

Clinicopathological and prognostic significance of Yes-associated protein expression in hepatocellular carcinoma and hepatic cholangiocarcinoma

Hao Wu¹ · Yan Liu² · Xiao-Wei Jiang³ · Wen-Fang Li⁴ · Gang Guo⁵ · Jian-Ping Gong¹ · Xiong Ding¹

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Abstract Hepatocellular carcinoma (HCC) and hepatic cholangiocarcinoma (CC) are the most aggressive malignancies with a poor prognosis in humans, and hepatic cholangiocarcinoma (CC) exhibits greater malignant behaviour. Yes-associated protein (YAP) is an important downstream target of the Hippo signalling pathway. As an oncogene, it plays a vital role in the occurrence and development of tumours. Our study focuses on the clinical significance of YAP protein expression in HCC and CC. Furthermore, we sought to explore the different survival rates between HCC and CC. A total of 137 patients with HCC and 122 with CC after resection were evaluated by immunohistochemistry for the expression of YAP. Our results showed that positive expression rates of YAP were more frequently noted in CC 67.2 % (82/122) than in HCC 56.9 % (78/137) ($P = 0.024$). High YAP expression in

HCC and CC was significantly associated with tumour size ($P < 0.001$ and $P = 0.019$, respectively), liver cirrhosis ($P = 0.002$ and $P = 0.009$, respectively), vascular invasion ($P = 0.047$ and $P = 0.018$, respectively), multiplicity ($P = 0.019$ and $P = 0.015$, respectively), and intrahepatic metastasis ($P = 0.015$ and $P = 0.047$, respectively). Importantly, recurrence-free survival and disease-specific survival rates were lower in CC with high YAP expression than in HCC with high YAP expression ($P < 0.001$ and $P < 0.001$, respectively). Overall, high YAP expression was more frequently found in CC than in HCC, and YAP overexpression was associated with poor survival rates in patients with HCC and CC. Targeting YAP treatment requires further prospective investigations in larger patient populations.

Keywords Hepatocellular carcinoma · Hepatic cholangiocarcinoma · Yes-associated protein(YAP) · Immunohistochemistry

Hao Wu and Yan Liu made equal contributions to this study.

Hao Wu and Yan Liu were the co-first authors.

✉ Xiong Ding
dinxiongqcq@163.com

¹ Department of Hepatobiliary Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China

² Department of Gastroenterology, The Fifth people's Hospital of Chengdu, Chengdu 611130, China

³ Department of General Surgery, The Fifth people's Hospital of Chongqing, Chongqing 400062, China

⁴ Department of General Surgery, The Taihe Hospital Affiliated to Hubei Medical University, Shiyan 442000, China

⁵ Laboratory of Pathology, West China Hospital of Sichuan University, Chengdu 610041, China

Introduction

Primary liver cancer (PLC) ranks as the second most common cancer worldwide and the leading cause of cancer-related deaths, approximately 745,000 deaths occur annually [1]. Hepatocellular carcinoma (HCC) and hepatic cholangiocarcinoma (CC) are two important pathological types of primary liver cancer. A previous study showed that CC has a poorer prognosis than HCC [2, 3]. There are many determining risk factors for PLC, such as age and sex (male), hepatitis B and C virus, exposure to toxins (aflatoxin), chronic alcohol abuse, and cirrhosis [4]. Despite the identification of many prognostic markers of PLC, such as alpha fetoprotein(AFP), vascular endothelial growth factor, and transforming growth factor β , in large-scale clinical and basic research projects, it is still

impossible unable to accurately predict the overall survival rates of patients with HCC [5, 6]. Therefore, it is very important and urgent to find an effective biomarker to identify PLC patients at high risk for recurrence or metastasis and provide personalized therapy according to the predicted risk of recurrence.

The Hippo pathway was originally identified in genetic mutant screens for tumour suppressors in *Drosophila melanogaster* [7, 8]. In mammals, the core molecules of the Hippo pathway include STE20-like protein kinase 1 (MST1 and MST2), SAV1 (Sav homologue), large tumour suppressor

1/2 LATS1/2 (Wts homologue), MOB1A/B (Mats homologue), and YAP (Yki homologue). As a transcription co-activator, YAP interacts with the PPXY motif-containing protein [9, 10]. If YAP is not inhibited by the Hippo pathway and remains in the nucleus, it will interplay with TEADs and activate the expression of several genes, such as CTGF, ErbB-4, and ITGB2 [8–13]. Therefore, Yes-associated protein (YAP) can influence tissue homeostasis, organ size, and cancer development and play a pivotal role in regulating cell proliferation. In recent years, functional studies have elucidated that YAP is an important oncogene and functions at a key

Table 1 Patient clinicopathological characteristics and YAP expression

Pathological Characteristics	Cases	HCC		P value	Cases	CC		P value
		YAP low expression (n = 59)	YAP high expression (n = 78)			YAP low expression (n = 40)	YAP high expression (n = 82)	
	137				122			
Age								
< 45	67	29	38	0.960	40	14	26	0.716
≥ 45	70	30	40		82	26	56	
Sex								
Female	20	9	11	0.857	22	7	15	0.915
Male	117	50	67		100	33	67	
Tumour size (cm)								
≤ 5	52	35	17	<0.001	46	21	25	0.019
> 5	85	24	61		76	19	57	
AFP (ng/ml)								
≤ 20	49	14	35	0.011	56	19	37	0.805
> 20	88	45	43		66	21	45	
Liver Cirrhosis								
Presence	106	53	53	0.002	71	30	41	0.009
Absence	31	6	25		51	10	41	
HBsAg								
Positive	130	56	74	0.991	94	31	63	0.934
Negative	7	3	4		28	9	19	
TNM stage								
I/II	37	18	19	0.422	34	10	24	0.622
III/IV	100	41	59		88	30	58	
Vascular invasion								
Presence	40	12	28	0.047	20	2	18	0.018
Absence	97	47	50		102	38	64	
Multiplicity								
Single	87	44	43	0.019	106	39	67	0.015
Multiple (≥2)	50	15	35		16	1	15	
Intrahepatic metastasis								
Presence	27	6	21	0.015	17	2	15	0.047
Absence	110	53	57		105	38	67	
Lymph node metastases								
Presence	25	10	15	0.732	37	17	20	0.041
Absence	112	49	63		85	23	62	

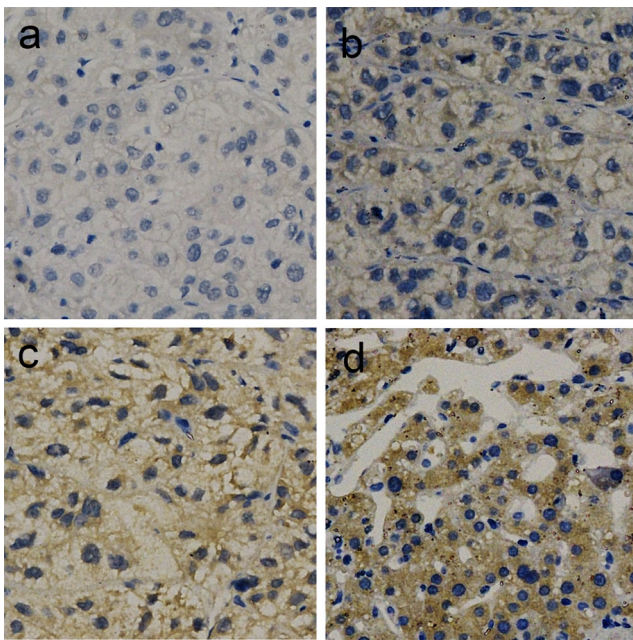


Fig. 1 Expression of YAP in HCC. **a** Negative expression. **b** Weak expression. **c** Moderate expression. **d** High expression. Original magnification, $\times 200$

crossroads of a complex network of cancer-causing signal pathways [14]. It has been demonstrated that the YAP gene exists in various human cancers, including pancreatic ductal adenocarcinoma, cutaneous melanoma, cervical cancer, and other malignant tumours [14–16].

However, YAP's clinical significance and expression patterns in HCC and CC have not been well explored. In the

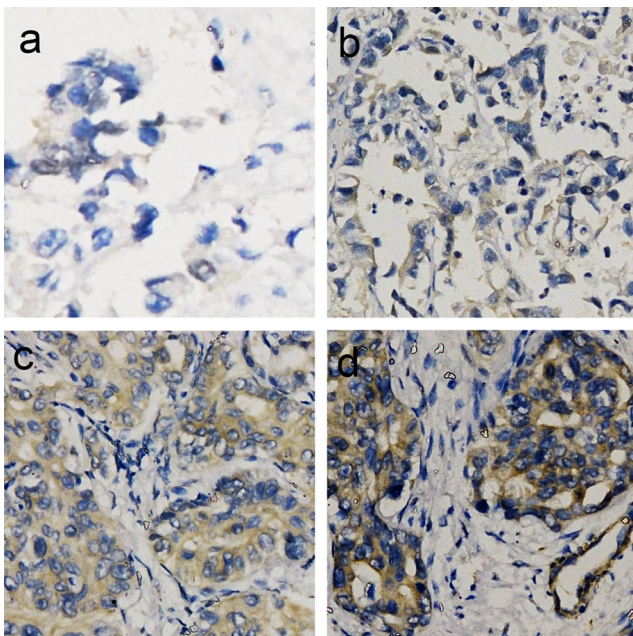


Fig. 2 Expression of YAP in CC. **a** Negative expression. **b** Weak expression. **c** Moderate expression. **d** High expression. Original magnification, $\times 200$

present study, we studied YAP expression in HCC and CC tissues through immunohistochemistry and explored the possibility of YAP as a prognostic factor for PLC.

Materials and methods

Patients and tissue specimens

In this study, all patient specimens were collected from July 2003 to July 2009 in the Second Hospital Affiliated to Chongqing Medical University. Eleven patients were excluded because their follow-up periods were interrupted for unclear reasons. In total, 259 PLC patients were analysed. The specimens included 137 HCC cases and 122 CC cases. All tissue samples were immediately processed after surgical removal, fixed with 4 % formalin (pH 7.0) and embedded in paraffin for no longer than 24 h. Finally, diagnoses of HCC and CC were identified histologically by two experienced pathologists in the Department of Pathology Archives of the Second Hospital Affiliated to Chongqing Medical University using haematoxylin and eosin (HE) staining. The complete clinical and prognostic data for each tumour tissue sample were recorded.

The diagnosis of PLC met the World Health Organization (WHO) criteria for the study of liver disease. The parameters of pathological analysis included age at diagnosis, sex, tumour size, distant metastases, cirrhosis, hepatitis B virus infection, and serum AFP levels (ng/ml), which were obtained from patient medical records. Before the deadline of May 2015, all patients with HCC and CC underwent follow-up. The follow-up time ranged from 6 to 142 months, and the median follow-up time was 40 months. The study was conducted in accordance with the protocol approved by the Declaration of Helsinki and the guidelines of the Ethics Review Committee of Second Hospital Affiliated with Chongqing Medical University.

Immunohistochemical staining

To determine YAP expression in HCC and CC, we used immunohistochemical staining to detect in the protein in paraffin-embedded sections (4 μm). Briefly, xylene was used to dewax the samples, which were rehydrated in a graded alcohol series. Antigen retrieval was performed in 10 mmol/L sodium citrate solution (PH 6.0) at 100 $^{\circ}\text{C}$ for 10 min, and the samples were cooled for 20 min. After rinsing in PBS (pH 7.2), endogenous peroxidase activity was inhibited with 3 % H_2O_2 for 15 min and closed with goat serum for 15 min at ambient temperature to avoid nonspecific protein binding. Thereafter, the slides were incubated with antihuman YAP rabbit monoclonal antibodies at a 1:100 dilution (rabbit monoclonal antibody,

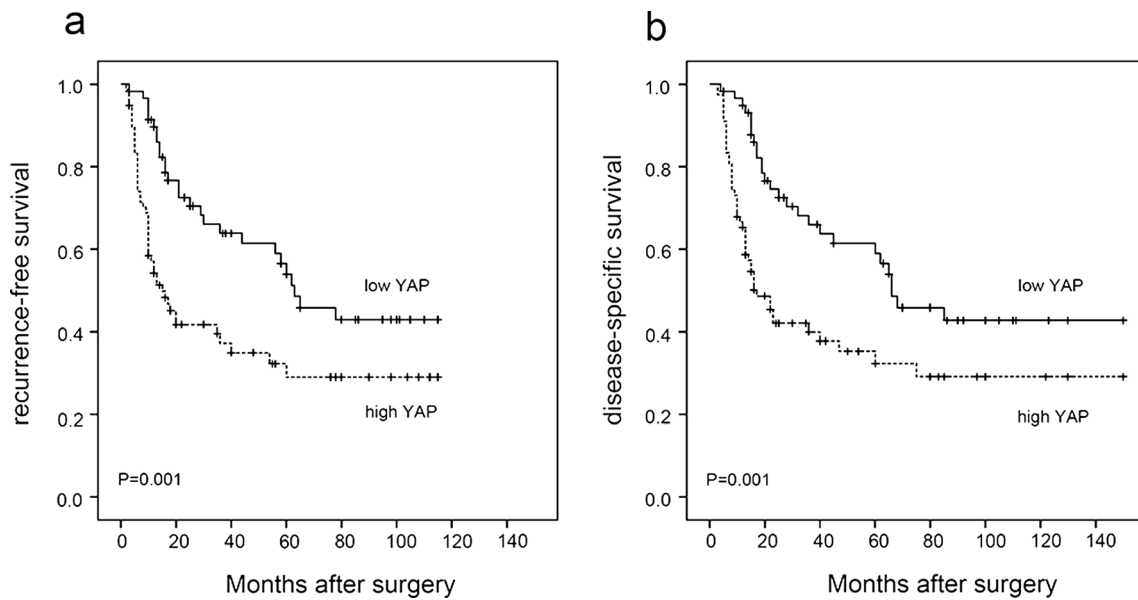


Fig. 3 **a** Kaplan–Meier survival curves of RFS in HCC patients according to YAP expression. **b** Kaplan–Meier survival curves of DSS in HCC patients according to YAP expression

EP1647Y, 1:100, Abcam Inc., Cambridge, CA, USA) at 4 °C overnight. Next, the samples were washed three times in PBS (pH 7.2), incubated with biotinylated secondary antibody at 37 °C for 30 min and hatched with avidin horseradish enzyme at 37 °C for 20 min. Colour development was carried out with DAB (3, 3- diaminobenzidine) and running water for 15 min. The slides were counterstained with 1 % Mayer's haematoxylin. A gradient ethanol series was used for dehydration, and the samples were sealed with neutral gum. Finally, the slides were cleaned and coverslipped.

Scoring systems for immunohistochemical staining

To evaluate the expression of YAP, all slides were assessed independently by two experienced pathologists with minimal interobserver variability, and we used a semi-quantitative assessment method of scoring. The scoring parameters included staining intensity (range 0–3: 0, negative; 1, weak; 2, moderate; and 3, strong) and the percentage of positive cells (range 0–4: 0, negative or <5 %; 1, 6 %–25 %; 2, 26 %–50 %; 3, 51 %–75 %; and 4, 76 %–100 %). We adopted the percentage of positive cells and the intensity to determine the final

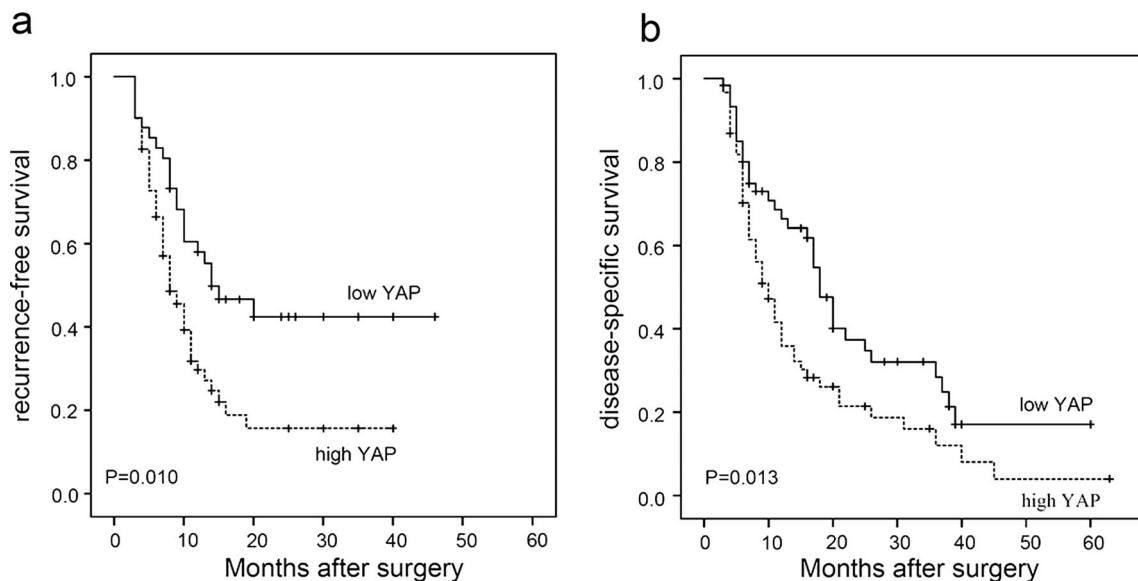


Fig. 4 **a** Kaplan–Meier survival curves of RFS in CC patients according to YAP expression. **b** Kaplan–Meier survival curves of DSS in CC patients according to YAP expression

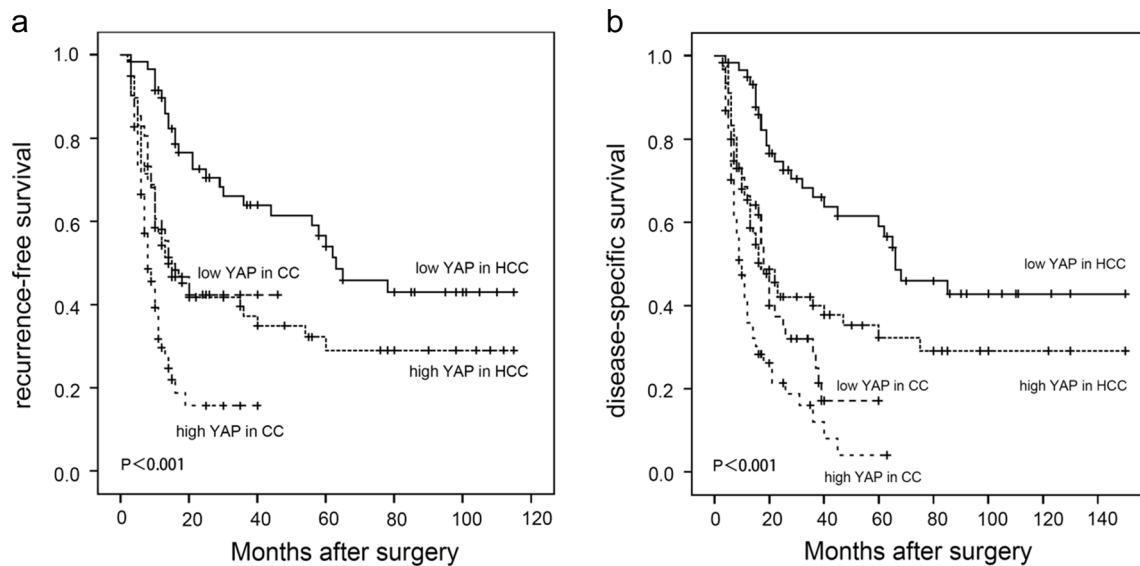


Fig. 5 **a** Kaplan–Meier survival curves of RFS between HCC and CC patients according to YAP expression. **b** Kaplan–Meier survival curves of DSS between HCC and CC patients according to YAP expression

staining scores. Slides with a total score <4 were defined as having low YAP expression, while slides with a score ≥ 4 were defined as having high YAP expression.

Statistical analysis

All data were analysed using SPSS 17.0 software (version 17.0; SPSS Inc., Chicago, IL, USA). The relationships between YAP expression and clinicopathological parameters in HCC and CC were analysed using Fisher's exact test or a χ^2 test. Tumour recurrence-free survival (RFS) was recorded as the time from liver tumour resection as clean as possible to liver tumour recurrence. Disease-specific survival (DSS) was recorded as the time from cancer diagnosis to death from cancer or the follow-up deadline. The Kaplan–Meier method was used to assess RFS and DSS, and the log-rank test was used to analyse the differences between the curves. The prognostic meaning of YAP expression in HCC and CC was calculated by univariate and multivariate Cox regression analysis. The threshold for statistical significance was $P < 0.05$.

Results

Clinical pathological features of patients and the expression of YAP

At the time of this analysis, 259 patients are with PLC, including 137 HCC and 122 CC. In HCC, there were 117 male patients (85.4 %) and 20 female patients (14.6 %). Sixty-seven patients < 45 , 70 patients is ≥ 45 years old. Eight-five patients have a size over 5 cm while the others $52 \leq 5$ cm. In CC, 100 patients is male (82.0 %), 22 patients of female

(18.0 %), 40 patients < 45 , 82 patients is ≥ 45 . The details of clinicopathological characteristics of HCC and CC are summarized in (Table 1). YAP expression mainly showed an incomplete dyeing in nuclear and cytoplasmic compartments in HCC and CC tissues. As above mentioned, YAP expression levels were graded as negative/low and high. The result indicated that high YAP expression was observed in HCC is 56.9 % (78/137) (Fig. 1) and CC is 67.2 % (82/122) (Fig. 2).

Relationship between YAP expression and the clinicopathologic characteristics of patients with HCC and CC

In our study, we analysed the association between YAP expression and the clinicopathologic characteristics of patients with HCC and CC by χ^2 (Table 1). High YAP expression in both HCC and CC had positive correlations with tumour size ($P < 0.001$ and $P = 0.019$, respectively), liver cirrhosis ($P = 0.002$ and $P = 0.009$, respectively), vascular invasion ($P = 0.047$ and $P = 0.018$, respectively), multiplicity ($P = 0.019$ and $P = 0.015$, respectively), and intrahepatic metastasis ($P = 0.015$ and $P = 0.047$, respectively). However, YAP status was not significantly associated with age ($P = 0.960$ and $P = 0.716$), patient gender ($P = 0.857$ and $P = 0.915$, respectively), HBV status ($P = 0.991$ and $P = 0.934$, respectively), and TNM stage ($P = 0.422$ and $P = 0.622$, respectively).

Association of YAP expression with recurrence-free survival in HCC and CC

The 1-year RFS rates in the low YAP expression groups with HCC and CC were 89.6 % and 63.9 %, respectively, and the 3-

Table 2 Multivariate analysis of different prognostic variables of RFS in HCC by the Cox proportional hazard model

Variables	Univariate analysis			Multivariate analysis model 1			Multivariate analysis model 2			Multivariate analysis model 3		
	HR	95 % CI	P value	HR	95 % CI	P value	HR	95 % CI	P value	HR	95 % CI	P value
Sex	0.841	0.406–1.745	0.643									
Age	0.884	0.554–1.411	0.606									
AFP (mg/ml)	1.065	0.638–1.777	0.810	1.043	0.652–1.667	0.861	1.233	0.764–1.990	0.391	1.041	0.637–1.699	0.874
HBS-Ag	2.493	0.578–10.752	0.221	3.060	0.742–12.626	0.122	3.135	0.762–12.892	0.113	2.604	0.619–10.956	0.192
Liver Cirrhosis	0.851	0.480–1.508	0.580	0.972	0.570–1.658	0.918	1.050	0.616–1.790	0.858	0.838	0.488–1.440	0.523
Tumour size (cm)	1.174	0.610–2.260	0.630									
Multiplicity	3.848	1.999–7.408	<0.001									
Intrahepatic	2.844	1.444–5.600	0.003									
Metastasis												
TNM stage	1.198	0.570–2.516	0.634									
Vascular invasion	3.295	1.815–5.982	<0.001									
Lymph node	1.632	0.721–2.955	0.502									
Metastases												
YAP expression	2.114	1.239–3.608	0.006				2.303	1.412–3.755	0.001	2.055	1.212–3.484	0.008

HR hazard rate, CI confidence interval

Vascular invasion is defined as microvascular invasion and liver veins invasion, YAP expression (high/low)

P < 0.05

year RFS rates were 55.5 % and 41.2 %, respectively. However, the 1-year RFS rates in the high YAP expression groups with HCC and CC were 54.2 % and 37.3 %, respectively. The 3-year RFS rates were 31.3 % and 17.4 %, respectively. The results of the log-rank test showed statistically significant differences in RFS between the two groups (HCC, $P = 0.001$, CC: $P = 0.010$) (Fig. 3a, Fig. 4a).

When HCC and CC were split into two groups according to YAP expression, the recurrence-free survival rate was relatively lower in the high YAP expression group with CC than in the high YAP expression group with HCC ($P < 0.001$) (Fig. 5a).

Univariate analysis revealed that multiplicity (HR = 3.848, $P < 0.001$), intrahepatic metastasis (HR = 2.844, $P = 0.003$), vascular invasion (HR = 3.295, $P < 0.001$), and YAP expression (HR = 2.114, $P = 0.006$) were significantly associated with RFS in HCC (Table 2). Liver cirrhosis (HR = 1.809, $P = 0.018$), tumour size (HR = 1.919, $P = 0.032$), multiplicity (HR = 2.545, $P = 0.041$), intrahepatic metastasis (HR = 2.534, $P = 0.028$), vascular invasion (HR = 2.365, $P = 0.010$), lymph node metastases (HR = 2.978, $P = 0.002$), and YAP expression (HR = 1.800, $P = 0.033$) were adverse prognostic factors that affected RFS in CC after resection (Table 3).

In multivariate analysis, alpha fetoprotein (AFP), liver cirrhosis, and hepatitis B virus infection were found to be essential parameters. Therefore, we used three different models for multivariate analysis (Table 2, Table 3). For model 1, the parameters included alpha fetoprotein (AFP), liver cirrhosis, and hepatitis B virus infection, and the results showed that alpha fetoprotein (AFP), liver cirrhosis, and hepatitis B virus infection were not prognostic factors for RFS in patients with HCC. Liver cirrhosis (HR = 1.581, $P = 0.048$) was an independent prognostic factor for RFS in patients with CC. For model 2, the parameters included alpha fetoprotein (AFP), liver cirrhosis, hepatitis B virus infection, and YAP expression, and the results indicated that YAP expression (HR = 2.303, $P = 0.001$) was an independent prognostic factor for RFS in patients with HCC. Liver cirrhosis (HR = 1.679, $P = 0.026$) and YAP expression (HR = 2.161, $P = 0.003$) were independent prognostic factors for RFS in patients with CC. Finally, alpha fetoprotein (AFP), liver cirrhosis, hepatitis B virus infection, YAP expression, intrahepatic metastasis, multiplicity, lymph node metastases, and vascular invasion were essential parameters in model 3. Multivariable analysis indicated that multiplicity (HR = 3.893, $P < 0.001$), intrahepatic metastasis (HR = 2.994, $P = 0.001$), vascular invasion (HR = 3.083, $P < 0.001$), and YAP expression (HR = 2.055, $P = 0.008$) were independent predictors for RFS in patients with HCC; liver cirrhosis (HR = 1.750, $P = 0.018$), multiplicity (HR = 2.460, $P = 0.041$), vascular invasion (HR = 2.148, $P = 0.021$), lymph node metastases (HR = 4.021, $P < 0.001$), and YAP expression (HR = 1.824, $P = 0.026$) were independent predictors for RFS in patients with CC.

Table 3 Multivariate analysis of different prognostic variables of RFS in CC by the Cox proportional hazard model

Variables	Univariate analysis			Multivariate analysis model 1			Multivariate analysis model 2			Multivariate analysis model 3		
	HR	95 % CI	<i>P</i> value	HR	95 % CI	<i>P</i> value	HR	95 % CI	<i>P</i> value	HR	95 % CI	<i>P</i> value
Sex	0.981	0.534–1.801	0.950									
Age	1.048	0.632–1.738	0.855									
AFP (ng/ml)	0.811	0.510–1.290	0.376	0.848	0.541–1.230	0.473	0.838	0.535–1.314	0.922	0.753	0.476–1.192	0.277
HBsAg	0.707	0.399–1.253	0.235	0.940	0.560–1.579	0.815	0.922	0.549–1.551	0.761	0.930	0.545–1.586	0.790
Liver Cirrhosis	1.809	1.108–2.953	0.018	1.581	1.003–2.492	0.048	1.679	1.062–2.653	0.026	1.750	1.101–2.781	0.018
Tumour size (cm)	1.919	1.057–3.484	0.032									
Multiplicity	2.545	1.037–6.248	0.041							2.460	1.039–5.824	0.041
Intrahepatic	2.534	1.107–5.799	0.028							1.998	0.931–4.287	0.075
Metastasis												
TNM stage	0.722	0.386–1.350	0.308									
Vascular Invasion	2.365	1.228–4.558	0.010							2.148	1.123–4.106	0.021
Lymph node	2.978	1.532–5.521	0.002							4.021	2.051–7.896	<0.001
Metastases												
YAP expression	1.800	1.050–3.086	0.033				2.161	1.300–3.593	0.003	1.824	1.074–3.099	0.026

HR hazard rate, CI confidence interval

Vascular invasion is defined as microvascular invasion and liver veins invasion, YAP expression (high/low)

$P < 0.05$

Association of YAP expression with disease-specific survival in HCC and CC

The 1-year DSS rates in the low YAP expression group with HCC and CC were 94.9 % and 60.4 %, respectively, and the 3-year DSS rates were 66.0 % and 44.7 %, respectively. However, the 1-year DSS rates in the high YAP expression group with HCC and CC were 65.3 % and 32.1 %, respectively. The 3-year DSS rates were 40.0 % and 18.2 %, respectively. In the log-rank test, the results were significantly different between the two groups for DSS (HCC: $P = 0.001$, CC: $P = 0.013$) (Fig. 3b, Fig. 4b).

When HCC and CC were split into two groups according to YAP expression, the disease-specific survival rate was relatively lower in the high YAP expression group with CC than in the high YAP expression group with HCC ($P < 0.001$) (Fig. 5b).

Univariate analysis revealed that multiplicity (HR = 4.045, $P < 0.001$), intrahepatic metastasis (HR = 3.050, $P = 0.001$), vascular invasion (HR = 3.281, $P < 0.001$), and YAP expression (HR = 1.902, $P = 0.017$) were significantly associated with DSS in HCC (Table 4). Liver cirrhosis (HR = 1.662, $P = 0.034$), tumour size (HR = 1.964, $P = 0.022$), multiplicity (HR = 2.478, $P = 0.037$), intrahepatic metastasis (HR = 2.323, $P = 0.024$), vascular invasion (HR = 2.265, $P = 0.015$), lymph node metastases (HR = 2.845, $P = 0.005$), and YAP expression (HR = 1.998, $P = 0.010$) were adverse prognostic factors affecting DSS in CC after resection (Table 5).

In multivariate analysis, we still used three different Cox models to analyse the significance of YAP for DSS in HCC and CC (Table 4, Table 5). For model 1, the parameters included alpha fetoprotein (AFP), liver cirrhosis, and hepatitis B virus infection, and the results showed that alpha fetoprotein (AFP), liver cirrhosis, and hepatitis B virus infection were not prognostic factors for DSS in patients with HCC. Liver cirrhosis (HR = 1.576, $P = 0.049$) was an independent prognostic factor for DSS in patients with CC. For model 2, the parameters included alpha fetoprotein (AFP), liver cirrhosis, hepatitis B virus infection, and YAP expression, and the results indicated that YAP expression (HR = 2.236, $P = 0.001$) was an independent prognostic factor for DSS in patients with HCC. Liver cirrhosis (HR = 1.677, $P = 0.027$) and YAP expression (HR = 2.134, $P = 0.003$) were independent prognostic factors for DSS in patients with CC. Finally, alpha fetoprotein (AFP), liver cirrhosis, hepatitis B virus infection, intrahepatic metastasis, multiplicity, vascular invasion, lymph node metastases, and YAP expression were essential model 3 parameters. Multivariable analysis indicated that multiplicity (HR = 4.042, $P < 0.001$), intrahepatic metastasis (HR = 3.179, $P = 0.001$), vascular invasion (HR = 3.089, $P < 0.001$), and YAP expression (HR = 1.823, $P = 0.024$) were independent predictors of DSS in patients with HCC; liver cirrhosis (HR = 1.657, $P = 0.031$), multiplicity (HR = 2.326, P

Table 5 Multivariate analysis of different prognostic variables of DSS in CC by the Cox proportional hazard model

Variables	Univariate analysis			Multivariate analysis model 1			Multivariate analysis model 2			Multivariate analysis model 3		
	HR	95 % CI	P value	HR	95 % CI	P value	HR	95 % CI	P value	HR	95 % CI	P value
Sex	0.843	0.467–1.522	0.571									
Age	1.077	0.647–1.795	0.775									
AFP (ng/ml)	0.800	0.500–1.278	0.350	0.871	0.556–1.365	0.547	0.862	0.550–1.350	0.516	0.742	0.467–1.179	0.206
HBsAg:	0.883	0.514–1.517	0.653	0.910	0.542–1.528	0.721	0.881	0.524–1.481	0.633	0.970	0.570–1.649	0.910
Liver Cirrhosis	1.662	1.039–2.659	0.034	1.576	1.000–2.483	0.049	1.677	1.061–2.651	0.027	1.657	1.047–2.622	0.031
Tumour size (cm)	1.964	1.102–3.502	0.022									
Multiplicity	2.478	1.055–5.824	0.037							2.326	1.017–5.319	0.046
Intrahepatic	2.323	1.119–4.819	0.024							2.135	1.054–4.321	0.035
Metastasis												
TNM stage	0.759	0.414–1.391	0.372									
Vascular Invasion	2.265	1.173–4.380	0.015							2.247	1.173–4.305	0.015
Lymph node	2.845	2.568–4.462	0.005							3.106	1.564–4.691	0.026
Metastases												
YAP expression	1.998	1.177–3.393	0.010				2.134	1.288–3.536	0.003	1.897	1.124–3.200	0.016

HR hazard rate, CI confidence interval

Vascular invasion is defined as microvascular invasion and liver veins invasion, YAP expression (high/low)

$P < 0.05$

expression rates were 56.9 % and 67.2 %, respectively. Moreover, we found that high YAP expression in HCC and CC was significantly associated with the tumour size, AFP, liver cirrhosis, vascular invasion, multiplicity, intrahepatic metastasis and lymph node metastases; however, there was no clear relationship between YAP expression, age, patient gender, HBV status, and TNM stage. According to the survival analysis of YAP expression in HCC and CC, we found that the high YAP expression group had lower 1-year and 3-year RFS and DSS rates. Further survival analysis showed that CC with high YAP expression had lower 1-year and 3-year RFS and DSS rates than HCC with high YAP expression. These data strongly imply that YAP participates in tumour progression in HCC and CC. High YAP expression was closely correlated with poor patient prognosis in HCC and CC. This may facilitate targeted therapy for elevated YAP in HCC and CC.

The crucial characteristic of malignant tumours is unrestricted cell division, which leads to cancer progression and tight connections with genetic alterations linked to the regulation of proliferation, the cell cycle, apoptosis, and genetic stability [26]. YAP is a potent oncogenic transcriptional co-activator that is opposed by the Hippo tumour suppressor pathway [27]. As a downstream effector gene of the Hippo pathway, in collaboration with the TEAD transcription factor, the overexpression of YAP represents the aberrant activation a group of target genes (CTGF, CCND1, ITGB2, and BCL2L1) responsible for cell proliferation, anti-apoptosis, survival, and migration. In

particular, TEAD1 and TEAD4 are most often associated with proliferation and cancer development [27–30]. YAP promoted the transcription of many genes that are normally associated with hepatocyte proliferation, such as Ki67, c-myc, SOX4, H19, and AFP. In addition, it also induced the expression of several negative regulators of apoptosis, such as the IAP family members BIRC5/survivin and BIRC2/cIAP1 and the BCL2 family gene MCL1 [27]. Remarkably, our results show that patients with high YAP expression exhibit increases in tumour volume, intrahepatic metastasis, and vascular invasion. In particular, CC, which is more malignant and invasive, exhibits high YAP expression and lower survival rates than HCC, which implies that YAP contributes to tumour cell proliferation and the metastasis of HCC and CC by promoting cell cycle progression, anti-apoptosis, and migration.

In conclusion, this is the first study of YAP expression in the comparison of HCC and CC. Our results suggested that YAP was more highly expressed in CC than in HCC. Moreover, YAP expression increased in HCC and CC tissues, which was strongly correlated with tumour size, liver cirrhosis, vascular invasion, multiplicity and lymph node metastases. Survival analysis results demonstrated that positive YAP expression was a poor prognostic factor for RFS and DSS. Additionally, high YAP expression in CC represents poorer survival than that in HCC. Our study showed that YAP increased the degree of malignancy and decreased patient survival. Of course, our research is limited, further studies should explore the specific mechanism of YAP's influence on the occurrence of PLC and the development of targeted therapy for PLC.

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Compliance with ethical standards

Conflicts of interest None

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