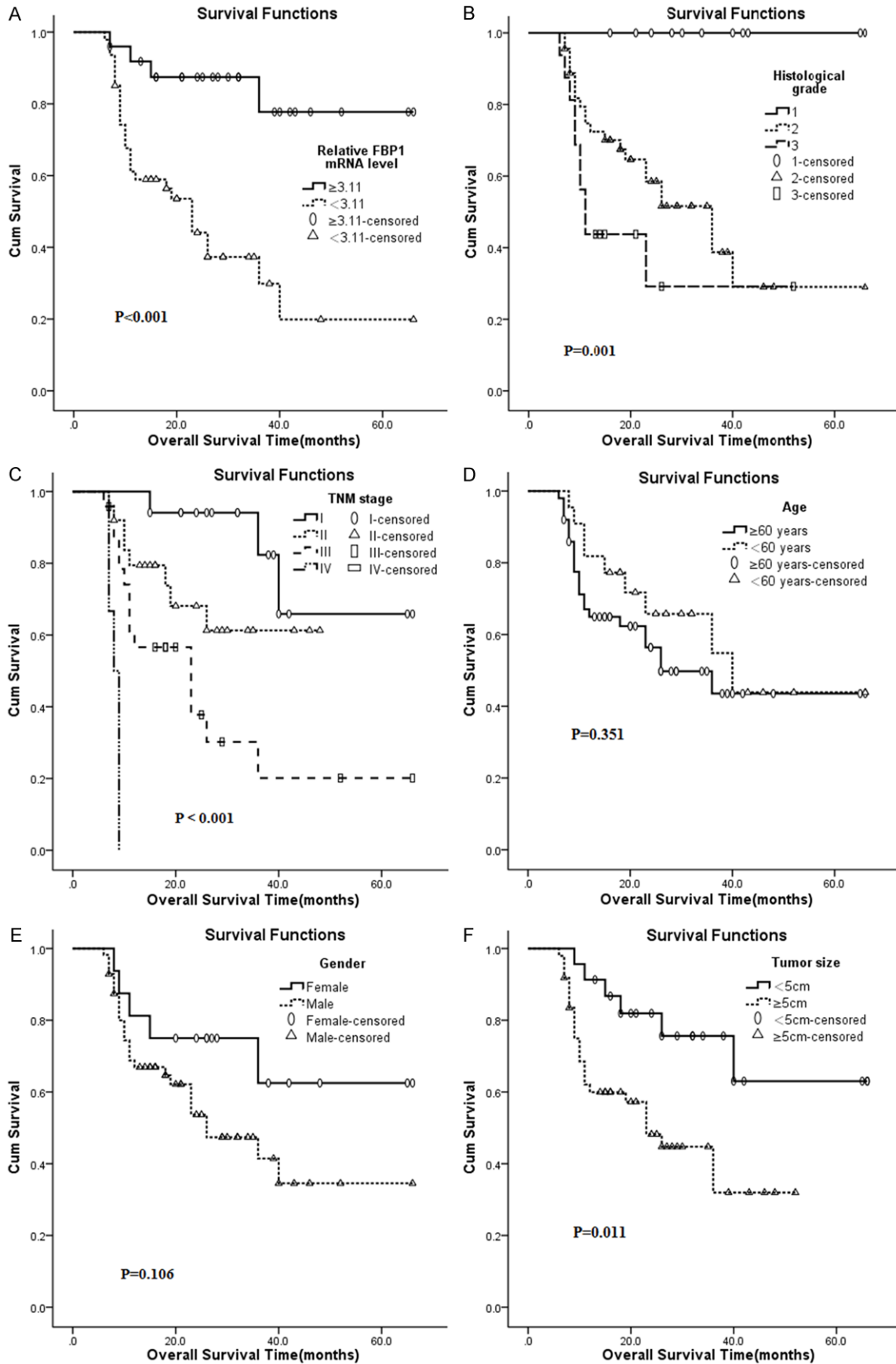


Figure 3. The impact of the clinicopathologic features on patients' disease-free survival after radical resection for HCC was evaluated using the Kaplan-Meier method; p value according to the log-rank test.

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**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.

**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)	$\beta$	<i>P</i> value	RR (95% CI)	$\beta$	<i>P</i> value
<b>Gender</b>							
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		
<b>Age</b>							
<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
<b>Tumor size</b>							
<5 cm	23	Reference			Reference		
≥5 cm	49	1.97 (0.98-3.97)	0.679	0.057	2.95 (1.21-7.21)	1.082	0.018
<b>Histological grade</b>							
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
<b>Tumor stage</b>							
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
<b>Relative FBP1mRNA level</b>							
<3.11	47	2.46 (1.22-4.96)	0.899	0.012	5.61 (1.95-16.18)	1.725	0.001
≥3.11	25	Reference			Reference		

RR: risk ratio; 95% CI: 95% confidence interval;  $\beta$ : 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; TNM stage: tumor-node-metastasis, according to the 7th edition of the AJCC (American Joint Committee on Cancer) cancer staging manual.

size and hepatic cirrhosis are not correlated with FBP1 mRNA levels (**Figure 1B, 1F**), which was inconsistent with the results in [Supplementary Table 1](#).

### *Diagnostic performance of FBP1 and determination of optimal cutoff value of FBP1 mRNA levels*

We considered death (yes vs. not) and recurrence (yes vs. not) as final diagnosis respectively; FBP1 expression was regarded as diagnostic test. Then ROC curve was plotted by software MedCalc11.4 to evaluate the predictive efficacy of FBP1 for HCC patients' survival (**Figure 2**). The optimal cutoff FBP1 expression values all were 3.11 according to the ROC curve for recurrence (**Figure 2A**) and for death (**Figure 2B**). Corresponding diagnostic indexes are as follows: sensitivity 76.19% and 90.62%, specificity 46.67% and 52.50%, negative likelihood ratio 0.51 and 0.18, positive likelihood ratio 1.43 and 1.91, AUC 0.637 and 0.745. For con-

venient to statistical analysis, patients were further categorized into two groups (≥3.11, high expression vs. <3.11, low expression) based on the optimal cutoff FBP1 expression values.

### *Relationship between disease-free survival, overall survival and clinicopathological factors in HCCs*

The bivariate correlation analysis was used again to sift the clinical factors that are related with DFS and OAS. The results showed that DFS and OAS respectively correlated with difference of gender, tumor size, age, histological grade, tumor stage and relative FBP1 mRNA level ([Supplementary Table 2](#)). Then 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 FBP1 expression (≥3.11) tended to have longer DFS and OAS compared with those with low FBP1 expression (<3.11).

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

Parameter	$\beta$	RR	95% CI	P
Relative FBP1 mRNA level (<3.11 vs. $\geq$ 3.11)	0.613	1.85	1.03-3.96	0.047
TNM stage (III or IV vs. I or II)	1.077	2.89	1.37-5.86	0.005
Histological grade (3 vs. 1 or 2)	0.645	1.97	1.05-4.03	0.041
Age ( $\geq$ 60 years vs. <60 years)	0.546	1.73	0.86-3.99	0.087
Gender (male vs. female)	0.018	1.02	0.44-2.35	0.966
Tumor size ( $\geq$ 5 cm vs. <5 cm)	0.313	1.37	0.647-2.94	0.426

RR: risk ratio; 95% CI: 95% confidence interval;  $\beta$ : 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; TNM stage: tumor-node-metastasis, according to the 7th edition of the AJCC (American Joint Committee on Cancer) cancer staging manual.

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

Parameter	$\beta$	RR	95% CI	P
Relative FBP1 mRNA level (<3.11 vs. $\geq$ 3.11)	1.503	4.49	1.38-14.67	0.013
TNM stage (III or IV vs. I or II)	1.047	2.85	1.24-6.55	0.014
Histological grade (3 vs. 1 or 2)	0.853	2.16	1.13-4.38	0.027
Age ( $\geq$ 60 years vs. <60 years)	0.153	1.17	0.48-2.86	0.738
Gender (male vs. female)	0.117	1.12	0.40-3.18	0.825
Tumor size ( $\geq$ 5 cm vs. <5 cm)	0.607	1.84	0.69-4.85	0.221

RR: risk ratio; 95% CI: 95% confidence interval;  $\beta$ : 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; TNM stage: tumor-node-metastasis, according to the 7th edition of the AJCC (American Joint Committee on Cancer) cancer staging manual.

Figures 3B, 4B and 3C, 4C showed that histologic grade and tumor stage were significantly correlated with DFS and OAS. Age, gender and tumor size showed no relevant with DFS (Figure 3D-F) and OAS (Figure 4D-F).

### Univariate analysis and multivariate analysis with Cox proportional hazards model

Furthermore, the univariate COX's Proportional Hazard Model, in which tumor size, age, gender, histologic grade, tumor stage, and FBP1 expression were respectively included, showed that low expression of FBP1 was an independent prognostic factor for DFS (RR=2.46, P=0.012) and OAS (RR=5.61, P=0.001) 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 analy-

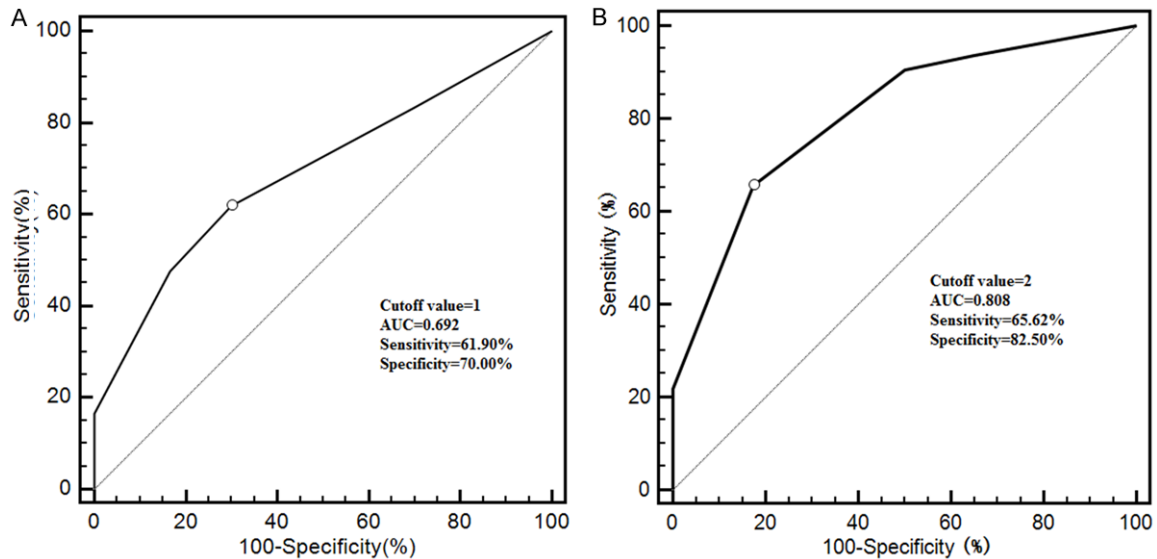
sis for DFS and OAS after radical resection for HCC is summarized in Tables 2 and 3. The expression of FBP1 was one of the independent risk factors in the multivariable analysis for DFS (P=0.047, RR=1.85; Table 2) and OAS (P=0.013, RR=4.49; Table 3). Tumor stage and histological grade were also significant correlated with DFS (Table 2) and OAS (Table 3), while poorer tumor stage appeared to have more significant impact on DFS (TNM stage III or IV vs. I or II, RR=2.89, P=0.005) and OAS (TNM stage III or IV vs. I or II, RR=2.85, P=0.014).

### A simple risk score for predicting the HCC patients' prognosis

Subsequently, a simple risk score devised by using significant variables in the Cox model with 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 (Supplementary Tables 3 and 4). The total score ranged from 0 to 4. HCC patients were divided into two groups by the endpoint of DFS (recurrence: yes or not) or endpoint of OAS (death: yes or not), and the total score was considered as diagnostic test. Then two ROC curves were performed by software MedCalc11.4. The optimal cutoff points of the two ROC curves were score 1 and score 2 severally. For clinical and informative application, patients were further categorized into two risk groups as low-risk (score  $\leq$ 1 or  $\leq$ 2) and high-risk group (score >1 or >2) to evaluate DFS and OAS. From the Figure 6, we can find that patients whose total score more than 2 are more likely to die and total score more than 1 are apt to relapse than patients whose score less than 2 and 1. By applying the cutoff point of the two ROC curves, the sensitivity and specificity to predict death



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**Figure 5.** ROC curves with simplified risk score to predict the HCCs' prognosis. A. ROC curve with simplified risk score to predict the recurrence of HCC patients after surgery. B. ROC curve with simplified risk score to predict the death of HCC patients after tumor resection.

of liver cancer patient after surgery were 65.62% and 82.50%, and to predict recurrence of HCC patient after operation were 61.90% and 70.00%. The AUC of the ROC curve for OAS was 0.808 (Figure 5B) and for DFS was 0.692 (Figure 5A).

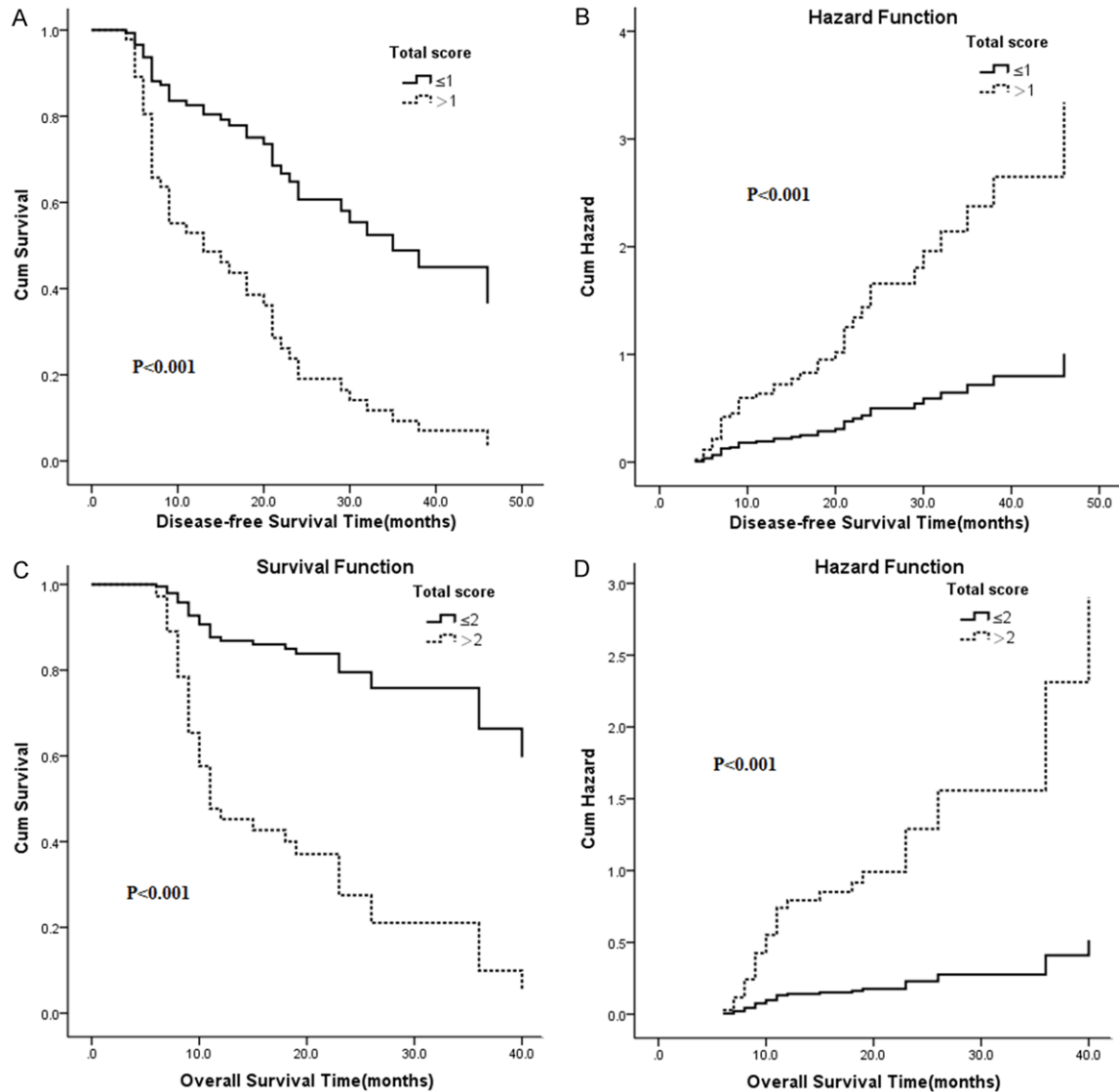
### Discussion

It has been verified [15] that FBP1 functions to antagonize glycolysis. As is well known, cancer cells have a higher rate of aerobic glycolysis, but not oxidative phosphorylation [5]. Fructose-1,6-bisphosphate is one of the most important intermediates in glycolysis and its level is mainly controlled by fructose-6-phosphate kinase and fructose-1,6-bisphosphatase. It was found that the production of lactate after FBP1 expression was significantly reduced [21], demonstrating the suppression of aerobic glycolysis by FBP1. Besides, cell cycle checkpoints are important control mechanisms that ensure the proper execution of cell cycle events. The growth suppression induced by ectopic FBP1 expression seems to be caused by cell cycle arrest since the numbers of cells with cell cycle blockage (G2-M phase arrest) were increased after FBP1 re-expression [14]. For a long time, ROS were considered oncogenic since it was implicated in cancer progression and metastasis [10]. Persistent oxidative stress has been associated with breast carcinoma and many

epithelial cancers such as colon and neck cancers [22]. However, it has been demonstrated that cisplatin apoptogenicity depends on formation of ROS and occurs independent of nuclear DNA damage, suggesting that apoptogenic oxidative stress is the crucial mechanism of cisplatin-induced cancer cell death [23]. In addition to causing cell cycle arrest at the S phase, the growth inhibitory effect of FBP1 as a tumor suppressor may also be mediated through enhancing the production of intracellular ROS [14].

Moreover, one research [24] published in *Nature* suggests that FBP1 is universally depleted in clear cell renal cell carcinoma (ccRCC), facilitating cancer progression by both suppressing gluconeogenesis and enhancing transactivation of glycolytic genes by hypoxia-inducible factors (HIFs). The research also identified that ectopic expression of FBP1 in ccRCC cell lines suppressed HIF activity and the extent of FBP1 suppression was significantly correlated with tumor stage and patient prognosis [24, 25]. In addition, FBP1 expression in basal-like breast cancer (BLBC) cells inhibited tumorigenicity in vitro and suppressed tumor formation in vivo [9]. Our previous study [14] found that FBP1 is frequently reduced by promoter hypermethylation in most liver cancer cell lines and primary tumor tissues, and suggested that epigenetic inactivation of FBP1 was an important

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**Figure 6.** The impact of total scoring system on disease-free survival and overall survival with Cox's regression analysis; *p* value was confirmed with Cox proportional hazards model.

factor in human liver carcinogenesis. Regrettably, the associations between prognosis of liver cancer patients and FBP1 expression have not been identified in our previous research.

In this study, we found that the FBP1 mRNA expression was significantly decreased in majority of primary HCCs that we examined compared with non-tumor liver tissues (**Figure 1A**). This result is consistent with previous report. We further investigated the correlations between FBP1 expression and clinicopathologic features of liver cancer. FBP1 expression was significantly correlated with age, tumor stage, and differentiation in histology (**Supplementary**

**Table 1** and **Figure 1C-E**). Compared with the later tumor stage and poorer histologic grade HCC patients, we found that early TNM stage and benign differentiation in histology seem to be associated with high expression of FBP1. Then two ROC curves of FBP1 expression to predict DFS and OAS were performed. The AUCs were 0.637 and 0.745, other corresponding diagnostic indexes like sensitivity values were 76.19% and 90.62%, specificity values were 46.67% and 52.50%, positive likelihood ratios were 1.43 and 1.91 (**Figure 2A, 2B**). These results implied that FBP1 can be an efficient biomarker for HCC prognosis. It is a pity that we only measured the FBP1 expression

level in tissue and neglected expression level of FBP1 in serum, which hinder the further study of FBP1's predictive efficacy for HCCs survival. Then 72 HCC patients were divided into FBP1 high expression and FBP1 low expression group according to the cutoff point 3.11. Further survival analysis with Kaplan-Meier method indicated that patients with high FBP1 expression have longer DFS and OAS compared to the others with low expression of FBP1 (**Figures 3A, 4A**). What's more, histologic grade, tumor stage and tumor size were also significantly correlated with DFS (**Figure 3B, 3C, 3F**) and OAS (**Figure 4B, 4C, 4F**) according to the Kaplan-Meier analysis. Apart from the impact of tumor size on DFS and OAS, other results were consistent with the study according to univariate Cox regression analysis (**Table 1**).

Traditionally, tumor size, histologic grade and tumor stage are 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 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 FBP1 expression, histological grade and tumor stage are the significant prognostic factors (**Tables 2 and 3**). Furthermore, we found that the hazard ratio (HR or RR) of FBP1 expression for DFS and OAS are respectively 1.85 ( $P=0.047$ , **Table 2**) and 4.49 ( $P=0.013$ , **Table 3**), indicating that the group with lower FBP1 expression may have about 1.85 times risk of liver cancer relapse and 4.49 times risk of death. In order to dig deeper to research the impact of FBP1 expression, histological grade and tumor stage on DFS and OAS, we developed a simple score composed of the three variables to predict the risk of HCC relapse and death after tumor resection. Patients with prediction score of  $\leq 1$  vs.  $>1$  had distinctly different risk of HCC relapse and with total score of  $\leq 2$  vs.  $>2$  had significantly different risk of HCC patients' overall survival. Notably, patients with total score  $\leq 1$  are low risk of HCC recurrence and with total score  $\leq 2$  are low risk for the death of HCC patients (**Figure 6**). Identification of patients' risk for their prognosis could initiate an individualized surveillance program for HCC patients after tumor resection.

Tumor occurrence and development can be considered as the accumulation of gene mutations and epigenetic modifications. The predominant consequence of this accumulation is the activation of proto-oncogenes or silencing of tumor-suppressor genes [26]. Consistent with previous reports that FBP1 can inhibit the occurrence or development of malignant tumors through various mechanisms, our results show the expression of FBP1 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 FBP1. More importantly, we found that the patients with higher FBP1 expression have better cumulative survival. These results together indicate that FBP1 acts as a tumor suppressor in the development of hepatic carcinoma and could well be considered as a novel biomarker for prognosis and therapy in liver cancer. The scoring system including FBP1 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 FBP1 in HCC leads to a better prognosis in terms of both DFS and OAS after radical resection. FBP1 can be a useful predictor of survival in hepatocellular carcinoma patients. What's more, the scoring system including FBP1 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.

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### Disclosure of conflict of interest

None.

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### References

- [1] Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. *Nat Rev Gastroenterol Hepatol* 2010; 7: 448-458.

## Fructose-1,6-bisphosphatase 1-a valuable predictor of survival in HCC patients

- [2] Sakamoto M. Early HCC: Diagnosis and molecular markers. *J Gastroenterol* 2009; 44 Suppl 19: 108-111.
- [3] Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis* 2013; 4: e532.
- [4] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell* 2011; 144: 646-674.
- [5] Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat Rev Cancer* 2011; 11: 325-337.
- [6] Ward PS, Thompson CB. Metabolic reprogramming: A cancer hallmark even warburg did not anticipate. *Cancer Cell* 2012; 21: 297-308.
- [7] Vander HM, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. *Science* 2009; 324: 1029-1033.
- [8] Locasale JW, Cantley LC. Metabolic flux and the regulation of mammalian cell growth. *Cell Metab* 2011; 14: 443-451.
- [9] Dong C, Yuan T, Wu Y, Wang Y, Fan TW, Miriyala S, Lin Y, Yao J, Shi J, Kang T, Lorkiewicz P, St CD, Hung MC, Evers BM, Zhou BP. Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell* 2013; 23: 316-331.
- [10] Bjorn ME, Hasselbalch HC. The role of reactive oxygen species in myelofibrosis and related neoplasms. *Mediators Inflamm* 2015; 2015: 648090.
- [11] Cabello CM, Bair WR, Wondrak GT. Experimental therapeutics: Targeting the redox Achilles heel of cancer. *Curr Opin Investig Drugs* 2007; 8: 1022-1037.
- [12] Fruehauf JP, Meyskens FJ. Reactive oxygen species: A breath of life or death? *Clin Cancer Res* 2007; 13: 789-794.
- [13] Kitanovic A, Wolf S. Fructose-1,6-bisphosphatase mediates cellular responses to DNA damage and aging in *Saccharomyces cerevisiae*. *Mutat Res* 2006; 594: 135-147.
- [14] Chen M, Zhang J, Li N, Qian Z, Zhu M, Li Q, Zheng J, Wang X, Shi G. Promoter hypermethylation mediated downregulation of FBP1 in human hepatocellular carcinoma and colon cancer. *PLoS One* 2011; 6: e25564.
- [15] Liu X, Wang X, Zhang J, Lam EKY, Shin VY, Cheng ASL, Yu J, Chan FKL, Sung JY, Jin HC. Warburg effect revisited: An epigenetic link between glycolysis and gastric carcinogenesis. *Oncogene* 2010; 29: 442-450.
- [16] Nagasue N, Kohno H, Chang YC, Taniura H, Yamanoi A, Uchida M, Kimoto T, Takemoto Y, Nakamura T, Yukaya H. Liver resection for hepatocellular carcinoma. Results of 229 consecutive patients during 11 years. *Ann Surg* 1993; 217: 375-384.
- [17] Edge SB, Compton CC. The American joint committee on cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; 17: 1471-1474.
- [18] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45: 228-247.
- [19] Gokcan H, Savas N, Oztuna D, Moray G, Boyvat F, Haberal M. Predictors of survival in hepatocellular carcinoma patients. *Ann Transplant* 2015; 20: 596-603.
- [20] Hwang S, Lee YJ, Kim KH, Ahn CS, Moon DB, Ha TY, Song GW, Jung DH, Lee SG. The impact of tumor size on Long-Term survival outcomes after resection of solitary hepatocellular carcinoma: Single-Institution experience with 2558 patients. *J Gastrointest Surg* 2015; 19: 1281-1290.
- [21] Adeva M, Gonzalez-Lucan M, Seco M, Donapetry C. Enzymes involved in L-lactate metabolism in humans. *Mitochondrion* 2013; 13: 615-629.
- [22] Aykin-Burns N, Ahmad IM, Zhu Y, Oberley LW, Spitz DR. Increased levels of superoxide and H<sub>2</sub>O<sub>2</sub> mediate the differential susceptibility of cancer cells versus normal cells to glucose deprivation. *Biochem J* 2009; 418: 29-37.
- [23] Berndtsson M, Hagg M, Panaretakis T, Havelka AM, Shoshan MC, Linder S. Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA. *Int J Cancer* 2007; 120: 175-180.
- [24] Li B, Qiu B, Lee DS, Walton ZE, Ochocki JD, Mathew LK, Mancuso A, Gade TP, Keith B, Nissim I, Simon MC. Fructose-1,6-bisphosphatase opposes renal carcinoma progression. *Nature* 2014; 513: 251-255.
- [25] Phillips R. Kidney cancer: FBP1 depletion feeds ccRCC. *Nat Rev Urol* 2014; 11: 482.
- [26] Hahn WC, Weinberg RA. Rules for making human tumor cells. *N Engl J Med* 2002; 347: 1593-1603.

## Fructose-1,6-bisphosphatase 1-a valuable predictor of survival in HCC patients

**Supplementary Table 1.** Correlation between FBP1 expression and clinical factors in liver cancer patients

Variable	FBP1 expression			
	$r_1$	<i>P</i> value (2-tailed)	$r_2$	<i>P</i> value (2-tailed)
Tumor or non-tumor	0.380	<0.001	0.312	<0.001
Gender	-0.114	0.340	-0.094	0.336
Tumor size (maximum diameter)	-0.336	0.004	-0.225	0.007
Age	0.235	0.046	0.180	0.027
HBsAg	-0.006	0.961	-0.005	0.961
HBeAg	0.102	0.392	0.084	0.388
Histological grade	-0.397	0.001	-0.314	0.001
AFP	0.020	0.871	0.018	0.827
Intrahepatic metastasis	-0.029	0.810	-0.024	0.808
TNM stage	-0.287	0.015	-0.211	0.019
lesion location	-0.072	0.548	-0.057	0.534
hepatic cirrhosis	0.257	0.029	0.212	0.030
DFST	0.462	<0.001	0.325	<0.001
OAST	0.482	<0.001	0.339	<0.001

$r_1$ : Spearman rank correlation coefficient;  $r_2$ : Kendall rank correlation coefficient; DFST: Disease-free survival time; OAST: Overall survival time; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; AFP: alpha fetoprotein; Histological grade: according to the three-tier grading scheme.

**Supplementary Table 2.** Relationship between disease-free survival, overall survival and clinicopathological factors in liver cancer patients

Variable	Disease-free survival		Over-all survival	
	<i>r</i>	<i>P</i> value (2-tailed)	<i>r</i>	<i>P</i> value (2-tailed)
Gender	-0.246	0.038	-0.250	0.034
Tumor size (maximum diameter)	-0.344	0.003	-0.351	0.003
Age	-0.240	0.043	-0.285	0.015
HBsAg	-0.151	0.206	-0.166	0.163
HBeAg	0.111	0.355	0.113	0.346
Histological grade	-0.423	<0.001	-0.444	<0.001
AFP	0.004	0.971	0.013	0.914
Intrahepatic metastasis	-0.221	0.062	-0.199	0.093
TNM stage	-0.584	<0.001	-0.523	<0.001
Lesion location	-0.231	0.051	-0.212	0.074
Hepatic cirrhosis	-0.048	0.691	-0.050	0.674
Relative FBP1 mRNA level	0.462	<0.001	0.482	<0.001

*r*: Spearman rank correlation coefficient; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; AFP: alpha fetoprotein; Histological grade: according to the three-tier grading scheme.

## Fructose-1,6-bisphosphatase 1-a valuable predictor of survival in HCC patients

**Supplementary Table 3.** Components of the disease-free survival prediction score

Factors	Score (rounded to nearest integer)	Score origin
Relative FBP1 mRNA level		
≥3.11	0	
<3.11	1	0.613/0.613
Tumor stage		
I or II	0	
III or IV	2	1.077/0.613
Histological grade		
1 or 2	0	
3	1	0.645/0.613

**Supplementary Table 4.** Components of the overall survival prediction score

Factors	Score (rounded to nearest integer)	Score origin
Relative FBP1 mRNA level		
≥3.11	0	
<3.11	2	1.503/0.853
Tumor stage		
I or II	0	
III or IV	1	1.047/0.853
Histological grade		
1 or 2	0	
3	1	0.853/0.853