Original Article

Establishment of a prognostic model for predicting short-term disease-free survival in cases of hepatitis B-related hepatocellular carcinoma with the TP53 249Ser mutation in southern China

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Background: Hepatitis B virus (HBV) infection and dietary aflatoxin exposure are two major and synergistic carcinogenic factors of hepatocellular carcinoma (HCC) in southern China. Mutation of the *TP53* gene at codon 249 (TP53 249Ser) is recognized as a fingerprint of aflatoxin B1 (AFB1) exposure.

Methods: A total of 485 HCC patients positive for serum hepatitis B surface antigen were enrolled. The clinicopathological information and survival time were collected. TP53 249Ser mutations in HCC were detected by Sanger DNA sequencing after PCR amplification. Immunohistochemical staining was used to evaluate TP53 expression. Propensity score matching (PSM) and Cox proportional hazards regression (CPHR) were conducted to identify independent risk factors for prognosis that were incorporated into the nomogram. Univariate logistic regression analysis was used to compare differences in clinical factors between the TP53 249Ser mutation group and the non-mutation group. A Kaplan-Meier plot, univariate and multivariate Cox proportional hazards models were used to assess the association between clinicopathological characteristics and survival outcomes.

Results: After PSM, a total of 322 cases were included in the analysis of clinical prognosis. Results of CPHR showed that the mutation group had a relatively higher risk of tumor recurrence within 2 years after undergoing hepatectomy (P=0.039, HR =1.47, 95% CI: 1.02–2.18). The prognostic model performed better in terms of 2-year DFS prediction than BCLC stage. Patients who had a nomogram score of more than 160 were considered to have a higher risk of recurrence within 2 years.

Conclusions: Our study found that the TP53 249Ser mutation may be a high risk factor of HBV-related HCC recurrence in the short term. And we initially established a nomogram scoring system for predicting 2-year recurrence in HBV-related HCC patients in southern China.

Keywords: Hepatocellular carcinoma (HCC); aflatoxin-B1; *TP53* gene; hepatitis B virus (HBV); clinical outcome

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Introduction

Primary liver cancer is one of the most prevalent malignant tumors, with almost 841,000 new cases and 782,000 deaths occurring worldwide in 2018, and hepatocellular carcinoma (HCC) is the pathological type which accounts for approximately 75–85% of primary liver cancer (1). The major risk factors of HCC include: chronic infection with hepatitis virus (mainly hepatitis B and C), alcoholic/non-alcoholic liver disease, liver fluke infection, environmental carcinogens (such as aflatoxin) and genetic factors (2). According to a previously-published study, approximately 80% of cases of HCC arise in patients with chronic hepatitis B virus (HBV) infection in China and Africa (3).

The Guangxi region is located in southern China, where the main primary epidemiological factors of HCC are chronic HBV infection and aflatoxin exposure, which are recognized as leading carcinogenic factors in human HCC. As shown in previous molecular epidemiological investigations, the G-T mutation of TP53 249Ser is a recognized fingerprint of aflatoxin B1 (AFB1) exposure in genetic material of the local population (4-6). Previous studies have shown that the TP53 249Ser mutation is a high-frequency mutation hotspot in HCC patients from high-exposure areas of AFB1, such as Qidong, Guangxi, and South Africa (6-8).

The *TP53* gene is known as a tumor suppressor, which plays an important role in cell growth and proliferation, cell cycle arrest, apoptosis, DNA repair, and senescence (9). *TP53* gene mutation is the most common genetic mutation and is related to alteration of biological activity in cancer (10). Several studies have found that the *TP53* gene mutation is also closely related to the clinical prognosis of HCC patients (11-13). However, there has been no research into the clinical prognostic value of TP53 249Ser in the HBV-related subtype of HCC in high-HBV infection and AFB1-exposure areas.

The nomogram is a commonly-used medical prediction model for predicting the likelihood of events such as DFS in an individual cancer patient (14,15). It has been reported that the nomogram achieved an optimal preoperative prediction of microvascular invasion (MVI) in HBV-related HCC (16). Therefore, in the present study, we mapped the TP53 249Ser mutation spectrum, and attempted to establish a prognostic model for predicting DFS in HCC cases in a high-HBV infection and high-aflatoxin exposure area. This is of great significance for improving the effect of therapy for HCC and predicting its clinical efficacy.

We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/tcr-19-2788).

Methods

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethical Review Committee of the First Affiliated Hospital of Guangxi Medical University [2016(K-Y-E-011)] and informed consent was taken from all the patients.

Study population

A total of 485 patients with HCC who underwent hepatectomy at the Hepatobiliary Surgery Department of the First Affiliated Hospital of Guangxi Medical University (Nanning, China) between 2001 and 2013 were enrolled. Serum hepatitis B surface antigen was positive in all patients. The clinicopathological features of patients were identified from medical records and pathology reports, including age, gender, smoking status, drinking status, pathological grade features, preoperative serum alpha fetoprotein (AFP) levels, hepatic cirrhosis, radical resection, adjuvant antiviral therapy, and adjuvant transcatheter arterial chemoembolization (TACE). The Barcelona Clinic Liver Cancer (BCLC) staging system (17) was applied to the clinical stage of HCC. Child-Pugh class (18), portal vein tumor thrombosis (PVTT) (19) and radical resection (20) were as previously defined.

Follow-up

All patients' follow-up information was obtained through outpatient interviews, telephone communication, and by reviewing medical records and hospital records. Follow-up continued until November 2015 unless terminated earlier due to death or recurrence events. Overall survival (OS) time was defined as from liver resection to HCC-related death. Disease-free survival (DFS) time was defined as from liver resection to HCC recurrence or distant metastasis. Patients still alive at the final follow-up were defined as censored.

DNA extraction and detection of TP53 249Ser mutation

All HCC specimens were collected within 1 hour of surgical resection and stored in an ultra-low temperature

freezer at –80 ℃ until DNA extraction. DNA was extracted according to our previous method (21). TP53 249Ser mutations were detected by Sanger DNA sequencing after PCR amplification using the following primers: forward primer 5'-CTTGCCACAGGTCTCCCCAA-3'; reverse primer 5'-AGGGGTCAGAGGCAAGCAGA-3'. All PCR products were subjected to bidirectional sequencing using the ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA), by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China).

Immunohistochemistry and scoring

All paraffin-embedded HCC tissues were used for immunohistochemical staining of TP53 according to a previously-described method (22). The stained HCC sections were reviewed and scored by two pathologists independently who were blinded to clinical characteristics. At least ten fields were randomly selected at high-power (×400 magnification) at regions distant from necrotic areas, and the percentage of positive cells was calculated using the following formula: number of positive cells/total number of cells ×100%. Positive cells had brown granules in their nuclei. Scoring was performed according to previous criteria (23,24), and positive TP53 expression was defined as the presence of ≥10% positive cancer cells.

Propensity score matching (PSM)

To reduce the selectivity bias and heterogeneity between the two groups, we included the additional factor of a P value less than 0.1 in univariate analysis for PSM analysis (25). A 1:1 matching requirement by the nearest-neighbor matching algorithm without replacement was performed to select matched pairs of HBV-related HCC patients. SPSS 18.0 statistical software with R version 2.8.1 was employed to complete the PSM analysis.

Establishment of the nomogram

We performed PSM and Cox proportional hazards regression (CPHR) to identify independent risk factors for DFS. Next, a nomogram was constructed based on the results of multivariate logistic regression analysis, and formulated using the rms package of R, version 3.0 (http://www.r-project.org/). Each variable in the nomogram was based on scaling each regression coefficient in multiple logistic regression on a scale of 0 to 100 points. Furthermore, we used receiver operating characteristic curve analysis to calculate maximizing the Youden index (sensitivity + specificity –1) to determine the optimal cutoff point. Finally, the accuracy of the prediction model was estimated by specificity, sensitivity and likelihood ratio.

Statistical analysis

Univariate logistic regression analysis was used to compare the differences in clinical factors between the TP53 249Ser mutation group and the non-mutation group. The odds ratio (OR) and 95% confidence interval (95% CI) were used to evaluate the association between clinical factors and TP53 249Ser mutation. A Kaplan-Meier plot, univariate and multivariate Cox proportional hazards models were used to assess the association between clinical factors and clinical outcomes. The hazard ratio (HR) and the 95% CI were used to assess the correlation between clinical factors and clinical outcomes. All of the statistical analyses used in this study were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA), and $P<0.05$ was considered statistically significant. GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA) was used to create statistical graphics.

Results

The spectrum of TP53 249Ser mutation in HBV-related HCC

As shown in *Figure 1*, we identified 165 (34.02%) cases of TP53 249Ser mutation among the 485 HCC patients in this study. The main mutation types were AGG > AGT (Arg > Ser) (159 cases, 32.78%) and AGG > AGC (Arg > Ser) (six cases, 1.24%). These results are similar to our previously-published results (6).

Patient characteristics and PSM

A total of 485 HCC patients were enrolled, comprising 430 men and 55 women. The median age was 46 years. The patients were divided into the mutation group (n=165) and the non-mutation group (n=320) according to the TP53 249Ser mutation status (*Table 1*). In order to minimize the selection bias between the mutation group and the non-mutation group, PSM was estimated using univariate regression analysis including the covariates with P values less

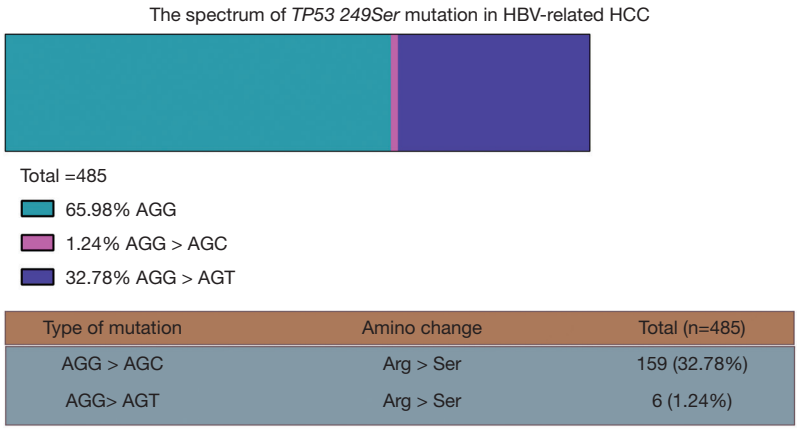

Figure 1 The TP53 249Ser mutation spectrum in 485 HBV-related HCC tissue samples. HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

Table 1 Clinicopathological characteristics of 485 HBV-related HCC patients

Variable	TP53 249Ser mutation			
	Non-mutation group (n=320)	Mutation group (n=165)	OR* (95% CI)	P value*
Age (years)				
≤46	178	82	1	
>46	142	83	1.17 (0.64–2.15)	0.605
Gender				
Male	282	148	1	
Female	38	17	0.79 (0.54–1.15)	0.215
Race				
Han	211	109	1	
Minority	109	56	0.72 (0.49–1.06)	0.940
BMI				
≤25	265	136	1	
>25	55	29	1.03 (0.63–1.69)	0.915
Smoking status				
None	211	107	1	
Ever	109	58	1.05 (0.71–1.56)	0.811
Drinking status				
None	198	97	1	
Ever	122	68	1.14 (0.78–1.67)	0.509
Child-Pugh class				
A	267	136	1	
B	53	29	1.07 (0.65–1.77)	0.778

Table 1 (*continued*)

Table 1 (*continued*)

Variable	TP53 249Ser mutation			
	Non-mutation group (n=320)	Mutation group (n=165)	OR* (95% CI)	P value*
BCLC stage				
A	189	95	1	
B	47	33	1.40 (0.84–2.32)	
C	84	37	0.88 (0.55–1.39)	0.238
TP53 expression[a]				
Negative	133	26	1	
Positive	132	115	4.46 (2.73–7.27)	<0.001
TACE status				
Before hepatectomy				
No	244	138	1	
Yes	76	27	0.63 (0.39–1.02)	0.061
After hepatectomy				
No	137	75	1	
Yes	183	90	0.90 (0.62–1.31)	0.578
Cirrhosis				
No	37	22	1	
Yes	283	143	0.83 (0.47–1.46)	0.511
Serum AFP[b]				
≤400 (ng/mL)	165	82	1	
>400 (ng/mL)	134	68	1.02 (0.69–1.51)	0.917
Radical resection[c]				
Yes	166	102	1	
No	146	59	1.52 (1.03–2.25)	0.035
Pathological grade[d]				
Good	22	5	2.28 (0.84–6.17)	
Moderate	245	127	1.96 (1.43–9.00)	0.262
Poor	9	4		
Antiviral therapy				
No	210	105	1	
Yes	110	60	1.09 (0.74–1.62)	0.664
Oncological behavior				
Tumor size				
≤5 cm	103	52	1	
>5 cm	217	113	1.03 (0.69–1.54)	0.880

Table 1 (*continued*)

Table 1 (*continued*)

Variable	TP53 249Ser mutation			
	Non-mutation group (n=320)	Mutation group (n=165)	OR* (95% CI)	P value*
No. of tumors				
Single (n=1)	238	118	1	
Multiple (n>1)	82	47	1.16 (0.76–1.76)	0.500
Capsule				
Complete	134	64	1	
Incomplete	129	76	0.92 (0.53–1.60)	
Absence	57	25	1.23 (0.82–1.86)	0.459
Regional invasion				
Absence	272	140	1	
Presence	48	25	1.01 (0.60–1.71)	0.965
Intrahepatic metastasis				
Absence	146	75	1	
Presence	174	90	1.01 (0.69–1.47)	0.972
Vascular invasion				
Absence	260	139	1	
Presence	60	26	0.81 (0.49–1.34)	0.414
PVTT				
No	269	140	1	
vp1	5	6	2.31 (0.69–7.69)	
vp2	14	3	0.41 (0.12–1.46)	
vp3	27	13	0.93 (0.46–1.85)	
vp4	5	3	1.15 (0.27–4.89)	0.414

[a], TP53 expression information was unavailable for 79 patients. [b], AFP information was unavailable for 36 patients. [c], radical resection information was unavailable for 12 patients. [d], pathological grade information was unavailable for 73 patients. *, OR and P value for univariate analysis of logistic regression model. AFP, alpha-fetoprotein; TACE, transarterial chemoembolization; BMI, body mass index; PVTT, portal vein tumor thrombus; MST, median survival time; MRT, median recurrence time; HR, hazard ratio; 95% CI, 95% confidence interval.

than 0.1 in *Table 1*. With a 1:1 ratio of propensity scoring, a total of 322 cases were included in the analysis, 161 cases from each group (*Table 2*). We found that median OS was 57 (non-mutation group) and 42 (mutation group) months. Median DFS was 11 (non-mutation group) and 6 months (mutation group). Our result showed that positive *TP53* gene expression was significantly associated with TP53 249Ser mutation (P<0.001, *Table 2*)

The results of Kaplan-Meier survival analysis (*Figure 2*) and univariate CPHR analysis only showed a statistically-significant difference in 2-year DFS between the two groups (P=0.033, HR =1.40, 95% CI: 1.01–1.94), but there was no significant difference in long-term DFS (P=0.351, HR =1.16, 95% CI: 0.83–1.63).

Prognostic model for 2-year DFS

The results of univariate CPHR analysis are shown in *Table 3*. All significant indicators were then incorporated into multivariate CPHR. As shown in *Table 4*, we found

Table 2 Clinicopathological characteristics of 322 HBV-related HCC patients after PSM

Variable	TP53 249Ser mutation			
	Non-mutation group (n=161)	Mutation group (n=161)	OR* (95% CI)	P value*
Age (years)				
≤46	81	81	1	
>46	80	80	1.00 (0.65–1.55)	1.000
Gender				
Male	140	144	1	
Female	21	17	0.79 (0.40–1.55)	0.490
Race				
Han	102	93	1	
Minority	59	68	1.26 (0.81–1.98)	0.305
BMI				
≤25	132	133	1	
>25	29	28	0.96 (0.54–1.70)	0.884
Smoking status				
None	106	104	1	
Ever	55	57	1.06 (0.67–1.67)	0.815
Drinking status				
None	102	93	1	
Ever	59	68	1.26 (0.81–1.98)	0.305
Child-Pugh class				
A	135	126	1	
B	26	35	1.43 (0.74–2.76)	0.288
BCLC stage				
A	105	95	1	
B	21	29	1.53 (0.82–2.85)	
C	35	37	1.17 (0.68–2.00)	0.402
TP53 expression[a]				
Negative	69	25	1	
Positive	67	112	4.61 (2.67–8.00)	<0.001
TACE status				
Before hepatectomy				
No	122	135	1	
Yes	39	26	0.60 (0.35–1.05)	0.073

Table 2 (*continued*)

Table 2 (*continued*)

Variable	TP53 249Ser mutation			
	Non-mutation group (n=161)	Mutation group (n=161)	OR* (95% CI)	P value*
After hepatectomy				
No	71	73	1	
Yes	90	88	0.95 (0.61–1.48)	0.823
Cirrhosis				
No	15	22	1	
Yes	146	139	0.65 (0.324–1.30)	0.224
Serum AFP[b]				
≤400 (ng/mL)	85	80	1	
>400 (ng/mL)	66	66	1.06 (0.67–1.68)	0.795
Radical resection				
Yes	102	102	1	
No	59	59	1.00 (0.64–1.57)	1.000
Pathological grade[c]				
Good	14	5	1	
Moderate	127	124	2.73 (0.96–7.82)	
Poor	7	4	1.60 (0.34–7.90)	0.130
Antiviral therapy				
No	111	103	1	
Yes	50	58	1.30 (0.82–2.06)	0.273
Oncological behavior				
Tumor size				
≤5 cm	56	50	1	
>5 cm	105	111	1.18 (0.74–1.88)	0.477
No. of tumors				
Single (n=1)	122	118	1	
Multiple (n>1)	39	43	1.14 (0.69–1.88)	0.609
Capsule				
Complete	63	61	1	
Incomplete	72	75	0.99 (0.52–1.91)	
Absence	26	25	1.08 (0.67–1.74)	0.945
Regional invasion				
Absence	139	136	1	
Presence	22	25	1.16 (0.63–2.16)	0.636

Table 2 (*continued*)

Table 2 (*continued*)

Variable	TP53 249Ser mutation			
	Non-mutation group (n=161)	Mutation group (n=161)	OR* (95% CI)	P value*
Intrahepatic metastasis				
Absence	70	73	1	
Presence	91	88	0.93 (0.60–1.44)	0.737
Vascular invasion				
Absence	135	135	1	
Presence	26	26	1.00 (0.55–1.81)	1.000
PVTT				
No	140	136	1	
vp1	0	6	NA	
vp2	8	3	0.39 (0.10–1.49)	
vp3	11	13	1.22 (0.53–2.81)	
vp4	2	3	1.54 (0.25–9.39)	0.657

[a], TP53 expression information was unavailable for 49 patients. [b], AFP information was unavailable for 25 patients. [c], pathological grade information was unavailable for 41 patients. *, OR and P value for univariate analysis of logistic regression model. AFP, alpha-fetoprotein; TACE, transarterial chemoembolization; BMI, body mass index; PVTT, portal vein tumor thrombus; OR, odds ratio; 95% CI, 95% confidence interval.

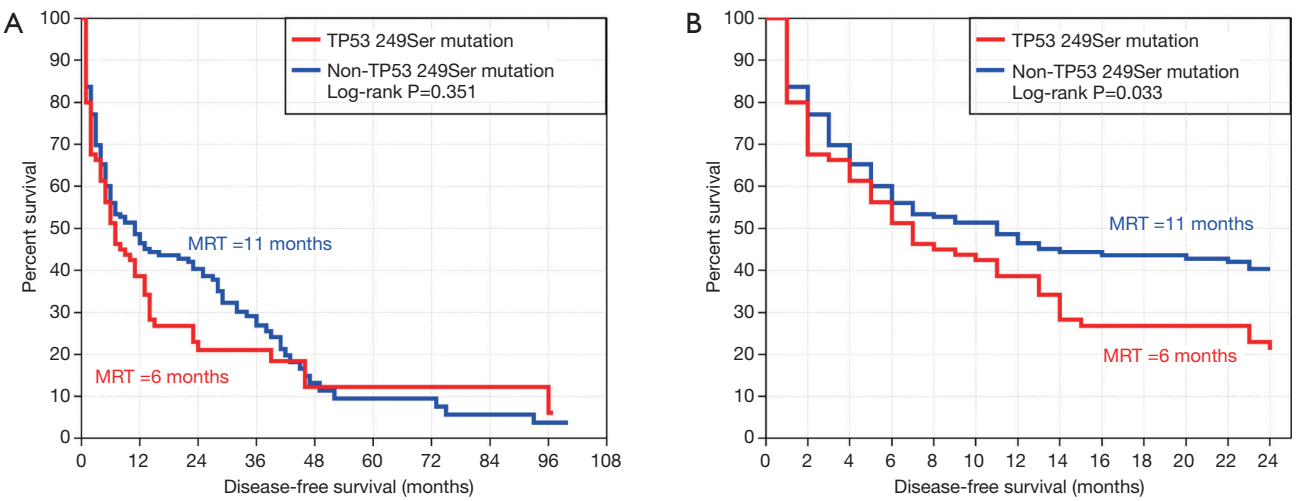

Figure 2 Kaplan-Meier plot of *TP53* gene 249th codon mutation and DFS in HBV-related HCC patients. (A) Kaplan-Meier plot of long-term DFS; (B) Kaplan-Meier plot of 2-year DFS. DFS, disease-free survival; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

that TACE after surgery, status of TP53 249Ser mutation, BCLC staging and tumor capsule were independent prognostic factors for 2-year DFS (P<0.05). We constructed the independent predictors above into a nomogram (*Figure 3*), and then used the bootstrap validation method to internally validate the resulting model (26) (*Figure 4*). The results indicated that the nomogram showed a good accuracy in assessing 2-year DFS, with a C-index of 0.718 (95% CI: 0.638–0.799), which was significantly greater than that of the BCLC staging system (C-index: 0.606,

Table 3 Univariate Cox proportional hazards analysis of clinicopathological characteristics and clinical outcomes in 322 HBV-related HCC patients after PSM

Variables	Patients (n=322)	Overall survival			Disease-free survival		
		MST (months)	HR* (95% CI)	P*	MRT (months)	HR* (95% CI)	P*
TP53 249Ser mutation							
Non-mutation	161	57	1		11	1	
Mutation	161	42	1.27 (0.92–1.75)	0.146	6	1.16 (0.83–1.63)	0.351
Age (years)							
≤46	162	57	1		7	1	
>46	160	48	0.88 (0.64–1.21)	0.438	12	0.78 (0.56–1.09)	0.151
Gender							
Male	284	48	1		7	1	
Female	38	51	0.66 (0.366–1.19)	0.166	12	0.89 (0.52–1.53)	0.673
Race							
Han	195	48	1		8	1	
Minority	127	51	1.03 (0.74–1.43)	0.868	8	1.22 (0.87–1.72)	0.243
BMI							
≤25	265	45	1		9	1	
>25	57	51	0.93 (0.61–1.40)	0.719	7	1.29 (0.88–1.89)	0.195
Smoking status							
None	210	61	1		9	1	
Ever	112	40	1.20 (0.86–1.67)	0.275	6	1.04 (0.73–1.47)	0.839
Drinking status							
None	195	57	1		8	1	
Ever	127	41	1.26 (0.91–1.74)	0.158	7	1.03 (0.73–1.45)	0.873
TP53 expression[a]							
Negative	94	45	1		12	1	
Positive	179	42	1.08 (0.75–1.58)	0.673	6	1.04 (0.71–1.52)	0.838
TACE status							
Before hepatectomy							
No	257	57	1		9	1	
Yes	65	44	1.07 (0.73–1.58)	0.720	6	1.37 (0.88–1.95)	0.190
After hepatectomy							
No	144	88	1		14	1	
Yes	178	42	1.16 (0.84–1.62)	0.368	6	1.64 (1.13–2.40)	0.010

Table 3 (*continued*)

Table 3 (*continued*)

Variables	Patients (n=322)	Overall survival			Disease-free survival		
		MST (months)	HR* (95% CI)	P*	MRT (months)	HR* (95% CI)	P*
BCLC stage							
A	200	84	1	<0.001	12	1	0.030
B	50	71	1.63 (1.05–2.53)	0.031	6	1.19 (0.75–1.90)	0.456
C	72	17	3.31 (2.29–4.78)	<0.001	2	1.70 (1.15–2.53)	0.008
Child–Pugh class							
A	261	57	1		7	1	
B	61	34	1.57 (1.02–2.43)	0.040	6	1.22 (0.75–2.00)	0.428
Cirrhosis							
No	37	88	1		38	1.	
Yes	285	45	1.55 (0.89–2.68)	0.122	7	1.79 (0.94–3.41)	0.077
Antiviral therapy							
No	214	58	1		6	1	
Yes	108	NA	0.54 (0.36–0.81)	0.003	13	0.62 (0.44–0.90)	0.010
AFP (ng/mL)[b]							
≤400	165	61	1		12	1	
>400	132	41	1.22 (0.87–1.72)	0.246	7	1.08 (0.76–1.54)	0.661
Radical resection							
Yes	204	71	1		11	1	
No	118	36	1.59 (1.15–2.19)	0.005	5	1.60 (1.14–2.24)	0.007
Pathological grade[c]							
Good	19	NA	1	0.496	6	1	0.164
Moderate	251	48	1.61 (0.71–3.66)	0.255	7	1.54 (0.67–3.56)	0.308
Poor	11	NA	1.33 (0.38–4.73)	0.656	3	3.18 (0.95–10.68)	0.061
Oncological features							
Tumor size							
≤5 cm	106	123	1		17	1	
>5 cm	216	41	1.9 (1.31–2.82)	0.001	5	1.81 (1.25–2.62)	0.002
No. of tumors							
Single (n=1)	240	51	1		11	1	
Multiple (n>1)	82	39	1.38 (0.97–1.96)	0.072	6	1.15 (0.79–1.68)	0.456
Capsule							
Complete	124	95	1	0.002	14	1	0.014
Incomplete	147	36	1.51 (0.93–2.46)	0.099	5	1.21 (0.71–2.06)	0.489
Absence	51	51	1.93 (1.33–2.78)	<0.001	8	1.72 (1.19–2.48)	0.004

Table 3 (*continued*)

Table 3 (*continued*)

Variables	Patients (n=322)	Overall survival			Disease-free survival		
		MST (months)	HR* (95% CI)	P*	MRT (months)	HR* (95% CI)	P*
Regional invasion							
Absence	275	57	1		11	1	
Presence	47	40	1.32 (0.84–2.08)	0.235	2	1.80 (1.17–2.77)	0.008
Intrahepatic metastasis							
Absence	179	75	1		12	1	
Presence	143	36	1.60 (1.16–2.20)	0.004	4	1.54 (1.10–2.15)	0.012
Vascular invasion							
Absence	270	73	Ref.		9	1	
Presence	52	12	3.40 (2.32–5.00	<0.001	2	1.57 (1.03–2.40)	0.036
PVTT							
No	276	71	Ref.	<0.001	11	1	0.014
vp1	6	7	4.67 (1.70–12.84)	0.003	1	2.29 (0.73–7.24)	0.158
vp2	11	17	4.16 (1.53–6.52)	<0.002	2	2.24 (0.82–6.13)	0.117
vp3	24	12	3.00 (1.80–5.00)	<0.001	3	1.43 (0.80–2.55)	0.226
vp4	5	8	6.52 (2.37–17.94)	<0.001	1	4.49 (1.61–12.55)	0.004

P<0.05 is statistically significant. [a], TP53 expression information was unavailable for 49 patients. [b], AFP information was unavailable for 25 patients. [c], pathological grade information was unavailable for 41 patients. *, HR and P value for univariate survival analysis of Cox proportional hazard regression model. AFP, alpha-fetoprotein; TACE, transarterial chemoembolization; BMI, body mass index; PVTT, portal vein tumor thrombus; MST, median survival time; MRT, median recurrence time; HR, hazard ratio; 95% CI, 95% confidence interval.

95% CI: 0.519–0.693) (*Figure 5*). The optimal cutoff point of the total nomogram scores was determined to be 160, and the specificity and sensitivity were 65.5% and 69.6% respectively.

Conclusions

HCC is one of the most common malignant tumors in the world, and more than half of new cases occur in China every year (1). HBV infection and AFB1 exposure are two major risk factors of HCC occurrence (2). The Guangxi area, located in southern China, has a higher incidence of HCC than the national average due to its unique environmental background of high exposure to AFB1 and high levels of endemic HBV, both of which are currently recognized risk factors for human HCC. In addition, HBV infection and AFB1 exposure are known to have synergistic carcinogenic effects (27).

Studies on HCC patients with a background of AFB1 exposure found that tumors exhibited a high frequency of a G-T mutation at codon 249 of the *TP53* gene, which is considered as the molecular fingerprint of AFB1 exposure during HCC pathogenesis in this region, revealing the interaction between environment and genes during hepatocarcinogenesis (6,28,29). Our previous studies also provided evidence that there was a hotspot for this characteristic TP53 mutation in HCC in Guangxi, in which the TP53 249Ser mutation rate in recurrent HCC was as high as 60% (30). Holmes *et al.* reported that the urine metabolism spectrum of a southern population, represented by Guangxi, was significantly different from that of northern China, indicating that diet and other environmental factors differed greatly between the northern and southern populations and that this in turn made a huge difference to metabolism (31). Previous study has shown that AFB1 contamination in food is an important factor underlying the high incidence of HCC in Guangxi (32). Previous reports also revealed that the exposure level

Table 4 Multivariate survival analysis of Cox proportional hazard regression model between variables and post-operative disease-free survival of HBV-related HCC patients

Variables	Patients (n=322)	2 years disease free survival		
		MRT (months)	HR* (95% CI)	P*
TP53 249Ser mutation				
Non-mutation	161	11	1	
Mutation	161	6	1.47 (1.02–2.18)	0.039
TACE after hepatectomy				
No	144	9	1	
Yes	178	6	1.65 (1.08–2.52)	0.020
Capsule				
Complete	124	14	1	0.044
Incomplete	147	5	1.27 (0.70–2.31)	0.438
Absence	51	8	1.71 (1.12–2.61)	0.013
BCLC stage				
A	200	12	1	0.012
B	50	6	1.35 (0.82–2.23)	0.234
C	72	2	1.92 (1.25–2.95)	0.003

P<0.05 is statistically significant. *, HR and P value for multivariate survival analysis of Cox proportional hazard regression model. MRT, median recurrence time; HR, hazard ratio; 95% CI, 95% confidence interval.

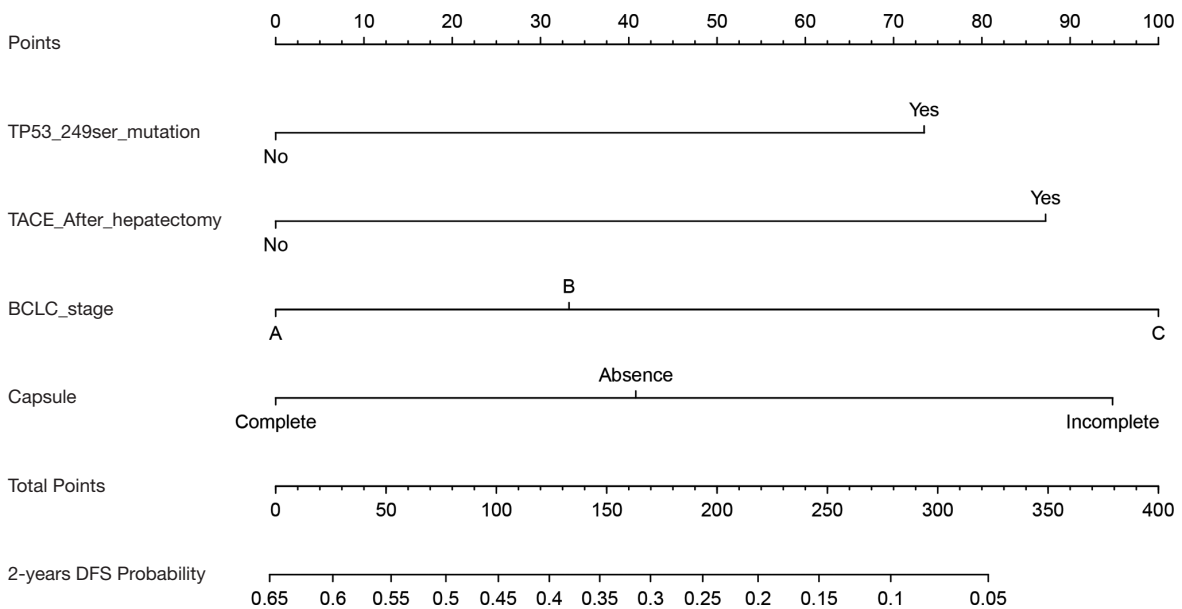

Figure 3 Nomogram to estimate the risk of 2-year DFS in HBV-related HCC. DFS, disease-free survival. HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

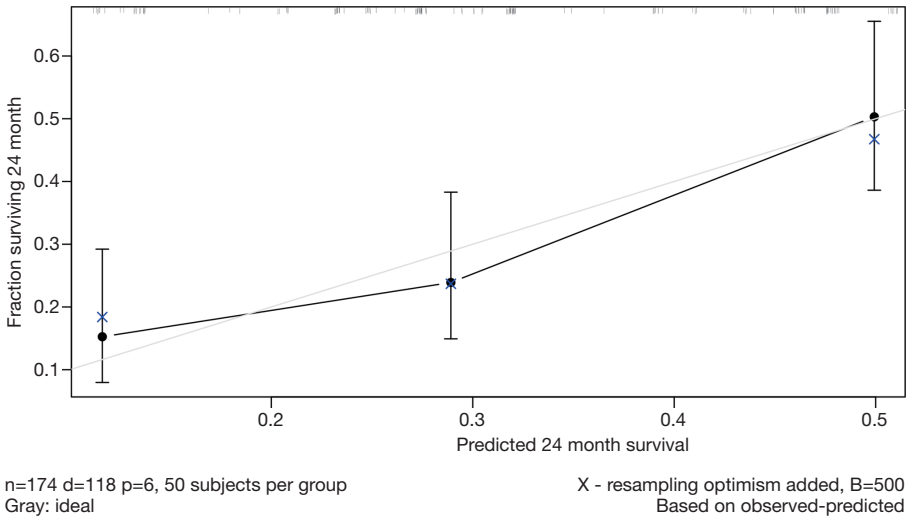

Figure 4 Validity of the predictive performance of the nomogram in estimating the risk of 2-year DFS. DFS, disease-free survival.

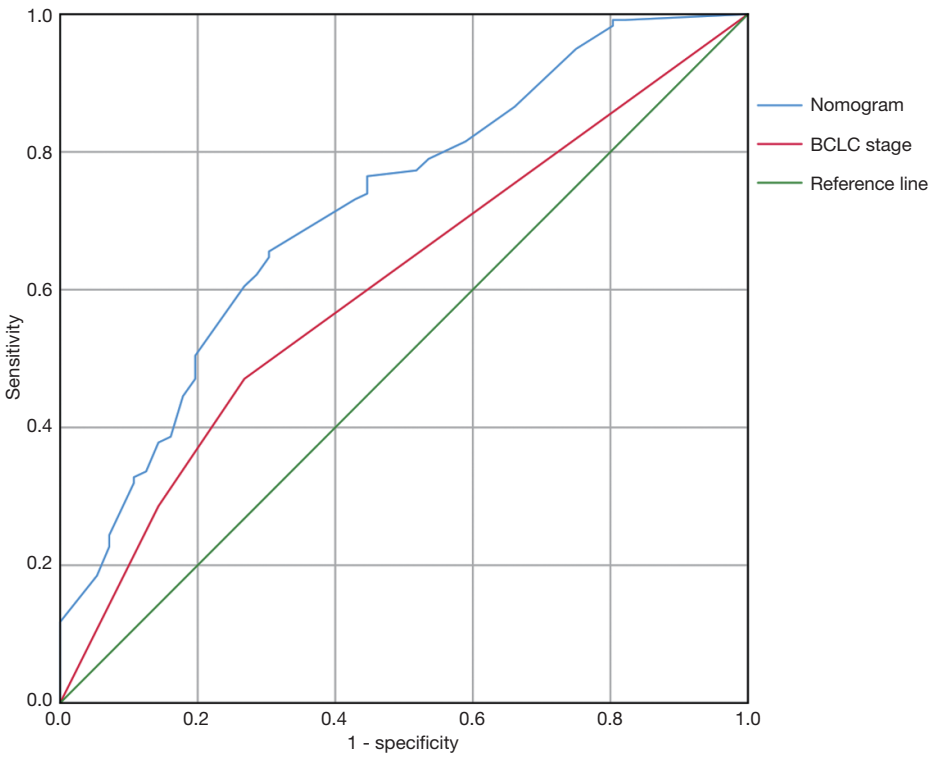

Figure 5 ROC curve for comparison of the nomogram and BCLC stage. ROC, receiver operating characteristic curve; BCLC, Barcelona Clinic Liver Cancer.

gradient of AFB1 paralleled the incidence of HCC (33-35) and exposure level of aflatoxin is closely related to TP53 249Ser mutation and the occurrence of HCC (36,37).

In our study, positive *TP53* gene expression was significantly associated with TP53 249Ser mutation (P<0.001, *Table 2*), and TP53 249Ser mutation was still significantly correlated with 2-year DFS of patients undergoing hepatectomy after correction of the clinical

factors significantly associated with recurrence (P=0.039, HR =1.47, 95% CI: 1.02–2.18). This result indicates that patients with the TP53 249Ser mutation will have a higher risk of tumor recurrence after surgery compared with patients without the mutation. Therefore, TP53 249Ser mutation may be an independent risk factor for tumor recurrence within 2 years after hepatectomy in patients with HBV-related HCC. Currently, several studies on Japanese HCC patients have shown that mutations in the exon region of the *TP53* gene are significantly correlated with short-term recurrence and pathological differentiation of tumors, a finding that is similar to the results of this study (37,38). The difference is that our localization is more specific, only targeting the codon 249 mutation of the *TP53* gene. A study of HCC patients in Guangdong, a region neighboring Guangxi, demonstrated that *TP53* gene 249Ser and V157F mutation hotspots were significantly correlated with short-term OS of HCC patients after surgery (11). Similarly, another report found that *TP53* gene mutation indicated poor prognosis among HCC patients in Taiwan (39).

As a medical tool to predict clinical events, in the field of HCC, the nomogram has been used in many studies to establish models to predict the clinical prognosis of patients with liver cancer (40,41). In the present study, through the clinical pathological characteristics of HCC patients and based on univariate and multivariate CPHR analysis, we preliminarily established a nomogram to predict the short-term DFS of HCC patients with AFB1 and HBV exposure. In this nomogram, TACE after surgery, status of TP53 249Ser mutation, BCLC staging and tumor capsule were independent prognostic factors for 2-year DFS. In order to make the model applicable to the clinic, we determined the cutoff value of 160 to evaluate prognosis by calculating the maximum Youden index, but the sensitivity and specificity were not satisfactory. Therefore a score of more than 160 points was considered a high-risk group for tumor recurrence within two years, which allows the nomogram to serve as a tool for making rough predictions about the prognosis of patients after hepatectomy. Further evaluation revealed that the C-index of the nomogram (C-index: 0.718, 95% CI: 0.638–0.799) exceeded that of the BCLC staging system (P=0.024) (C-index: 0.606, 95% CI: 0.519–0.693). The reason may be that the liver disease background and environmental background of HCC patients in the region of this study were different from those in Europe. In addition the risk factors incorporated in the prediction model may be more geographically representative.

There were still some limitations in our research that need further improvement. The cases included in the study were from a single center and may not be sufficiently representative. The nomogram we established was based on the patient's clinical characteristics. Furthermore, *in vitro* and *in vivo* studies are necessary to elucidate the molecular mechanism by which TP53 249Ser mutation affects the clinical prognosis of HBV-related HCC patients.

In this study, we found that the TP53 249Ser mutation may be a high risk factor of HBV-related HCC recurrence in the short term. Then we established a preliminary prediction model for 2-year DFS, based on regional characteristics, high AFB1 exposure and high incidence of chronic hepatitis B. This study not only fills a gap in the relevant research field but will also help with the implementation of active prevention and treatment strategies for high-risk populations in areas with high incidence of liver cancer.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethical Review Committee of the First Affiliated Hospital of Guangxi Medical University [2016(K-Y-E-011)] and informed consent was taken from all the patients.

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