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ORIGINAL RESEARCH

C8B in Complement and Coagulation Cascades Signaling Pathway is a predictor for Survival in HBV-Related Hepatocellular Carcinoma Patients

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Objective: The role of the complement and coagulation cascades signaling pathway in the pathogenesis of cancers remains uncertain. This study aimed to investigate the associations between enriched differentially expressed genes (DEGs) in this pathway and hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) patients.

Materials and Methods: Clinical and gene expression data of the Gene Expression Omnibus (GEO) series profile GSE14520 were downloaded. The "Limma" package was used to screen the DEGs and the "clusterProfiler" package was used to identify the complement and coagulation cascades pathway and enriched significant genes. Cox regression analysis, the Kaplan–Meier method, and the nomogram model were used to address the correlations between significantly enriched DEGs in the complement and coagulation cascades pathway and HCC survival.

Results: A total of 220 HBV-related HCC patients were enrolled in this study. The complement and coagulation cascades pathway was significantly enriched by 37 DEGs (p-value < 0.05 and adjusted p-value < 0.05). Complement 8 beta chain (C8B) expression levels had protective effects on overall survival (OS) and recurrence-free survival (RFS) in HBV-related HCC patients. High levels of C8B contributed to favorable OS and RFS in this population (both $p < 0.01$), even after adjustment of clinicopathological characteristics including tumor node metastasis (TNM) staging, Barcelona Clinic liver cancer (BCLC) staging, gender, and fibrinogen beta chain (FGB) expression (all $p < 0.05$).

Conclusion: C8B in the complement and coagulation cascades signaling pathway serves as a predictive candidate for survival in HBV-related HCC patients.

Keywords: C8B, FGB, complement and coagulation cascades, hepatocellular carcinoma, survival

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Introduction

The International Agency for Research on Cancer (IARC) report released in 2020 indicated that liver cancer is the third leading cause of cancer death.[1] Hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) is the most frequent form of liver cancer and the major cause of cancer-related deaths worldwide.[2–4] During the past decades, comprehensive therapeutic approaches including immunotherapy and novel targeting agents have prolonged the survival of HCC patients.[5,6] Even though antiviral treatment for HBV, regular monitoring, and early screening for HCC have been proven beneficial for alleviating the burden of this disease,[2,4,7] the incidence of liver cancers has continued to increase and will rise over the next two

decades, and the mortality related to liver cancer has increased by more than 2% annually since 2007.[8,9]

Recently, public data and bioinformatic analysis methods have provided us with invaluable resources to outline the underlying mechanisms and pathogenesis of HCC development.[10–12] Complement and coagulation cascades signaling, interacting at multiple levels in thrombosis and inflammatory diseases,[13] was one of the most frequently enriched functional pathways in the bioinformatic studies.[14,15] In soft tissue sarcoma patients, differentially expressed genes (DEGs) in complement and coagulation pathways are correlated with chemotherapy resistance and survival.[16] Downregulation of alpha-2-macroglobulin (A2M), coagulation factor XIII A chain (F13A1), and G protein subunit gamma 11 (GNG11) in the complement and coagulation cascades pathway might contribute to the progression of bladder cancer.[17] Dysregulation of the complement and coagulation pathway is usually triggered by dysfunctions of the innate immune system,[18,19] which is mainly composed of complement, contact/coagulation, and fibrinolytic systems. Activation of these systems subsequently induces activation of endothelial cells, leukocytes, and platelets, which ultimately lead to thrombosis and inflammation reactions.[19,20] Emerging evidence has indicated that thrombo-inflammation accounts for cancer progression.[21–23] Hence, we conducted a bioinformatics analysis to address the potential candidates correlated with survival of HBV-related HCC patients, to draw a diagram for understanding the roles of the complement and coagulation cascades signaling pathway in the development of HCC.

Materials and Methods

Ethics Statement

This study is a secondary analysis based on the public database. As presented by Roessler et al,[24,25] all the HBV-related subjects received radical resection therapy between 2002 and 2003 in Zhongshan Hospital, Fudan University. Written informed consent was provided by all participants, and reviewed and approved by the Institutional Review Board of the participating institutes.[24,25]

Patients

All clinical and gene expression data of HBV-related HCC patients are publicly available in the Gene Expression Omnibus (GEO) Repositories (https://www.ncbi.nlm.nih.gov/geo/, accession number: GSE14520). In this series, 247 HBV-related HCC patients were diagnosed by two independent pathologists who had detailed information on manifestations and pathological features. Outcomes including overall survival (OS) and recurrence-free survival (RFS), as well as other clinicopathological characteristics, were available for 242 subjects.[24] Among these 242 cases, 22 had no gene expression data. Thus, 220 HBV-related HCC patients were finally recruited to this study. Clinicopathological data including age, gender, HBV status, alanine aminotransferase, cirrhosis status, main tumor size, alpha-fetoprotein (AFP), Barcelona Clinic liver cancer (BCLC) staging, Cancer of the Liver Italian Program (CLIP) staging, and Tumor Node Metastasis (TNM) staging were obtained.

Microarray Processing and DEGs Identification

The microarray processing approaches were presented in detail by Roessler et al.[24] All the tumor and nontumor samples with good RNA quality were profiled separately using a single channel array platform. Affymetrix human genome U133A 2.0 array and Affymetrix HT human genome U133A array platforms were used to determine the fluorescent intensities of samples. Relative log expression (RLE) and normalized unscaled standard errors (NUSE) tests by the "affyPLM" package in the R program were used for quality assessment of each sample.[26] The Robust Multi-array Average (RMA) method and global median centering were used to normalize the raw gene expression data.[24] Missing gene expression data were imputed with the k-Nearest Neighbor (KNN) method by impute index in the R program.[27] The mean gene expression was calculated for genes with more than one probe. DEGs between tumor and nontumor samples, with the criterion of a $|\log2FC| > 1.0$ and adjusted p-value < 0.05, were addressed by the "Limma" package in the R program.[26,28]

Gene Set Enrichment Analysis (GSEA)

The R package "clusterProfiler"[29] was used to address complement and coagulation cascades signaling pathway enrichment of DEGs between tumor and nontumor tissues in HBV-related HCC patients from GSE14520. Significant genes enriched in the complement and coagulation cascades pathway were transferred to gene symbol by "clusterProfiler", "org.Hs.eg.db", and "AnnotationDbi" packages in the R program.[29]

Outcome Definitions

The survival data of OS and RFS were available in this study. OS was defined as the period from radical resection to death

from any causes. RFS was defined as new lesions found in the abdominal computed tomography (CT)/magnetic resonance imaging (MRI) examinations and an abnormal AFP value greater than 300 ng/mL; subjects with a high pretreatment AFP value that had not decreased to normal or had increased again after returning to normal were included.[24,25]

Nomogram Model Establishment

Variables significantly correlated with OS and/or RFS in HBV-related HCC patients in the multivariate Cox proportional hazards model were included in the risk prediction model by nomogram with the "rms" package in the R program. According to the Cox model, the "survival" package in the R program is used to calculate the cumulative risk of death. The "survcomp" package was used to calculate the concordance index and its 95% confidence intervals (CIs). The bootstrap method was used for repeated sampling for internal verification of the model. Calibration curved addressed by the "rms" package were presented for evaluating the performance of the nomogram.

Statistical Analysis

Mean ± standard deviation (SD) was used for the statistical description for normally distributed continuous data, and frequency with percentage was used for the description of the enumeration data. Parameters associated with the outcomes were assessed by univariate analysis and multivariate analysis using Cox regression. Only covariates significantly associated with outcomes according to the univariate analysis (two-sided p-value < 0.10) are shown and included in the multivariate model. The Kaplan–Meier analysis with log rank test was used to compare survival between different groups. Results were reported as hazard ratios (HR) with a 95% CI. Stata software version 16.0 (Stata Corp LLC, Texas, USA) was used for other statistics; $p < 0.05$ (two-sided) was considered significant for all tests.

Results

Patient Characteristics

As summarized in Table 1, of 220 HBV-related HCC patients, 190 (86.4%) cases were male, and the average age was 50.8 ± 10.6 years. Of these 220 subjects, 155 (70.5%) were HBV chronic carriers, less than half (91/220, 41.4%) had alanine aminotransferase > 50 U/L, and more than 90% had HBV-induced cirrhosis. There were 99 (45.6%) cases with an AFP > 300 ng/mL. The distribution of cancer stages, that is, BCLC staging, TNM staging, and CLIP staging, are also presented in Table 1.

Complement and Coagulation Cascades Signaling Pathway Enrichment

In the GSE14520 dataset, the complement and coagulation cascades signaling pathway was significantly enriched with DEGs between tumor and nontumor samples in HBV-related HCC patients (p-value < 0.05 and adjusted p-value < 0.05; Figure 1A). In total 37 DEGs, PROS1, FGA, C8A, C8B, C6, FGG, C4BPA, PLG, A2M, F9, SERPINC1, MASP2, C9, CPB2, MBL2, SERPINF2, PROC, C1S, C7, CFI, KLKB1, F12, FGB, F7, CFB, C5, SERPINA5, KNG1, SERPING1, VTN, F2, CLU, C1QB, C1R, CFH, C3, and F11, were significantly enriched in the complement and coagulation cascades signaling pathway ($p < 0.0001$ or $p < 0.05$; Figure 1B).

Table 1 Baseline Characteristics of HCC Patients Included in This Study

Variables	N = 220
Male, n (%)	190 (86.4)
Age, years, mean ± SD	50.8 ± 10.6
HBV status, n (%)	
Chronic carriers	155 (70.5)
Active viral replication chronic carrier	56 (25.5)
NA	9 (4.1)
Alanine aminotransferase > 50 U/L, n (%)	91 (41.4)
Cirrhosis, n (%)	202 (91.8)
Main tumor size > 5 cm, n (%)	80 (36.5)
AFP > 300 ng/mL, n (%)	99 (45.6)
BCLC staging, n (%)	
0-A	170 (77.3)
B	22 (10.0)
C	28 (12.7)
TNM staging, n (%)	
I	95 (43.2)
II	77 (35.0)
III	48 (21.8)
CLIP staging, n (%)	
0	100 (45.5)
1	74 (33.6)
2	33 (15.0)
3–5	13 (5.9)

Abbreviations: HBV, hepatitis B virus; BCLC, Barcelona Clinic liver cancer; TNM, tumor node metastasis; CLIP, Cancer of the Liver Italian Program; AFP, alpha-fetoprotein; NA, not available.

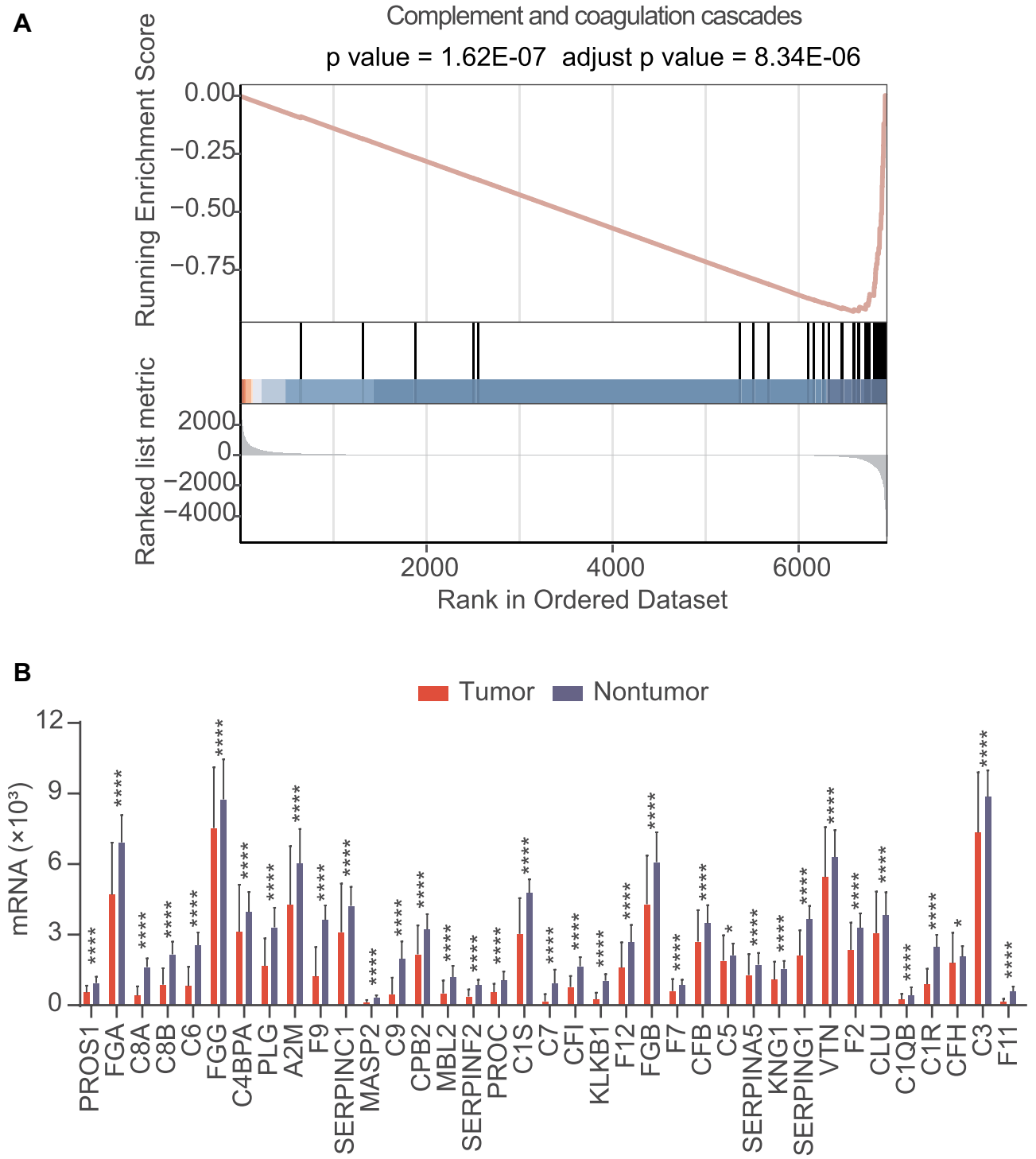

Figure 1 In GSE14520 dataset, the complement and coagulation cascades signaling pathway was significantly enriched with differentially expressed genes (DEGs) in HBV-related HCC patients (p-value < 0.05 and adjusted p-value < 0.05, (**A**)); in total 37 DEGs were significantly enriched in the complement and coagulation cascades signaling pathway (p < 0.0001 or p < 0.05, (**B**). *p < 0.05, ****p < 0.0001.

Parameters Correlated with OS in HCC Patients

When the 37 significant genes in the complement and coagulation cascades pathway andthe clinicopathological characteristics (age, gender, HBV status, cirrhosis, main tumor size, AFP level, and cancer stages) were included in the univariate Cox regression model, main tumor size, cirrhosis, BCLC staging, TNM staging, CLIP staging,

AFP, C8B, PLG, SERPINC1, MASP2, CPB2, KLKB1, F12, CFB, KNG1, SERPING1, VTN, F2, and F11 were correlated with OS in HCC patients (all $p < 0.10$; Table 2). After adjustment in the multivariate Cox model, high levels of C8B in tumor tissues contributed to favorable OS in HCC patients compared to low levels of C8B (HR = 0.51, 95% CI = 0.29–0.92, p = 0.024; Table 2). The Kaplan–Meier plot indicated that HCC patients with high C8B in tumors had better OS than those with low C8B (p = 0.0013; Figure 2A), and similar results were obtained after adjusting TNM staging (p = 0.0108; Figure 2B).

As summarized in Figure S1, C8B mRNA was significantly downregulated in tumor samples compared to that in non-tumor samples in four GEO series (all $p < 0.05$; Figure S1A). In line with the results in GSE14520, high C8B levels contributed to significantly favorable OS in HCC patients both in the Gene Expression Profiling Interactive Analysis (GEPIA) database[30] and Kaplan–Meier plotter database[31,32] (both $p < 0.05$; Figure S2).

Parameters Correlated with RFS in HCC Patients

When the parameters mentioned above were included in the univariate Cox regression model for assessing predictors of RFS in HCC patients, gender, main tumor size, cirrhosis, BCLC staging, TNM staging, CLIP staging, C8B, PLG, SERPINC1, MASP2, CPB2, KLKB1, F12, KNG1, VTN, F11, FGB, and SERPINA5 were potential biomarkers for RFS (all $p < 0.10$; Table 2). When these candidates were included in the multivariate Cox model, C8B, FGB, together with gender, BCLC staging, and TNM staging showed significance for predicting RFS in HCC patients (all $p < 0.05$; Table 2). The Kaplan–Meier method revealed that high levels of C8B in tumor tissues were significantly associated with favorable RFS in HBV-related HCC patients (p = 0.0012; Figure 3A). In addition, high levels of C8B were significantly correlated with better RFS in HBV-related HCC patients, after adjusting BCLC staging (p = 0.0081; Figure 3B), TNM staging (p = 0.0067; Figure 3C), gender (p = 0.0013; Figure 3D), and FGB expression (p = 0.0006; Figure 3E). Even when adjusted by BCLC, TNM, gender, and FGB levels, C8B upregulation also contributed to favorable RFS in this population (p = 0.0185; Figure 3F). Similarly, HCC patients with high C8B levels had significantly better RFS both in GEPIA and Kaplan–Meier plotter databases (p = 0.034 and p = 0.028, respectively; Figures S3A and S3B).

On the other side, FGB was identified as a risk factor by univariate and multivariate Cox regression analysis (HR = 1.98, 95% CI = 1.32–2.97, p = 0.001; Table 2). The Kaplan–Meier method showed that high FGB expression was associated with worse RFS in HBV-related HCC patients (p = 0.0188; Figure 4A). High levels of FGB were significantly correlated with unfavorable RFS in HBV-related HCC patients, after adjusting BCLC staging (p = 0056; Figure 4B), TNM staging (p = 0.0192; Figure 4C), gender (p = 0.0219; Figure 4D), and C8B expression (p = 0.0062; Figure 4E). Even when adjusted by BCLC, TNM, gender, and C8B levels, FGB upregulation also contributed to unfavorable RFS in this population (p = 0.0136; Figure 4F). Consistent with our results, FGB was downregulated in HCC tumors in five GEO series (all $p < 0.05$; Figure S1B). Conversely, FGB upregulation might account for favorable RFS in HCC patients both in GEPIA and Kaplan–Meier plotter databases (p = 0.0011 and p = 0.049, respectively; Figures S3C and S3D). Considering the results above, the associations between FGB and HCC recurrence are still controversial.

Nomogram Model for Evaluating Candidates for OS in HCC Patients

Nomogram risk models for OS were established according to independent parameters screened by multivariate Cox regression analysis (Figure 5). According to the upper scale of each independent parameter, the corresponding score could be determined and the total score was calculated by adding the scores of each parameter. Projecting downward from the total score, the corresponding mortality or recurrence risk prediction probability value could be obtained.

In the nomogram for OS in HCC patients, for example, in a patient with TNM stage III and low C8B expression, his/her total score was 1.24, and his/her 1-year, 3-year, and 5-year mortality risk was 0.308, 0.694, and 0.825, respectively (Figure 5A). The concordance index (C-index) of this model was 0.73 (95% CI = 0.66–0.79). One-year, 3-year, and 5-year calibration curves for internal verification of this nomogram with bootstrapping are shown in Figures 5B–D, respectively. The 1-year, 3-year, and 5-year calibration curves displayed good consistency between actual OS and predicted OS (Figures 5B–D).

Table 2 Univariate and Multivariate Cox Regression Models for Screening Parameters Correlated with OS and RFS in HCC Patients

Variables[#]	OS				RFS			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Gender, male vs female	ns	ns	ns	ns	2.15 (1.12–4.11)	0.021	2.05 (1.03–4.06)	0.04
Tumor size > 5 cm, yes vs no	1.97 (1.28–3.03)	0.002	1.2 (0.67–2.17)	0.541	1.41 (0.98–2.04)	0.066	1.09 (0.68–1.73)	0.723
Cirrhosis, yes vs no	4.58 (1.13–18.61)	0.034	4.17 (0.96–18.1)	0.057	2.17 (0.95–4.93)	0.065	1.82 (0.77–4.33)	0.173
BCLC staging								
0-A	Reference	1.0	Reference	1.0	Reference	1.0	Reference	1.0
B	2.40 (1.28–4.53)	0.007	1.03 (0.4–2.63)	0.948	2.08 (1.19–3.64)	0.01	1.83 (0.87–3.85)	0.111
C	4.60 (2.72–7.76)	< 0.001	2.22 (0.75–6.6)	0.15	3.26 (2.02–5.25)	< 0.001	4.43 (1.73–11.37)	0.002
TNM staging								
I	Reference	1.0	Reference	1.0	Reference	1.0	Reference	1.0
II	2.08 (1.21–3.59)	0.008	1.83 (1.01–3.33)	0.047	1.97 (1.29–3.02)	0.002	1.59 (1.001–2.53)	0.049
III	4.93 (2.82–8.6)	< 0.001	2.03 (0.74–5.57)	0.171	3.06 (1.91–4.9)	< 0.001	0.97 (0.42–2.22)	0.941
CLIP staging								
0	Reference	1.0	Reference	1.0	Reference	1.0	Reference	1.0
1	1.42 (0.83–2.42)	0.198	1.42 (0.67–3.0)	0.357	1.23 (0.81–1.88)	0.333	1.09 (0.68–1.75)	0.711
2	2.95 (1.66–5.25)	< 0.001	1.42 (0.46–4.34)	0.538	1.94 (1.17–3.22)	0.01	0.88 (0.45–1.7)	0.695
3–5	6.24 (3.01–12.93)	< 0.001	1.94 (0.43–8.7)	0.385	3.07 (1.55–6.07)	0.001	0.74 (0.27–2.02)	0.558
AFP > 300 ng/mL, yes vs no	1.60 (1.04–2.45)	0.032	0.93 (0.41–2.09)	0.86	ns	ns	ns	ns
C8B, high vs low	0.49 (0.32–0.77)	0.002	0.51 (0.29–0.92)	0.024	0.55 (0.38–0.79)	0.001	0.61 (0.4–0.96)	0.031
PLG, high vs low	0.61 (0.39–0.94)	0.025	1.13 (0.64–2.0)	0.684	0.66 (0.46–0.94)	0.023	1.01 (0.61–1.69)	0.958
SERPINC1, high vs low	0.48 (0.31–0.75)	0.001	0.99 (0.5–1.94)	0.968	0.59 (0.41–0.84)	0.004	0.9 (0.51–1.59)	0.724
MASP2, high vs low	0.49 (0.31–0.76)	0.001	0.88 (0.47–1.64)	0.687	0.63 (0.44–0.9)	0.011	0.99 (0.59–1.67)	0.983
CPB2, high vs low	0.55 (0.35–0.85)	0.007	0.8 (0.46–1.4)	0.443	0.68 (0.48–0.98)	0.038	0.99 (0.62–1.57)	0.96
KLKB1, high vs low	0.5 (0.32–0.78)	0.002	0.68 (0.36–1.3)	0.243	0.72 (0.5–1.03)	0.074	1.04 (0.63–1.71)	0.883
F12, high vs low	0.44 (0.28–0.68)	< 0.001	0.72 (0.4–1.28)	0.26	0.55 (0.39–0.8)	0.001	0.97 (0.61–1.56)	0.915
CFB, high vs low	0.64 (0.41–0.98)	0.04	1.12 (0.64–1.97)	0.698	ns	ns	ns	ns

KNG1, high vs low	0.53 (0.34–0.83)	0.005	0.97 (0.55–1.73)	0.925	0.58 (0.4–0.83)	0.003	0.72 (0.45–1.14)	0.159
SERPING1, high vs low	0.63 (0.41–0.98)	0.038	1.52 (0.83–2.76)	0.173	ns	ns	ns	ns
VTN, high vs low	0.62 (0.40–0.96)	0.032	0.85 (0.5–1.47)	0.568	0.7 (0.49–1.01)	0.055	1.05 (0.65–1.69)	0.836
F2, high vs low	0.63 (0.41–0.98)	0.04	1.27 (0.74–2.19)	0.384	ns	ns	ns	ns
F11, high vs low	0.57 (0.37–0.88)	0.011	1.06 (0.57–1.96)	0.865	0.7 (0.49–1.01)	0.056	1.0 (0.63–1.57)	0.98
FGB, high vs low	ns	ns	ns	ns	1.54 (1.07–2.22)	0.02	1.98 (1.32–2.97)	0.001
SERPINA5, high vs low	ns	ns	ns	Ns	0.66 (0.46–0.94)	0.022	0.99 (0.63–1.57)	0.98

Notes: #Only parameters significantly associated with OS/RFS in HCC patients screened by univariate Cox regression ($p < 0.10$) were presented and included in the multivariate model.
Abbreviations: BCLC, Barcelona Clinic liver cancer; TNM, tumor node metastasis; CLIP, Cancer of the Liver Italian Program; AFP, alpha-fetoprotein; ns, no significance.

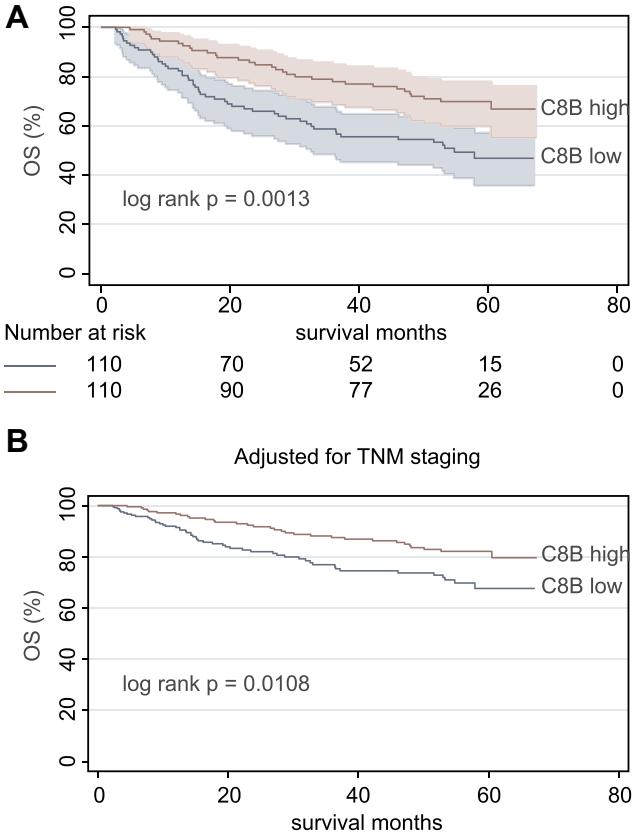

Figure 2 Kaplan–Meier method indicated that HBV-related HCC patients with high levels of C8B in tumor tissues had significantly better overall survival (OS) than those with low C8B ($p = 0.0013$, (**A**)); After adjustment of TNM staging, C8B overexpression in tumor tissues also contributed to favorable OS in HBV-related HCC patients compared to C8B downregulation ($p = 0.0108$, (**B**)).

Discussion

The constituent of innate immunity, complement, is present in the tumor microenvironment. The functions of complement could be anti-tumoral and pro-tumoral depending on the cancer type, even for the same type of cancer.[33,34] As a member of the complement gene family, complement C8 consists of three subunits encoded by individual genes: C8A, C8B, and C8G. C8 serves as a main component of the membrane attack complex.[35,36] Complement deficiency represents about 1–6% of all primary immunodeficiencies, but in some communities this proportion may be as high as 10%.[37] Notably, C8 deficiency accounts for 8% of complement deficiency in Europe.[38] A single C-T exchange in exon 9 and 3 leading to a premature stop codon is considered as the main cause of C8B deficiency.[39,40] Currently, the C8 gene is mainly investigated in immunodeficiency diseases.[41,42] One report revealed that type 2 diabetic patients had markedly increased levels of C8 compared with healthy individuals.[43] In a recent bioinformatic study, C8B in

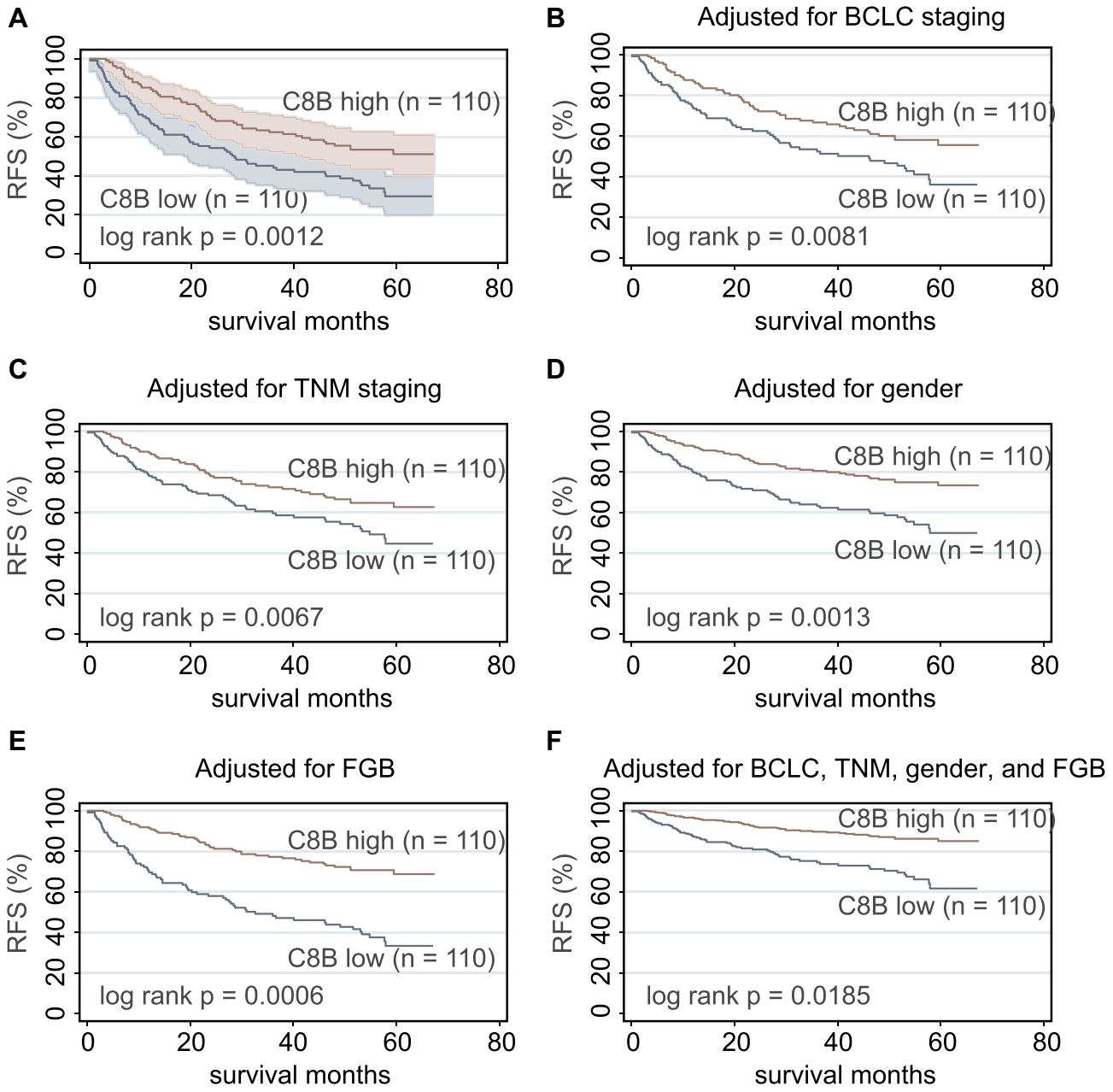

Figure 3 Kapan–Meier plot revealed that high levels of C8B in tumor tissues were significantly associated with favorable recurrence-free survival (RFS) in HBV-related HCC patients (p = 0.0012, (**A**); high levels of C8B were significantly correlated with better RFS in HBV-related HCC patients, after adjusting BCLC staging (p = 0.0081, (**B**), TNM staging (p = 0.0067, (**C**), gender (p = 0.0013, (**D**), and FGB expression (p = 0.0006, (**E**); even adjusted by BCLC, TNM, gender, and FGB levels, C8B upregulation also contributed to favorable RFS in this population (p = 0.0185, (**F**)).

peripheral blood mononuclear cells was found to have no significance in relation to normal and HCC patients, and the diagnostic value of C8B for HCC was not satisfied.[44] Unfortunately, very little research has focused on the correlations between C8B and cancer survival. Our results using the multiple analysis approach demonstrated that C8B downregulation in tumor tissues accounts for unfavorable OS and RFS in HCC patients. Since complement can enhance the silent clearance of tumor cells, and play dual roles in promoting and inhibiting tumor growth, and differ between tumor types,[34,45,46] we assumed that a better understanding of C8B in HCC tumor biology and pathology should be validated in larger prospective cohorts and experimental trials.

Current consensus indicated that the determination of fibrinogen content and fibrin lysate in plasma is helpful for the diagnosis of cancer, as well as evaluation of both therapeutic effects and prognosis.[47] The average pre-

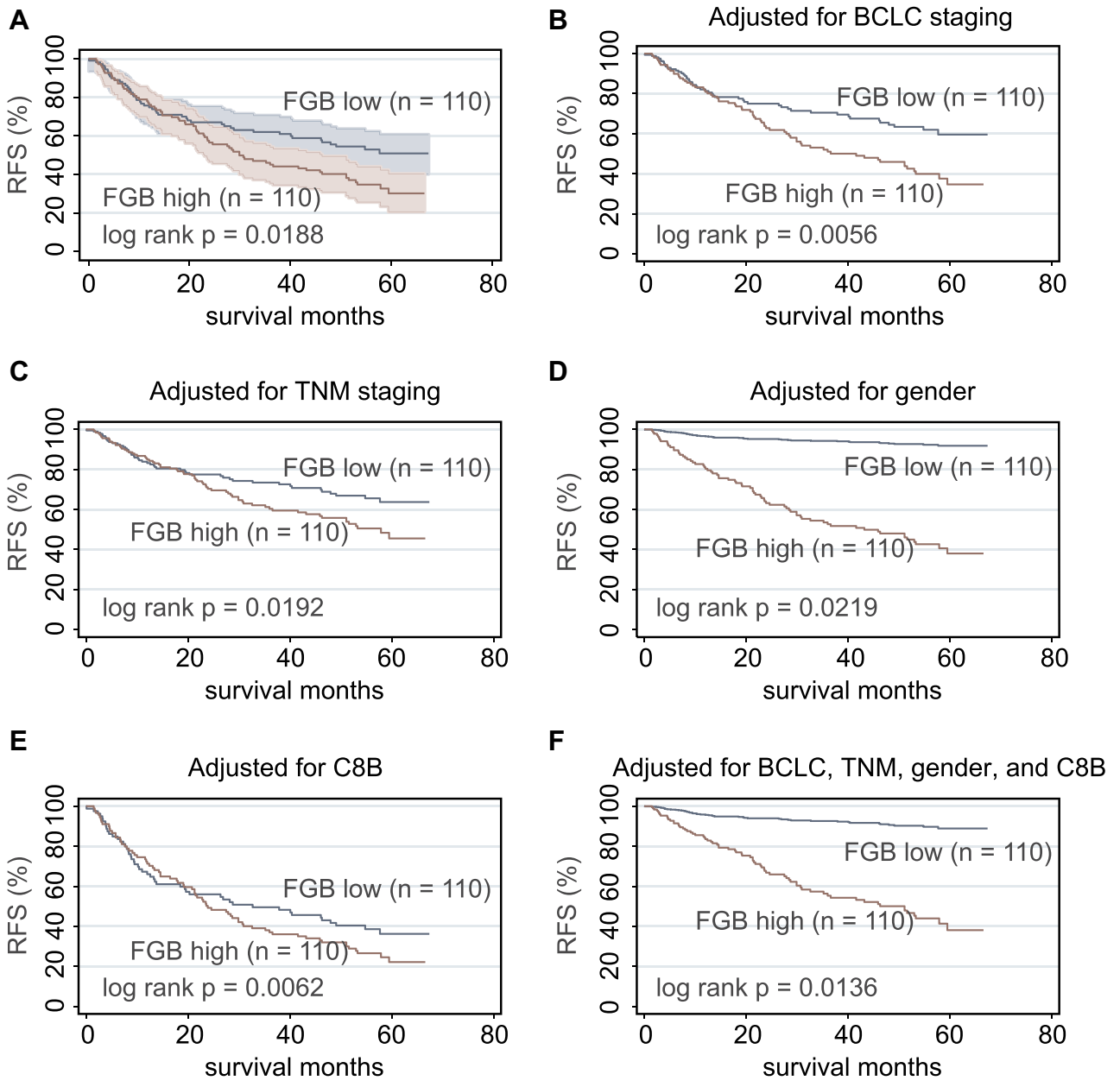

Figure 4 Kapan–Meier plot showed that high FGB expression was associated with worse RFS in HBV-related HCC patients (p = 0.0188, (**A**)); high levels of FGB were significantly correlated with unfavorable RFS in HBV-related HCC patients, after adjusting BCLC staging (p = 0056, (**B**), TNM staging (p = 0.0192, (**C**), gender (p = 0.0219, (**D**), and C8B expression (p = 0.0062, (**E**); even adjusted by BCLC, TNM, gender, and C8B levels, FGB upregulation also contributed to unfavorable RFS in this population (p = 0.0136, (**F**)).

treatment plasma fibrinogen level has been linked with a hypercoagulable status, tumor progression, and prognosis of several types of human cancers.[48–51] For instance, the preoperative plasma fibrinogen content could predict cancer metastasis, tumor progression, as well as tumor stage and survival in gastric cancer.[52] A model combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR) showed significant associations with the presence of tumor thrombus, larger tumor size, vascular invasion, and advanced BCLC stage. Additionally, F-NLR was indicated as an independent predictor for OS and disease-free survival in HCC following surgical resection.[53] Another cohort study found that hyperfibrinogenemia was also correlated with advanced tumor stage, portal vein invasion, larger tumor size, multiple tumors, nonresponse to transarterial chemoembolization therapy, and poor survival in HCC patients.[54] A bioinformatic study revealed that FGB is involved in the advanced tumor stage and hepatic

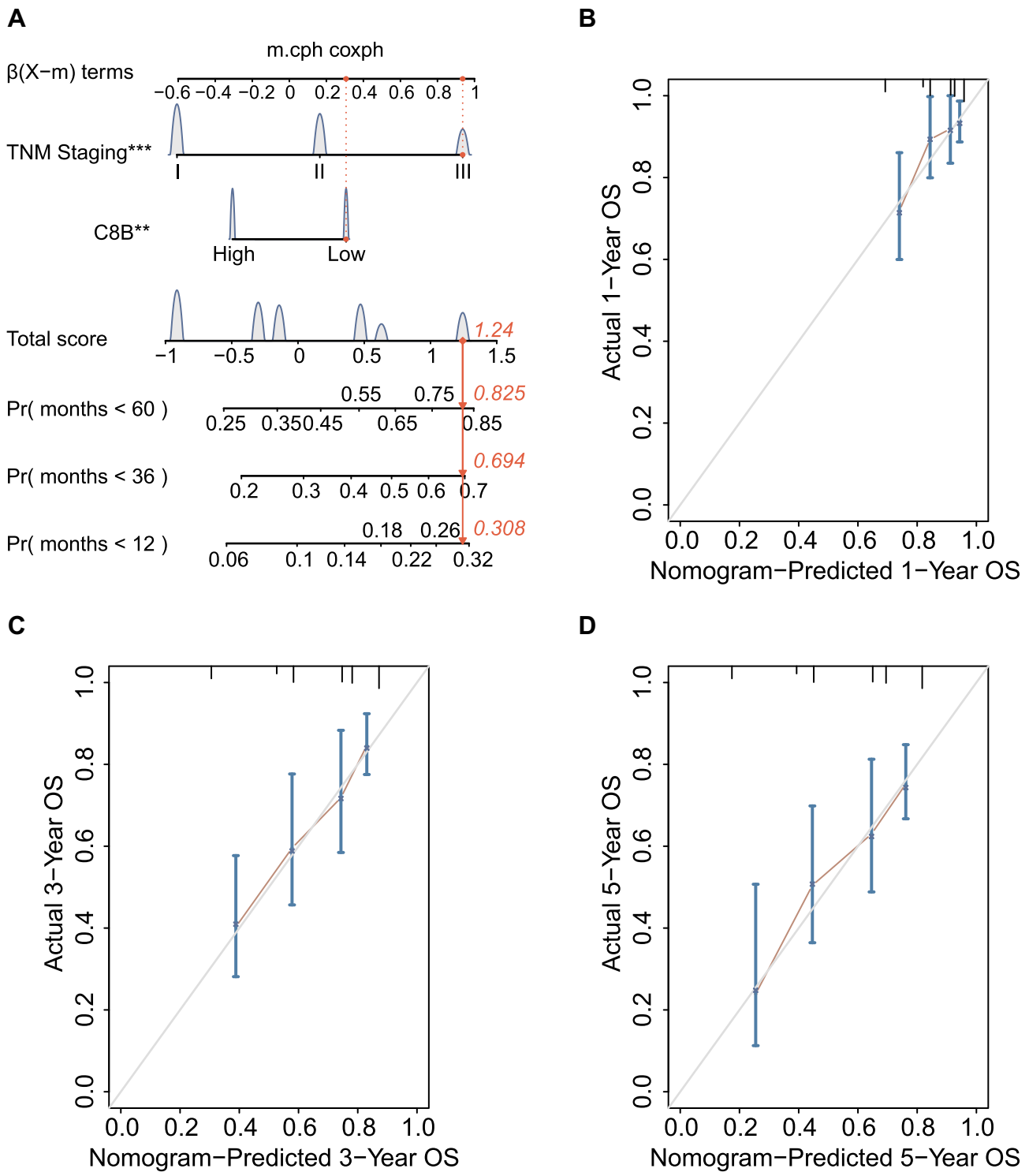

Figure 5 Screened by Cox regression model, TNM staging and C8B were included in competing risk model with nomogram for OS from HBV-related HCC patients in GSE14520 (**A**); the 1-year (**B**), 3-year (**C**), and 5-year (**D**) calibration curves for internal verification of this nomogram with bootstrapping displayed good consistency between actual OS and predicted OS.**p < 0.01, ***p < 0.001.

metastasis of colorectal cancer.[55] The serum peptides derived from FGB increased significantly in HCC patients with bone metastasis compared to those without bone metastasis.[56] All these reports have investigated plasma levels of fibrinogen in HCC patients. Since inconsistent results existed in GSE14520, GEPIA and Kaplan–Meier plotter databases, and the FGB level in tumor tissues was significantly lower than that in nontumor tissues in our

analysis, we suggest further research to address the roles of FGB in the progression of HCC at multiple levels.

Our research has some limitations. Firstly, no experiments were performed to address the impact of the complement and coagulation cascades pathway on the hepatoma cellular functions of our subjects. Secondly, follow-up data on our HCC patients was not available, and our results from public datasets were not verified by prospective cohorts. However, despite these limitations, our preliminary analysis provides a clue for a deep understanding of the complement and coagulation cascades signaling pathway in HCC biology and pathology.

Data Sharing Statement

Datasets of the current study are available from the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/, GSE14520). All the datasets are available from the corresponding author (Z.Y) in response to a reasonable request.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest in this work.

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