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ORIGINAL RESEARCH

Prognostic value of increased expression of RACO-1 in patients with hepatitis B-related hepatocellular carcinoma

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Abstract: RING domain AP-1 coactivator-1 (RACO-1) is a coactivator that links c-Jun to growth factor signaling and is essential for AP-1 function. This study aimed to investigate the expression and clinical significance of RACO-1 protein in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) in China. A total of 136 tissue samples of HBV-related HCC were detected by immunohistochemistry (including 76 patients in training cohort and 60 patients in validation cohort). Correlation between RACO-1 expression and clinicopathologic features of HBV-related HCC was analyzed in both the cohorts. RACO-1 expression was significantly higher in HBV-related HCC tissues than in adjacent non-tumor liver tissues. All the patients were divided into two groups: the low expression group and the high expression group. RACO-1 expression was significantly related to vascular invasion (P=0.021), tumor numbers (P=0.046), International Union for Cancer Control/American Joint Committee on Cancer stage (P=0.006), cirrhosis (P=0.046), capsular (P=0.039), and Barcelona Clinic Liver Cancer stage (P=0.041) in training cohort. The validation cohort showed the same results. The high RACO-1 expression was the independent prognostic factor for HBV-related HCC patients in both training cohort and validation cohort. Our data implicate RACO-1 as a novel prognostic marker and a potential therapeutic target for HBV-related HCC.

Keywords: RACO-1, hepatitis B, hepatocellular carcinoma, prognosis, BCLC stage, vascular invasion, cirrhosis, UICC/AJCC stage

Background

Hepatocellular carcinoma (HCC) is one of the most common carcinomas in the world, especially in China. Most of the HCC in China is hepatitis B virus (HBV)-related which is caused by the high HBV infection rates.[1–3] Although the overall survival of patients with HBV-related HCC had been improved, the survival still remains unsatisfactory.[4,5] Therefore, it is very important to find novel prognostic markers and potential therapeutic target for HBV-related HCC.

Previous studies have shown that AP-1 transcription factor c-Jun is overexpressed in many human cancers.[6–8] RING domain AP-1 coactivator-1 (RACO-1) is a c-Jun coactivator that is regulated by growth factor signaling. RACO-1 depletion decreased the expression of several AP-1 target genes, such as *cdc42*, *cyclinD1*. RACO-1 also has an important relationship with Wnt signaling and Ras.[9,10] However, the role of RACO-1 in HBV-related HCC has not been demonstrated. Whether RACO-1 has an effect on the prognosis of HBV-related HCC is still unknown. This study aimed to investigate the effects of RACO-1 on HBV-related HCC and find out the relationship between RACO-1 and the prognosis of HBV-related HCC.

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Methods

Patients and specimens

In this study, HCC was diagnosed and staged according to both the Barcelona Clinic Liver Cancer (BCLC) stage system and International Union for Cancer Control (UICC)/American Joint Committee on Cancer (AJCC) stage system. HCC and the adjacent non-tumor liver tissue (ANLT) specimens were obtained from 136 HBV-related HCC patients during surgical resection without any preoperative treatment at the Department of Hepatobiliary Surgery, Shaoxing Second Hospital, and Department of Surgery, Zhuzhou Clinical Institute, Central South University (CSU) School of Medicine from March 2008 to March 2014. The follow-up status and any recurrence were regularly updated in the database for each patient. Our research was in compliance with the Helsinki Declaration. Prior written informed consent was obtained from all patients. The ethics committees of Shaoxing Second Hospital, Zhuzhou Clinical Institute of CSU, and the Fourth Affiliated Hospital of Zhejiang University School of Medicine gave ethics approval for this study and approved the consents. Before the operations, we evaluated the complete blood count, liver function, computed tomography (CT) scan of liver and other tests if necessary. Liver resection was undertaken in patients with Pugh–Child grades A or B.

Immunohistochemistry (IHC)

Formalin-fixed paraffin sections were stained for RACO-1 using anti-human RACO-1 antibody (Abcam, Cambridge, UK). The expression level of RACO-1 was scored as: 1, 1%–25% positive; 2, 26%–50% positive; 3, >50% positive. The protein expression of RACO-1 was thus considered low expression if scored 1; score 2 or 3 was considered as high expression. Correlation between RACO-1 expression and clinicopathologic features of HBV-related HCC were analyzed in both the cohorts.

Statistical analysis

Data in the study was analyzed by the Statistical Package for the Social Sciences (SPSS) 19 for Windows (SPSS Inc., Chicago, IL, USA). Fisher's exact test was used for the statistical analysis of categorical data, whereas independent *t*-tests were used for continuous data. The log-rank test was used to compare the overall and disease-free survival. The Cox proportional hazards regression model was established to identify factors that were independently associated with the survival of HCC patients. $P<0.05$ was considered to be statistically significant.

Results

Clinical features of patients and the correlations of RACO-1 expression with the clinical characteristics of HBV-related HCC

A total of 136 tissue samples of HBV-related HCC were detected by IHC, including 76 patients in training cohort and 60 patients in validation cohort (Figure 1). There is no significant difference between training cohort and validation cohort (Table 1). RACO-1 expression was significantly high in HBV-related HCC tissues in both training cohort and

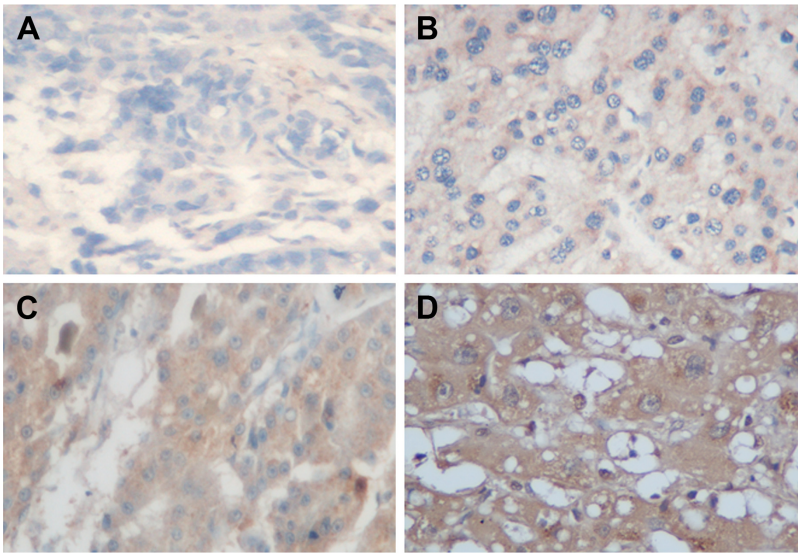

Figure 1 Immunohistochemistry of RACO-1 expression in HBV-related HCC tissues.
Notes: (**A**–**D**) These representative images show negative control-isotype control (scored as 0, **A**), 1%–25% of cancer cells (scored as 1+, **B**), 26%–50% of cancer cells (scored as 2+, **C**), and >51% of cancer cells (scored as 3+, **D**); original magnification ×400.
Abbreviations: RACO-1, RING domain AP-1 coactivator-1; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

Table 1 Clinicopathologic variables of training cohort and validation cohort

Clinicopathologic variables	Training cohort (n)	Validation cohort (n)	*P*-value
Gender			
Female	24	19	0.642
Male	52	41	
Age (years)			
≤60	60	50	0.665
>60	16	10	
Liver cirrhosis			
Presence	40	31	0.645
Absence	36	29	
Tumor number			
Solitary	36	30	0.168
Multiple	40	30	
Tumor size			
≤5 cm	36	28	0.635
>5 cm	40	32	
Capsular formation			
Presence	35	29	0.804
Absence	41	31	
Vascular invasion			
Presence	30	26	0.715
Absence	46	34	
Edmondson-Steiner grade			
Low grade (I–II)	45	32	0.803
High grade (III–IV)	31	28	
UICC/AJCC stage			
I	30	25	0.682
II–III	46	35	
BCLC stage			
0–A	37	27	0.811
B–C	39	33	

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; UICC, International Union for Cancer Control.

Table 2 Correlations between RACO-1 expression and clinicopathologic variables of 76 cases of HBV-related HCC in training cohort

Clinicopathologic variables	Patients (n)	RACO-1 expression levels		*P*-value
		Low	High	
Gender				
Female	24	7	17	0.502
Male	52	17	35	
Age (years)				
≤60	60	19	41	0.678
>60	16	5	11	
Liver cirrhosis				
Presence	40	7	33	**0.046**
Absence	36	17	19	
Tumor number				
Solitary	36	17	19	**0.046**
Multiple	40	7	33	
Tumor size				
≤5 cm	36	9	27	0.217
>5 cm	40	15	25	
Capsular formation				
Presence	35	17	18	**0.039**
Absence	41	7	34	
Vascular invasion				
Presence	30	3	27	**0.021**
Absence	46	21	25	
Edmondson-Steiner grade				
Low grade (I–II)	45	11	34	0.185
High grade (III–IV)	31	13	18	
UICC/AJCC stage				
I	30	17	13	**0.006**
II–III	46	7	39	
BCLC stage				
0–A	37	18	19	**0.041**
B–C	39	6	33	

Note: $P<0.05$ was considered to be statistically significant; significant values are shown in bold.

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; UICC, International Union for Cancer Control; RACO-1, RING domain AP-1 coactivator-1; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

validation cohort (Tables 2 and S1). It was found that RACO-1 expression negatively correlates with capsular in both training cohort and validation cohort (P=0.039; 0.041). The RACO-1 expression in training cohort was positively related to liver cirrhosis (P=0.046), tumor number (P=0.046), vascular invasion (P=0.021), UICC stage (P=0.006), and BCLC stage (P=0.041) (Table 2). While in validation cohort, RACO-1 expression was positively related to liver cirrhosis (P=0.041), tumor number (P=0.046), vascular invasion (P=0.005), UICC stage (P=0.048), and BCLC stage (P=0.003) (Table S1).

Correlations of RACO-1 expression with the overall survival of HBV-related HCC

Overall survival was analyzed according to the expression of RACO-1 both in training cohort and validation cohort. The high level of RACO-1 had worse overall survival rates in both the training cohort and the validation cohort ($P<0.001$; $P<0.001$) (Figure 2). In training cohort, we found that RACO-1 expression is one of the important prognostic factors (Table 3). The multivariate cox regression analysis showed the same result in the training cohort. RACO-1 expression is one of the independent prognostic factors for overall survival of HBV-related HCC. Furthermore, in the validation cohort, the results from the univariate cox regression analysis associated with overall survival also indicated that RACO-1 expression is one of the important prognostic factors (Table S2). The multivariate cox regression analysis also showed that RACO-1 is one of the independent prognostic factors for overall survival of HBV-related HCC.

Correlations of RACO-1 expression with the disease-free survival of HBV-related HCC

Disease-free survival was detected in both training cohort and validation cohort. RACO-1 high expression group had worse disease-free survival in both training cohort and validation cohort ($P<0.001$; $P<0.001$) (Figure 2). In training cohort, it was found that RACO-1 expression is one of the

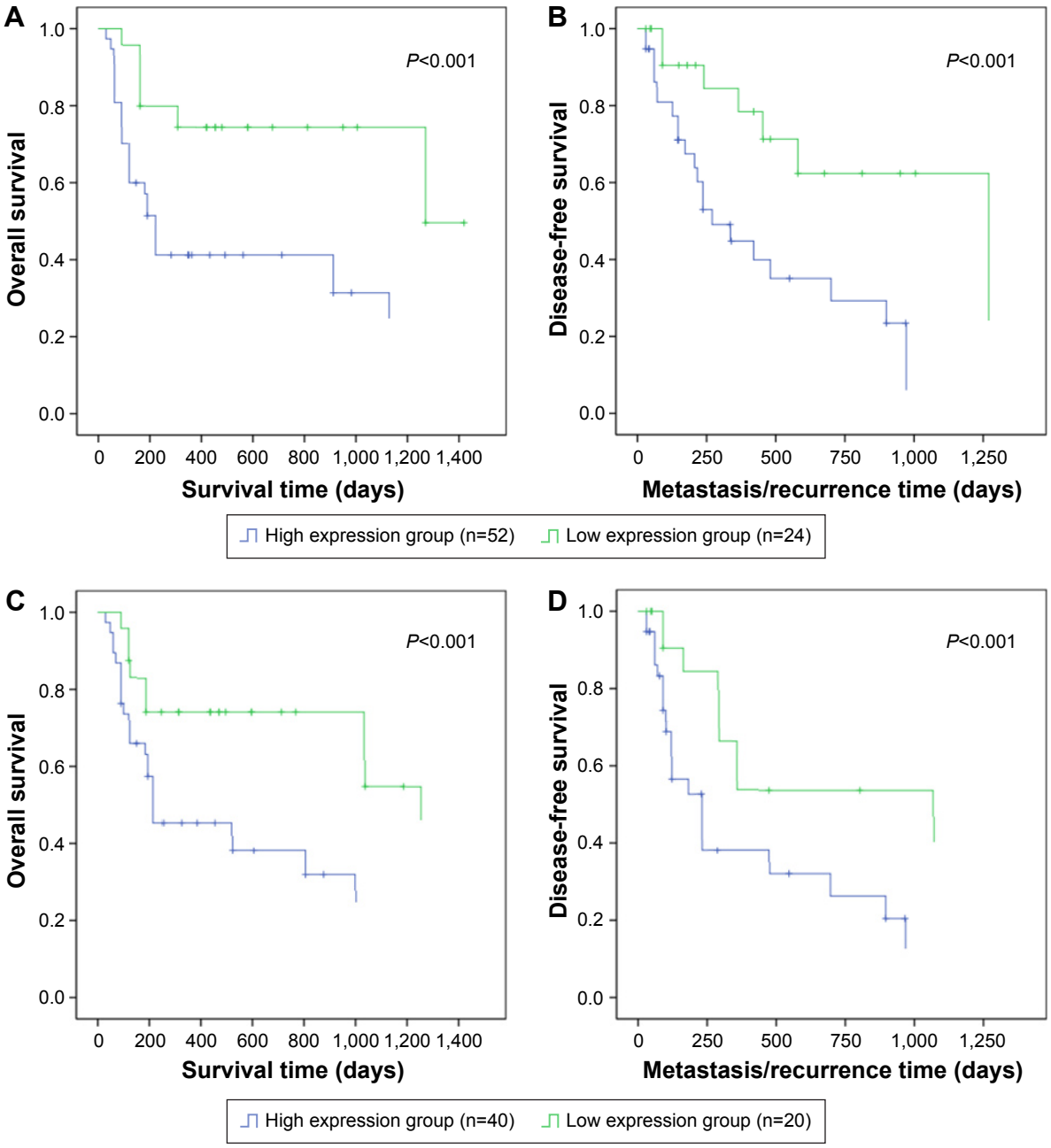

Figure 2 Correlations of RACO-1 expression with the overall survival and disease-free survival of HBV-related HCC.
Notes: (**A**) Overall survival was analyzed according to the expression of RACO-1 in training cohort ($P<0.001$). (**B**) Kaplan–Meier survival curves of disease-free survival in training cohort ($P<0.001$). (**C**) Overall survival was analyzed according to the expression of RACO-1 in validation cohort ($P<0.001$). (**D**) Kaplan–Meier survival curves of disease-free survival in validation cohort ($P<0.001$).
Abbreviations: RACO-1, RING domain AP-1 coactivator-1; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

important factors together with UICC/AJCC stage, and so on (Table 4). The multivariate cox regression analysis showed RACO-1 expression together with vascular invasion, UICC/AJCC stage and BCLC stage were independent prognostic factors for disease-free survival of HBV-related HCC. In validation cohort, the result is similar. RACO-1 expression together with UICC/AJCC stage and some other factors were important for prognosis (Table S3). The multivariate cox regression analysis showed that RACO-1 expression is one of the independent prognostic factors for disease-free survival of HBV-related HCC.

Discussion

This study aimed to investigate the effects of RACO-1 on HBV-related HCC. Interestingly, it was found that RACO-1 expression was significantly high in HBV-related HCC tissues

Table 3 Univariable and multivariable analysis of factors in training cohort associated with overall survival

Variables	Patients (n)	Univariable analysis		Multivariable analysis	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender					
Female	24	1			
Male	52	1.654 (0.689–6.426)	0.268	NA	NA
Age (years)					
≤60	60	1			
>60	16	1.125 (0.895–2.102)	0.468	NA	NA
Liver cirrhosis					
Absent	40	1			
Present	36	1.339 (0.902–3.125)	0.425	NA	NA
Tumor number					
Solitary	36	1			
Multiple	40	2.932 (0.856–3.258)	0.258	NA	NA
Tumor size					
≤5 cm	36	1			
>5 cm	40	1.568 (0.957–2.569)	0.368	NA	NA
Capsular formation					
Presence	35	1		1	
Absence	41	1.654 (1.181–3.544)	**0.039**	1.258 (1.258–3.985)	**0.041**
Vascular invasion					
Absent	30	1		1	
Present	46	2.802 (1.258–4.533)	**0.013**	2.698 (1.288–5.010)	**0.017**
Edmondson-Steiner grade					
Low grade (I–II)	45	1			
High grade (III–IV)	31	1.398 (0.862–2.358)	0.099	NA	NA
UICC/AJCC stage					
I	30	1		1	
II–III	46	2.685 (1.658–3.968)	**0.019**	2.125 (1.377–4.457)	**0.022**
BCLC stage					
0–A	37	1			
B–C	39	1.698 (1.212–3.682)	**0.037**	1.525 (1.137–3.878)	**0.046**
RACO-1 expression					
Low	24	1		1	
High	52	2.388 (1.529–6.587)	**0.019**	2.149 (1.689–5.776)	**0.022**

Note: $P<0.05$ was considered to be statistically significant; significant values are shown in bold.

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; UICC, International Union for Cancer Control; RACO-1, RING domain AP-1 coactivator-1; HR, hazard ratio; CI, confidence interval; NA, not applicable.

in both training cohort and validation cohort. RACO-1 high expression group had worse overall and disease-free survival rates than the low expression group in both training cohort and validation cohort. This indicates that RACO-1 may play an important role in the development of HBV-related HCC and could be a potential target for the treatment of HCC. However, we still do not know the mechanism. This part of work remains to be done in our further research.

Subsequently, the RACO-1 expression was positively related to liver cirrhosis in both training cohort and validation cohort. Most of HCC occurring in China would pass through three steps: HBV infection, liver cirrhosis, and HCC.[11–15] RACO-1 may play an important role in the development of HCC by regulating HBV-related cirrhosis.

Interestingly, it is found that RACO-1 expression also has a significant relationship with tumor number, capsular formation, and vascular invasion. Tumor number together with capsular formation and vascular invasion play an important role in the invasion and metastasis of HCC.[16–18] The present study also showed that RACO-1 expression together with vascular invasion, UICC/AJCC stage, and BCLC stage were independent prognostic factor for overall and disease-free survival of HBV-related HCC. Accordingly, it was speculated that RACO-1 may play an important role in the invasion and metastasis of HBV-related HCC. New research into the potential mechanism of RACO-1 in the invasion and metastasis of HCC has been undertaken.

Conclusion

In conclusion, it has been shown for the first time that increased expression of RACO-1 is related to worse overall survival in patients with HBV-related HCC. The study

Table 4 Univariable and multivariable analysis of factors in training cohort associated with disease-free survival

Variables	Patients (n)	Univariable analysis		Multivariable analysis	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender					
Female	24	1			
Male	52	1.258 (0.785–6.125)	0.339	NA	NA
Age (years)					
≤60	60	1			
>60	16	1.950 (0.818–4.58)	0.415	NA	NA
Liver cirrhosis					
Absent	40	1			
Present	36	1.308 (0.785–3.988)	0.329	NA	NA
Tumor number					
Solitary	36	1			
Multiple	40	2.058 (0.820–3.158)	0.208	NA	NA
Tumor size					
≤5 cm	36	1			
>5 cm	40	1.512 (0.688–2.155)	0.312	NA	NA
Capsular formation					
Presence	35	1		1	
Absence	41	1.398 (1.117–3.128)	**0.039**	1.258 (0.689–3.023)	0.089
Vascular invasion					
Absent	30	1		1	
Present	46	2.125 (1.286–3.786)	**0.018**	2.133 (1.365–4.013)	**0.026**
Edmondson-Steiner grade					
Low grade (I–II)	45	1			
High grade (III–IV)	31	1.188 (0.890–2.318)	0.123	NA	NA
UICC/AJCC stage					
I	30	1		1	
II–III	46	2.339 (1.205–3.683)	**0.025**	2.056 (1.237–4.384)	**0.037**
BCLC stage					
0–A	37	1			
B–C	39	1.288 (1.012–3.127)	**0.039**	1.512 (1.087–3.233)	**0.041**
RACO-1 expression					
Low	24	1		1	
High	52	2.072 (1.268–4.658)	**0.011**	2.233 (1.304–4.285)	**0.029**

Note: $P<0.05$ was considered to be statistically significant; significant values are shown in bold.
Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; UICC, International Union for Cancer Control; RACO-1, RING domain AP-1 coactivator-1; HR, hazard ratio; CI, confidence interval; NA, not applicable.

implicates RACO-1 as a novel prognostic marker and a potential therapeutic target for HBV-related HCC.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Correlations between RACO-1 expression and clinicopathologic variables of 60 cases of HBV-related HCC in validation cohort

Clinicopathologic variables	Patients (n)	RACO-1 expression levels		
		Low	High	*P*-value
Gender				
Female	19	9	10	0.133
Male	41	11	30	
Age (years)				
≤60	50	15	35	0.098
>60	10	5	5	
Liver cirrhosis				
Presence	31	6	25	**0.041**
Absence	29	14	15	
Tumor number				
Solitary	30	14	16	**0.046**
Multiple	30	6	24	
Tumor size				
≤5 cm	28	8	20	0.688
>5 cm	32	12	20	
Capsular formation				
Presence	29	14	15	**0.041**
Absence	31	6	25	
Vascular invasion				
Presence	26	3	23	**0.005**
Absence	34	17	17	
Edmondson-Steiner grade				
Low grade (I–II)	32	12	20	0.721
High grade (III–IV)	28	8	20	
UICC/AJCC stage				
I	25	12	13	**0.048**
II–III	35	8	27	
BCLC stage				
0–A	27	15	12	**0.003**
B–C	33	5	28	

Note: $P<0.05$ was considered to be statistically significant, and the significant values are shown in bold.

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; UICC, International Union for Cancer Control; RACO-1, RING domain AP-1 coactivator-1; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

Table S2 Univariable and multivariable analysis of factors in validation cohort associated with overall survival

Variables	Patients (n)	Univariable analysis		Multivariable analysis	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender					
Female	19	1			
Male	41	1.743 (0.646–4.703)	0.274	NA	NA
Age (years)					
≤60	50	1			
>60	10	1.264 (0.842–1.898)	0.418	NA	NA
Liver cirrhosis					
Absent	31	1			
Present	29	1.208 (0.367–3.976)	0.528	NA	NA
Tumor number					
Solitary	30	1			
Multiple	30	2.683 (0.732–4.156)	0.089	NA	NA
Tumor size					
≤5 cm	28	1			
>5 cm	32	1.874 (0.876–4.009)	0.427	NA	NA
Capsular formation					
Presence	29	1		1	
Absence	31	2.287 (1.732–3.020)	**0.048**	1.327 (0.931–1.891)	0.076
Vascular invasion					
Absent	26	1		1	
Present	34	2.359 (1.265–4.400)	**0.029**	2.539 (1.873–3.442)	**0.021**
Edmondson-Steiner grade					
Low grade (I–II)	32	1			
High grade (III–IV)	28	1.325 (0.856–1.663)	0.068	NA	NA
UICC/AJCC stage					
I	25	1		1	
II–III	35	2.464 (1.905–3.187)	**0.021**	2.643 (1.735–4.026)	**0.031**
BCLC stage					
0–A	27	1		1	
B–C	33	2.532 (1.456–4.403)	**0.039**	1.753 (1.231–2.496)	**0.041**
RACO-1 expression					
Low	20	1		1	
High	40	1.936 (1.533–4.238)	**0.031**	2.052 (1.435–2.934)	**0.029**

Note: $P<0.05$ was considered to be statistically significant; significant values are shown in bold.

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; UICC, International Union for Cancer Control; RACO-1, RING domain AP-1 coactivator-1; HR, hazard ratio; CI, confidence interval.

Table S3 Univariable and multivariable analysis of factors in validation cohort associated with disease-free survival

Variables	Patients (n)	Univariable analysis		Multivariable analysis	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender					
Female	19	1			
Male	41	1.328 (0.395–3.542)	0.329	NA	NA
Age (years)					
≤60	50	1			
>60	10	1.681 (0.787–2.252)	0.535	NA	NA
Liver cirrhosis					
Absent	31	1			
Present	29	1.268 (0.688–3.127)	0.631	NA	NA
Tumor number					
Solitary	30	1			
Multiple	30	2.281 (0.868–3.689)	0.535	NA	NA
Tumor size					
≤5 cm	28	1			
>5 cm	32	1.431 (0.627–2.758)	0.595	NA	NA
Capsular formation					
Presence	29	1		1	
Absence	31	1.658 (1.217–3.268)	**0.045**	1.212 (0.919–3.278)	0.078
Vascular invasion					
Absent	26	1		1	
Present	34	2.215 (1.364–3.182)	**0.008**	2.378 (1.468–4.271)	**0.013**
Edmondson-Steiner grade					
Low grade (I–II)	32	1			
High grade (III–IV)	28	1.188 (0.890–2.685)	0.275	NA	NA
UICC/AJCC stage					
I	25	1		1	
II–III	35	2.209 (1.338–3.817)	**0.017**	2.129 (1.210–4.644)	**0.039**
BCLC stage					
0–A	27	1			
B–C	33	1.367 (1.121–3.017)	**0.022**	1.415 (1.123–3.684)	**0.037**
RACO-1 expression					
Low	20	1		1	
High	40	2.175 (1.198–4.128)	**0.016**	2.156 (1.338–3.985)	**0.028**

Note: $P<0.05$ was considered to be statistically significant; significant values are shown in bold.

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; UICC, International Union for Cancer Control; RACO-1, RING domain AP-1 coactivator-1; HR, hazard ratio; CI, confidence interval.