Table 3 Factors associated with the time between HCV infection and the development of HCC ($n = 166$)

Variable	Median	1st–3rd Quartile	*P*-value	
			Univariate	Multivariate†
PNPLA3 genotype			0.47	0.008
GG ($n = 40$)	39.96	33.43–45.84		
CC/CG ($n = 126$)	40.85	33.52–46.76		
Sex			0.04	<0.001
Male	38.54	31.95–44.93		
Female	42.45	35.67–47.25		
BMI			0.75	–
>25 kg/m^2	37.94	32.91–45.60		
≤25 kg/m^2	40.85	33.70–46.87		
Alcohol consumption			0.26	–
>50 g/day	40.13	28.55–45.33		
≤50 g/day	40.87	33.79–46.76		
HCV genotype			0.09	–
Genotype 1	41.46	34.20–46.92		
Genotype 2	37.80	28.70–45.44		
Viral load			0.008	0.11
High‡	41.81	35.18–48.28		
Low§	38.53	30.79–45.12		

†Stepwise regression analysis of age at onset of hepatocellular carcinoma (HCC; the dependent variable) using *PNPLA3* genotype, sex, body mass index (BMI), alcohol consumption, hepatitis C virus (HCV) genotype, HCV viral load and the age at blood transfusion as independent variables.
‡At or above the median value.
§Below the median value.

histological findings for CC, CG and GG genotypes. The increment in the G allele was significantly associated with a higher prevalence of steatosis, as demonstrated by the Cochran–Armitage trend test (CC 13.11% vs CG 28.45% vs GG 40.00%, respectively; $P = 0.004$).

DISCUSSION

IN THIS STUDY, we found that the risk allele of *PNPLA3*, which was strongly correlated with significant liver steatosis, also may be a risk factor for hepatocarcinogenesis in CHC patients. Median age at onset of HCC was significantly younger ($P < 0.001$), and the median interval between blood transfusion and the onset of HCC was significantly shorter ($P = 0.008$) in patients with the rs738409 GG genotype than in those with non-GG genotypes after adjustment for sex, BMI, alcohol consumption, HCV genotype and HCV viral load.

Earlier age at HCC onset or shorter time between HCV infection and the development of HCC in the GG genotype was thought to be caused by the acceleration of liver fibrosis. The patients with the rs738409 GG genotype may reach the stage of advanced cirrhosis and develop HCC in their early age or shorter time after HCV infection. Previous studies reported hepatic steatosis as a risk factor for progressed fibrosis and HCC in CHC patients.[4,42] The *PNPLA3* polymorphism was originally reported as a determinant of liver fat content,[23] and a significant association between rs738409 SNP and histological evidence of steatosis (≥5%) was identified in the present study. The *PNPLA3* polymorphism was thought to affect the susceptibility to HCC in CHC patients via alteration of lipid accumulation in the liver.

Although this was not confirmed histologically, the *PNPLA3* GG genotype was also significantly associated with higher AST level and tended to be associated with a higher prevalence of progressed histological fibrosis compared to the non-GG genotypes (74.0% vs 60.5%, $P = 0.11$) at the time of HCC onset. Moreover, the GG genotype was associated with a lower prothrombin time, which suggests depressed liver function. Increased lipid accumulation in the *PNPLA3* GG genotype may enhance the risks of hepatic inflammation, fibrosis and impairment of liver function in CHC patients.

Table 4 Associations between *PNPLA3* genotype and clinical findings at the time of HCC onset ($n = 358$)

Variable	Median/number (1st–3rd quartile)		*P*-values	
	GG	Non-GG	*P*-value	Adjusted *P*-value†
Platelet count ($\times 10^4/\mu L$)	10.05 (7.73–12.78)	10.30 (7.68–13.35)	0.53	–
AST (IU/L)	69.5 (49.0–88.5)	59.0 (43.0–83.5)	0.048	0.02§
ALT (IU/L)	59.0 (42.0–93.3)	55.0 (37.0–86.3)	0.29	–
TB (mg/dL)	0.8 (0.6–1.1)	0.8 (0.6–1.1)	0.85	–
Albumin (g/dL)	3.7 (3.3–3.9)	3.7 (3.4–3.9)	0.41	–
PT (%)	73.0 (67.3–79.0)	78.0 (69.0–90.0)	0.004	0.008§
Viral load (log IU/mL)	4.73 (4.51–4.94)	4.75 (4.35–5.20)	0.90	–
LDL cholesterol (mg/dL)	77.2 (63.1–90.3)	74.7 (57.6–93.6)	0.77	–
Triglyceride (mg/dL)	82.0 (59.0–108.0)	87.0 (66.0–114.0)	0.32	–
Fasting plasma glucose (mg/dL)	100.0 (88.5–116.0)	103.0 (91.3–121.8)	0.20	–
Plasma insulin (μg/mL)	12.0 (8.0–18.0)	12.0 (9.0–19.0)	0.67	–
Histological findings ($n = 235$)				
Fibrosis				
F0–3	13	73	0.11	–
F4	37	112		
Activity				
A0–1	30	112	0.93	–
A2–3	20	73		
Steatosis‡				
<5%	30	144	0.02	0.01¶
≥5%	20	41		

†Adjusted for sex, BMI and alcohol consumption (independent variables). The dependent variables of each *P*-value are the items in the leftmost fields of the corresponding row (e.g. platelet count, AST, ALT).
‡Odds ratio (95% CI) for the GG allele was 2.43 (1.24–4.77), and the 95% CI of each proportion is shown in parentheses for this outcome.
§*P*-value by stepwise regression analysis.
¶*P*-value by stepwise logistic regression analysis.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HCC, hepatocellular carcinoma; LDL, low-density lipoprotein; PT, prothrombin time; TB, total bilirubin.

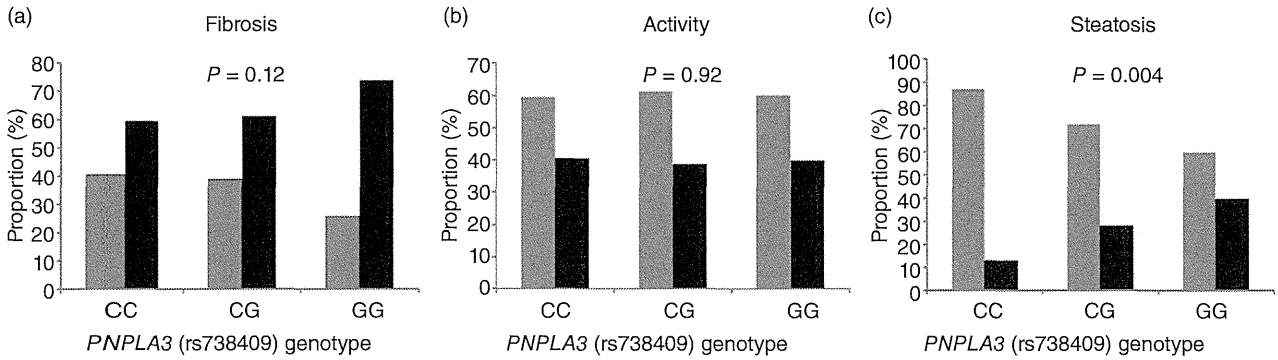

Figure 2 Bar plot: prevalence of fibrosis (F1–3 vs F4, a), necroinflammation (A1 vs A2–3, b) and steatosis (<5% vs ≥5%, c) in 235 patients with chronic hepatitis C. The proportions are shown on the Y axis. *P*-values of the frequency distributions are shown (Cochran–Armitage trend test). ▒, F1–3; ■, F4; ▒, A1; ■, A2–3; ▒, <5%; ■, ≥5%.

One study investigated the impact of the *PNPLA3* polymorphism on liver steatosis and fibrosis in CHC patients.[36] In this study, the cumulative incidence of HCC during the follow-up period was significantly higher in patients with the GG genotype.[36] The *PNPLA3* polymorphism is also associated with susceptibility to HCC in patients with other causes of hepatitis.[34,43] Our data suggest that the *PNPLA3* rs738409 polymorphism may provide important information that will assist identification of patients at particular risk for HCC.

In the present study, early age at onset of HCC was also independently associated with male sex and higher BMI, and the median interval between blood transfusion and the onset of HCC was significantly associated with male sex. These results are consistent with previous reports of male sex and higher BMI as independent risk factors for HCC development in CHC patients.[9,44,45]

A limitation of the present study is its retrospective design. The histology samples at the time of initial treatment were obtained via ultrasound-guided aspiration at the time of percutaneous tumor ablation or surgical resection. To minimize the risk of bleeding, ultrasound-guided aspiration was not performed for patients with a platelet count of less than 6 ($\times 10^4/\mu L$). Therefore, the histological samples were collected from a biased group of patients. Another limitation is the cross-sectional study design and the lack of controls without HCC. We are unable to confirm whether the age at onset of HCC (primary outcome of the present study) is an adequate indicator of susceptibility to HCC from the current study alone. Further prospective study is needed to validate the current results.

In conclusion, the *PNPLA3* rs738409 C>G polymorphism may play a significant role in hepatocarcinogenesis in CHC patients. Thus, this genetic factor should be taken into consideration when determining a treatment strategy intended to prevent the future development of HCC in CHC patients.

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Research Article

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Surgical treatment of hepatocellular carcinoma associated with hepatic vein tumor thrombosis

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See Editorial, pages 462–463

Background & Aims: Presence of hepatic vein tumor thrombosis (HVTT) in patients with hepatocellular carcinoma (HCC) is regarded as signaling an extremely poor prognosis. However, little is known about the prognostic impact of surgical treatment for HVTT.

Methods: Our database of surgical resection for HCC between October 1994 and December 2011 in a tertiary care Japanese hospital was retrospectively analysed. We statistically compared the patient characteristics and surgical outcomes in HCC patients with tumor thrombosis in a peripheral hepatic vein, including microscopic invasion (pHVTT), tumor thrombosis in a major hepatic vein (mHVTT), and tumor thrombosis of the inferior vena cava (IVCTT). Among 1525 hepatic resections, 153 cases of pHVTT, 21 cases of mHVTT, and 13 cases of IVCTT were identified.

Results: The median survival time (MST) in the pHVTT and mHVTT groups was 5.27 and 3.95 years, respectively ($p = 0.77$), and the median time to recurrence (TTR) was 1.06 and 0.41 years, respectively ($p = 0.74$). On the other hand, the MST and TTR in the patient group with IVCTT were 1.39 years and 0.25 year respectively; furthermore, the MST of Child-Pugh class B patients was significantly worse (2.39 *vs.* 0.44 years, $p = 0.0001$). Multivariate analyses revealed IVCTT (risk ratio [RR] 2.54, $p = 0.024$) and R 1/2 resection (RR 2.08, $p = 0.017$) as risk factors for the overall survival.

Conclusions: Hepatic resection provided acceptable outcomes in HCC patients with mHVTT or pHVTT when R0 resection was feasible. Resection of HCC may be attempted even in patients with IVCTT, in the presence of good liver function.

Keywords: Hepatocellular carcinoma; Hepatic vein tumor thrombosis; Tumor thrombosis of the inferior vena cava; Hepatic resection.

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Abbreviations: HCC, hepatocellular carcinoma; PVTT, portal vein tumor thrombosis; HVTT, hepatic vein tumor thrombosis; IVC, inferior vena cava; AASLD/BCLC, American Association for the Study of the Liver Diseases/Barcelona Clinic for Liver Cancer; MST, median survival time; pHVTT, tumor thrombosis in a peripheral hepatic vein; mHVTT, tumor thrombosis in a major hepatic vein; IVCTT, tumor thrombosis in the inferior vena cava; TACE, transcatheter arterial chemoembolization; IQR, interquartile range; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; TTR, time to recurrence; RR, risk ratio; CI, confidence interval; EMT, epithelial-mesenchymal transition.

Introduction

Patients with advanced hepatocellular carcinoma (HCC) showing macrovascular invasion, i.e., tumor thrombosis in the portal vein (PVTT) or hepatic vein (HVTT), have been reported to carry a poor prognosis [1]. PVTT can cause intrahepatic metastasis and increased portal pressure leading to esophagogastric variceal hemorrhage. HVTT can extend into the inferior vena cava (IVC) leading to the formation of thrombi in the right atrium, intrapulmonary dissemination, pulmonary embolism, or even sudden death [2]. There is no concrete evidence yet for establishing an optimal treatment strategy for HCC with macrovascular invasion, and in most previous reports, cases of PVTT and HVTT are mixed as cases of vascular invasion, with cases of HVTT always being in the minority.

In the American Association for the Study of the Liver Diseases/Barcelona Clinic for Liver Cancer (AASLD/BCLC) Staging System and treatment guidelines, major vascular invasion, including PVTT and HVTT, is regarded as indicating an advanced stage of the disease [3]. The only proposed treatment option for this group of patients is sorafenib. However, the reported median survival time (MST) of patients with advanced HCC treated with sorafenib is as short as 10.7 months [4], and there may be a role of surgical intervention for selected patients.

As a result of recent advances in surgical techniques and perioperative management, liver resection has become a reasonably safe treatment option with an acceptable mortality rate [5,6]. Aggressive surgical resection for HCC with vascular invasion has been proposed from some tertiary centers [7,8]. Recently, we reported an acceptable long-term outcome of surgical resection for HCC in patients with PVTT [9,10]. However, little is known about the impact of surgical treatments in HCC patients

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with HVTT. In this study, we analysed the patient characteristics of HCC patients with HVTT, and the short-term and long-term outcomes of surgical resection in these patients.

Patients and methods

All elective liver resections for HCC carried out from October 1994 to December 2011 were included in this study. The demographic, clinical and pathological data were recorded prospectively and analysed retrospectively. The extent of hepatectomy required was evaluated according to the extent of disease progression, liver function status, and general condition of the patient. The status of disease progression and resectability were assessed by imaging studies such as contrast-enhanced computed tomography, magnetic resonance imaging, hepatic arterial angiography, and ultrasonography. Liver function impairment associated with underlying liver disease was assessed by liver biochemistry tests, Child-Pugh classification, and the indocyanine green retention rate at 15 min [11]. Hepatic resection was performed under intraoperative ultrasonographic guidance with inflow occlusion (Pringle's maneuver).

Diagnostic criteria for PVTT and HVTT

The presence of PVTT and/or HVTT was determined according to the final pathological findings. PVTT was categorized into main trunk/contralateral branch (Vp4), first-order branch (Vp3), second-order branch (Vp2), and third-order branch or microscopic invasion (Vp1), according to the Japanese staging system. HVTT was categorized as tumor thrombosis in a peripheral hepatic vein, including microvascular invasion (pHVTT, Vv1), in a major hepatic vein (mHVTT, Vv2), or in the inferior vena cava (IVCTT, Vv3) according to the Japanese staging system [12]. The major hepatic vein indicates the first branch of the hepatic vein branching from the IVC, including the right, left and middle hepatic vein, the inferior right hepatic vein, or the short hepatic veins. Involvement of Vp 2-4 or Vv 2-3 is considered as major vascular invasion. The remnant liver volume and liver function were assessed and R0 resection was attempted. The resection criteria for mHVTT and IVCTT are shown in Fig. 1. During the study period, we identified 28 mHVTT and 28 IVCTT patients diagnosed by preoperative computed tomography. In cases of macroscopic HVTT, poor liver function and multiple extrahepatic metastases were considered as contraindications for surgery. In cases where R1/0 resection was judged to be difficult preoperatively, surgical resection was considered only for preventing embolic complications or rupture. IVCTT extending to the right atrium was not considered as an indication for surgery. During the study period, a total of 1525 hepatic resections were performed for HCC. Pathological examination revealed 153 cases of pHVTT, 21 cases of mHVTT, and 13 cases of IVCTT in this study group.

Patient characteristics

The clinical features were compared among the pHVTT, mHVTT, and IVCTT groups. The clinical variables analysed were divided into the baseline characteristics, operative procedures, and outcome variables, as shown in Tables 1 and 2. More than 90% of the liver resections performed in this study group represented the primary surgical treatment for HCC (pHVTT; 140 [92%], mHVTT; 21 [100%], IVCTT; 13 [92%]). Previous history of radiofrequency ablation was present in 5 cases of the pHVTT group, but none of the cases of the mHVTT or IVCTT group. Portal hypertension was defined according to the criteria proposed by the Barcelona group for patients in whom the hepatic venous pressure gradient was not measured [13]. Postoperative complications were recorded and graded according to the Dindo-Clavien classification [14]. Prophylactic drainage of pleural effusion was considered as Grade II. Postoperative mortality was defined as any death during postoperative hospitalization or death within 90 days of surgery.

Follow-up

All patients were followed up at our outpatient clinic in a standardized manner, including tumor marker tests every 1–2 months, ultrasonography every 3 months, and computed tomography or magnetic resonance imaging every 6 months [15]. Recurrence was confirmed based on the findings in more than two different imaging modalities, including magnetic resonance imaging. Repeat resection was performed for recurrences where possible, and transcatheter arterial chemoembolization (TACE) or systemic chemotherapy was selected for unresectable cases [16]. Sorafenib was administered for recurrence in 5 cases of the pHVTT group, 1 case of the mHVTT group, and 2 cases of the IVCTT group.

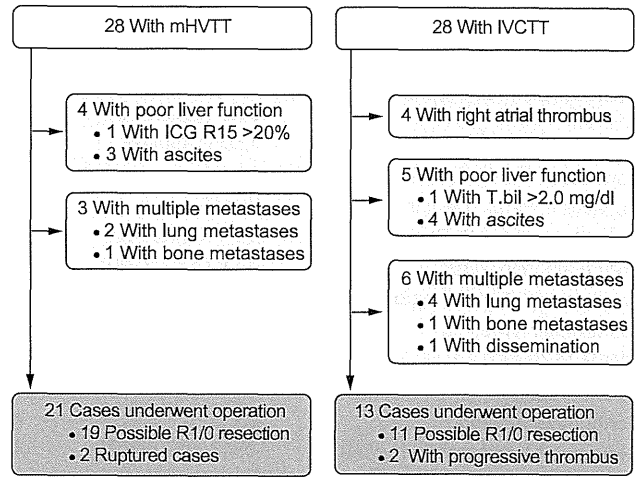

Fig. 1. Resection criteria of patients with mHVTT and IVCTT.

Statistical analysis

Statistical analyses were performed using the JMP 10 software (SAS Institute Inc., Cary, NC). Categorical variables were analysed using the χ^2 test or Fisher's exact test, as appropriate. Continuous variables were analysed using the Student's *t* test or the Mann-Whitney U test, as appropriate. A *p* value less than 0.05 was considered to indicate statistical significance. The overall and recurrence-free survival curves were determined using the Kaplan-Meier method and compared using the log-rank test. A multivariate analysis was performed using a Cox proportional hazards model and the backward elimination procedure. A *p* value of less than 0.10 was set as the cut-off value for the elimination. The following 13 variables were examined as potential risk factors: age >65 y/o, Child-Pugh class B, serum alpha-fetoprotein >10,000 (ng/ml), number of tumors ⩾3, recurrent HCC, presence of IVCTT, presence of PVTT, R1/2 resection, liver cirrhosis, portal hypertension, satellite nodules, moderate to poor cancer cell differentiation, and tumor size >10 cm. All statistical analyses were 2-tailed and based on the intention-to-treat concept.

Results

Characteristics of the pHVTT and mHVTT groups

The median ages of the patients in the pHVTT and mHVTT groups were similar (62.8 (interquartile range [IQR] 60.8–64.8) and 64.0 (IQR 58.6–69.3) years, respectively). Analysis of the preoperative characteristics (Table 1) showed a significantly higher serum level of alpha-fetoprotein (AFP) [ng/ml] (49666, [IQR 0–100360] *vs.* 5508, [IQR 2537–8479]; $p < 0.0001$), a higher positivity rate for des-gamma-carboxy prothrombin (DCP, >40 mAU/ml, 90 *vs.* 67%, $p = 0.02$), a larger tumor size [mm] (90.4, [IQR 71.4–110] *vs.* 59.8, [IQR 52.8–67.0]; $p = 0.003$), and a higher incidence of satellite nodules (52 *vs.* 27%; $p = 0.02$) and PVTT (95 *vs.* 56%; $p = 0.0005$) in the mHVTT group as compared to the pHVTT group. As listed in Table 2, the intraoperative characteristics were similar, except for the higher incidence of major hepatic resection (67% *vs.* 42%, $p = 0.037$) in the mHVTT group. In the mHVTT group, extended right or left hepatectomy was performed in 4 patients, and 1 case needed partial resection of the IVC. The postoperative courses were similar in the two groups, including in terms of the median hospital stay, frequency of complications, and the mortality rate (Table 2). The MST was 5.27 years in the pHVTT group and 3.95 years in the mHVTT group (Fig. 2), the difference not being statistically significant ($p = 0.77$). The median time to recurrence (TTR) was not statistically significantly different

Table 1. Patient baseline characteristics.

Patient characteristics	pHVTT group n = 53	mHVTT group n = 21	IVCTT group n = 13	*p* values (pHVTT *vs.* mHVTT)
Age (yr)*	62.8 (60.8-64.8)	64.0 (58.6-69.3)	61.8 (57.4-66.2)	0.69
Sex (male/female)**	122/31 (80/20)	18/3 (86/14)	10/3 (77/23)	
Hepatitis B virus infection	39 (25)	8 (38)	6 (46)	0.22
Hepatitis C virus infection	70 (46)	10 (48)	5 (38)	0.87
Child-Pugh class (A/B)	137/16 (90/10)	17/4 (81/19)	11/2 (85/15)	0.25
Serum albumin (g/dl)	3.68 (3.62-3.74)	3.50 (3.33-3.67)	3.65 (3.33-3.98)	0.05
Serum total bilirubin (mg/dl)	0.81 (0.73-0.89)	0.69 (0.62-0.75)	0.75 (0.62-0.88)	0.26
Prothrombin time (%)	82.9 (80.3-85.5)	78.4 (71.3-85.6)	82.5 (73.6-91.4)	0.25
ICG R 15 (%)	14.5 (13.2-15.8)	15.0 (11.5-18.5)	11.4 (7.6-15.2)	0.78
Liver cirrhosis	61 (40)	10 (48)	3 (23)	0.50
Portal hypertension	26 (17)	2 (10)	3 (23)	0.38
Serum alpha-fetoprotein (ng/ml)	5508 (2537-8479)	49,666 (0-100,360)	22,812 (0-47,220)	<0.0001
Serum DCP (positive/negative)	102/51 (67/33)	19/2 (90/10)	10/3 (77/23)	0.02
Number of tumors				0.30
1-2	130 (85)	16 (76)	9 (69)	
≥3	23 (15)	5 (24)	4 (31)	
Satellite nodules	42 (27)	11 (52)	7 (54)	0.02
Tumor differentiation				0.15
Well	14 (9)	0 (0)	0 (0)	
Moderate or poor	139 (91)	21 (100)	13 (100)	
Tumor size (mm)	59.8 (52.8-67.0)	90.4 (71.4-110)	87.9 (63.7-112)	0.003
Primary/recurrence	140/13 (92/8)	21/0 (100/0)	12/1 (92/8)	0.17
Extent of PVTT				
Main trunk/contralateral branch	3 (2)	2 (10)	0 (0)	
First-order branch	9 (6)	3 (14)	0 (0)	
Second-order branch	10 (7)	7 (33)	4 (31)	
Third-order branch	63 (41)	8 (38)	6 (46)	
PVTT Total	85 (56)	20 (95)	10 (77)	0.0005

*Median (IQR).
**Number (%).
IQR, interquartile range; ICG R 15, indocyanine green retention rate at 15 min; PVTT, portal vein tumor thrombus, DCP, Des-gamma-carboxy thrombin.

between the two groups (Fig. 3) either, being 1.06 and 0.41 years, respectively ($p = 0.74$).

Characteristics of the IVCTT group

The median age was 61.8 (IQR 57.4–66.2) years. The incidence of PVTT in the IVCTT group was 10 (77%). Major hepatic resection was performed in 10 patients (77%). Extended right hemihepatectomy was performed in 3 patients, and thrombectomy of IVCTT was performed in all patients. R0 resection was achieved in 9 patients (69%). The median hospital stay was 24.1 (IQR 15.8–32.3) days and mortality within 90 days was observed in 1 case (7.6%). The MST and TTR in the IVCTT patients were 1.39 years and 0.25 year, respectively (Figs. 2 and 3). The MST was statistically significantly shorter as compared to that in the other two HVTT groups. The MST was also significantly worse in the Child-Pugh class B patients of the IVCTT group ($p = 0.001$, Fig. 4).

Risk factors for survival and recurrence

Multivariate analysis carried out to determine the risk factors for overall survival identified IVCTT (risk ratio [RR] 2.54, 95% confidence interval [CI] 1.14–5.08, $p = 0.024$) and R 1/2 resection (RR 2.08, 95% CI 1.15–3.63, $p = 0.017$) as significant risk factors. Concerning the TTR, Child-Pugh class B (RR 1.92, 95% CI 1.08–3.19, $p = 0.027$) and number of tumors ⩾3 (RR 2.10, 95% CI 1.26–3.33, $p = 0.005$) were identified as risk factors by the multivariate analysis (Table 3). Despite the high rate of coexistence, presence of PVTT was not identified as a risk factor.

Type of recurrence and treatment of HVTT

As listed in Table 4, the most frequent location of recurrence was intrahepatic in all types of HVTT (pHVTT 89 [79%], mHVTT 9 [56%], IVCTT 5 [45%]). Of note, lung metastasis was also frequent in the mHVTT group, while multiple metastases, including to other organs, were more frequent in the IVCTT group. The most frequently employed treatment for recurrence was TACE, followed by repeated resection in patients with any type of HVTT.

Discussion

The current study revealed that patients with mHVTT have a similar prognosis to those with pHVTT, which indicates that mHVTT is not a contraindication for surgical resection. The mHVTT group had

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Table 2. Operative procedures and outcomes.

	pHVTT group n = 153	mHVTT group n = 21	IVCTT group n = 13	*p* values (pHVTT *vs.* mHVTT)
Operative time (min)*	447 (421-474)	466 (396-537)	583 (491-675)	0.62
Blood loss (ml)	1281 (1065-1497)	1320 (737-1902)	1788 (1105-2470)	0.90
RBC transfusion required**	27 (18)	7 (33)	2 (15)	0.09
FFP transfusion required	59 (39)	10 (48)	7 (54)	0.43
Major hepatectomy§	65 (42)	14 (67)	10 (77)	0.037
Extent of resection				0.76
R0	114 (75)	15 (71)	9 (69)	
R1/2	39 (25)	6 (29)	4 (31)	
Median hospital stay (d)	20 (IQR 18-21)	20 (IQR 15-24)	24 (IQR 16-32)	0.92
Complication§§				0.60
I	11 (7)	1 (5)	0 (0)	
II	41 (27)	8 (38)	5 (38)	
III	6 (4)	0 (0)	1 (8)	
IV	0 (0)	0 (0)	0 (0)	
90-day mortality	3 (2)	1 (5)	1 (8)	0.42

*Median (IQR).
**Number (%).
§More than 3 Couinaud's segments.
§§According to Dindo *et al.* Classification [14].
IQR, interquartile range; RBC, Red blood cells; FFP, Fresh frozen plasma.

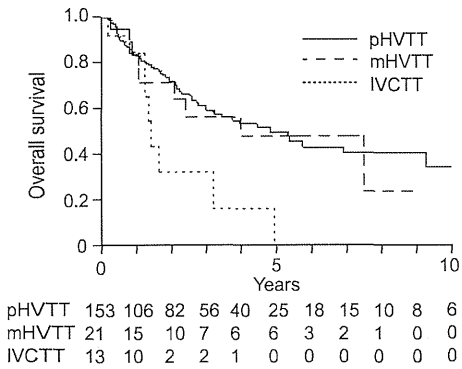

Fig. 2. Overall survival according to the type of HVTT. Numbers below the x-axis indicate the number of patients at risk.

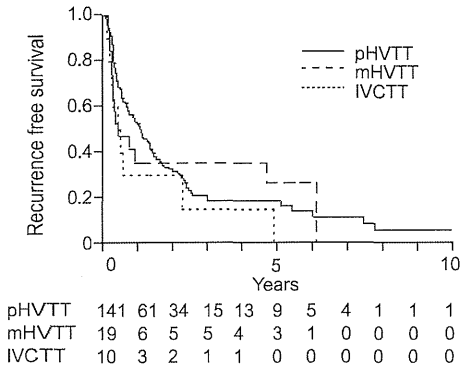

Fig. 3. Recurrence-free survival according to the type of HVTT. Numbers below the x-axis indicate the number of patients at risk.

a higher serum AFP level, a higher positivity rate for DCP, a larger tumor size, and a higher incidence of satellite nodules and PVTT. R1/2 resection was identified as a significant impact on the prognosis and R0 resection should be attempted, so it is not surprising that major hepatic resection was more frequently required for patients of the mHVTT group than for those of the pHVTT group. IVCTT had a worse prognosis as compared to the other two types of HVTT, but the results of this study suggest that surgery should still be considered in patients categorized as Child-Pugh class A.

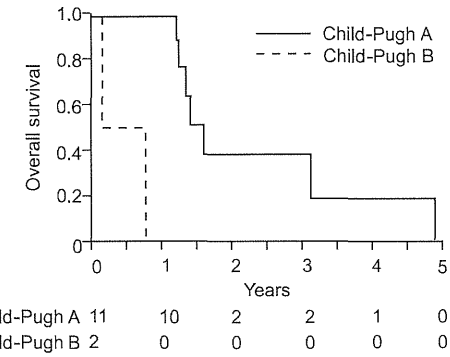

Fig. 4. Overall survival of patients with IVCTT according to the Child-Pugh grade. Numbers below the x-axis indicate the number of patients at risk.

There is no worldwide consensus on the management of HCC associated with macroscopic vascular tumor thrombi. Multiple strategies have been attempted to treat PVTT and we have proposed TACE and subsequent surgical resection as a promising strategy [17]. On the other hand, little is known about HVTT. This is probably because HVTT is relatively rare as compared to PVTT in patients with HCC [18]. Although the number of patients with mHVTT and IVCTT was relatively small in this study, this was one of the largest single-center series of HVTT in which the clinical outcomes of surgical resection under standardized criteria and follow-up protocol were evaluated. Although the MST and TTR in this population were worse than those in patients without HVTT, i.e., 6.42 years and 1.58 years, respectively (unpublished

Table 3. Multivariate analysis to identify prognostic factors associated with survival and recurrence.

Risk factors	*p* value	Risk ratio (95% CI)
Overall survival		
IVCTT	0.024	2.54 (1.14-5.08)
Age >65 yr	0.073	0.64 (0.39-1.04)
Child-Pugh class B	0.072	1.72 (0.95-2.95)
Recurrence	0.134	1.89 (0.81-3.89)
R1/2 resection	0.017	2.08 (1.15-3.63)
Time to recurrence		
Child-Pugh class B	0.027	1.92 (1.08-3.19)
Number of tumors ≥3	0.005	2.10 (1.26-3.33)
Satellite nodules	0.090	1.41 (0.95-2.05)
R1 resection	0.134	1.46 (0.89-2.29)

PVTT, portal vein tumor thrombi; IVCTT, tumor thrombosis of the inferior vena cava.

data), the long-term outcome after surgical resection of HCC patients with HVTT may be acceptable.

Presence of HVTT is classified as advanced-stage HCC in the AASLD/BCLC staging system, and surgical resection is not recommended, however, the MST after surgical resection for mHVTT was significantly better than that in the unresectable population treated by sorafenib (47.4 *vs.* 10.7 months) [4]. We have to admit that there was probably a selection bias for patients who were suitable candidates for surgical resection, however, even in the IVCTT group, with possibly the poorest prognosis, the MST was better than that in patients treated with sorafenib (12.7 *vs.* 10.7 months). Although surgical treatment for mHVTT and IVCTT is technically demanding and major hepatectomy is often required, we encountered only one mortality in each of these groups, and the length of hospital stay and frequency of complications did not differ in either of these groups from those in the pHVTT group. These findings may justify the consideration of surgical treatment for mHVTT and IVCTT.

Presence of mHVTT indeed represented an advanced stage of the disease and early recurrence after surgical resection was common (TTR 0.41 year). Discrepancy between the relatively favorable MST of 3.95 years and the very short TTR may be explained by the impact of the treatment given for tumor recurrence. Of note, the most frequent pattern of recurrence after surgical resection in cases with mHVTT was liver-only recurrence (56%). As long as the recurrence is confined to the liver, we can at least select from one of the several effective treatment options available, including repeated hepatectomy, radiofrequency ablation, and TACE.

Coexistence of PVTT was observed at a high incidence in the HVTT groups in our study. Recently, epithelial-mesenchymal transition (EMT), the mechanism of invasion in a variety of cancers, has been shown to be involved in the vascular invasion in HCC [19]. Thus, EMT underlying the formation of both PVTT and HVTT may explain the high rate of co-existence of the two. This may explain why in HVTT-positive patients, PVTT was not identified as a predictor of poor prognosis. However, the reason why PVTT is found more commonly than HVTT remains unclear and needs further investigation.

Available evidence for the most suitable treatment strategies for IVCTT is even more limited, possibly attributable to the technical difficulty of surgical treatment for IVCTT [2,8,20]. A retrospective review of the data of 56 IVCTT patients conducted by Wang *et al.* showed significantly longer MSTs in the 25 patients treated by surgery [18]. The MST of 19 months in this study (similar to our results) was good, considering the advanced tumor status in cases with IVCTT. Based on the results of the studies conducted by Wang and our own studies, surgical resection can be safely performed in selected patients, especially in those with good liver function, and may yield a better prognosis, as compared to other treatments.

In a comparison between the IVCTT and mHVTT groups, while the TTR was similar, the MST was significantly poorer in the IVCTT group. As shown in Tables 1 and 2, there were no major differences in the patient characteristics or incidence of R0 resection between the two groups. The incidence of intrahepatic recurrence was also similar in both groups (Table 4), although there was significant difference in other types of recurrence; in the mHVTT group, the most frequent type of recurrence was lung-only metastases, while multiple-organ recurrences including both intrahepatic recurrence and metastasis to the lung and/or other organs were the most common in the IVCTT group. Recently, presence of a limited number of lung metastases in the absence of intrahepatic HCC was shown as an indication for surgery, which yielded an acceptable survival benefit [21–26]. In the present study, surgical resection of lung metastasis with curative intent could be performed for 3 patients in the mHVTT group, but none of the patients in the IVCTT group. This may explain the significant difference in the MST between the mHVTT and IVCTT groups.

Sorafenib has become established as a new standard treatment option for advanced HCC [4,27]. There have also been reports showing the effectiveness of sorafenib for HCC patients

Table 4. Type of recurrence and treatment.

Number of patients with recurrence	pHVTT group n = 113**	mHVTT group n = 16	IVCTT group n = 11
Site of the first recurrence*			
Intrahepatic only	89 (79)	9 (56)	5 (45)
Lung only	11 (10)	5 (31)	1 (9)
Intrahepatic and lung	5 (4)	1 (6)	3 (27)
Other organs	8 (5)	1 (6)	2 (18)
Treatment for the first recurrence*			
Repeat resection	34 (30)	4 (25)	3 (27)
TACE	38 (34)	7 (44)	4 (36)
Systemic chemotherapy	18 (16)	3 (19)	2 (18)
Best supportive care	23 (20)	2 (13)	2 (18)

*Number (%).
**1 case was lost to follow-up.
TACE, Transcatheter arterial chemoembolization.

with PVTT [28,29]. In our study, there were hardly any patients who received sorafenib as adjuvant or neoadjuvant chemotherapy. Sorafenib as adjuvant chemotherapy could be a promising strategy for HCC patients with mHVTT or IVCTT. We are now conducting a clinical study to test this hypothesis.

Because the hepatic vein finally flows into the pulmonary vessels, lung metastasis would be expected to be frequent in patients with HVTT. However, the most frequent type of recurrence encountered in our study subjects was intrahepatic metastasis. This suggests that HVTT by itself is not a systemic disease, and that control of intrahepatic recurrence should be undertaken in these cases as in cases of HCC without vascular invasion. Since there are several effective treatments for intrahepatic metastasis, including repeat resection and TACE, aggressive surgical treatment may be justified for HVTT.

One of the limitations of our study is that all the patients included in the study were suitable candidates for liver resection. Indeed, the frequency of mHVTT was 1.4% and that of IVCTT was 0.9% among all cases of liver resection performed at our institution for HCC, which are much lower than the corresponding rates reported previously (11% and 3–4%, respectively) [18,30]. However, in properly selected cases of HVTT, although the TTR was less than one year, the MST was much better than that obtained with non-surgical treatments, including sorafenib. This implies that at least in cases of mHVTT with good liver function, surgical treatment should be recommended, especially if R0 resection is possible.

In conclusion, surgical resection is associated with a good prognosis in HCC patients with mHVTT just as in that of HCC patients with pHVTT, yielding an acceptable MST especially when R0 resection can be achieved. Patients with IVCTT can also be considered for surgery in the presence of good liver function.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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ORIGINAL SCIENTIFIC REPORT

Above 5 cm, Size Does Not Matter Anymore in Patients with Hepatocellular Carcinoma

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Abstract

Background Solitary hepatocellular carcinoma (HCC) is a good candidate for surgical resection. However, the significance of the size of the tumor in solitary HCC remains unclear.

Objective The aim of this study was to evaluate the impact of tumor size on overall and recurrence-free survival of patients with solitary HCC.

Materials We retrospectively reviewed 616 patients with histologically confirmed solitary HCC who underwent curative surgical resection between 1994 and 2010. The characteristics and prognosis of patients with HCC were analyzed stratified by tumor size.

Results A total of 403 patients (65 %) had tumors <5 cm, 172 (28 %) had tumors between 5 and 10 cm, and 41 (7 %) had tumors >10 cm. The incidence of microvascular invasion, satellite nodules, and advanced tumor grade significantly increased with tumor size. The 5-year overall and recurrence-free survival rates of HCC <5 cm were 69.6 % and 32 %, respectively, which were significantly better than those of HCC between 5 and 10 cm (58 % and 26 %, respectively) and HCC >10 cm (53 % and 24 %, respectively). On multivariate analysis, cirrhosis ($p = 0.0307$), Child–Pugh B ($p = 0.0159$), indocyanine green retention rate at 15 min >10 % ($p = 0.0071$), microvascular invasion ($p < 0.0001$), and satellite nodules ($p = 0.0009$) were independent predictors of poor survival, whereas tumor size >5 cm was not.

Conclusion Although recurrence rates are high, surgical resection for solitary HCC offers good overall survival. Tumor size was not a prognostic factor. Solitary large HCC >10 cm would be a good candidate for hepatectomy as well as solitary HCC between 5 and 10 cm.

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Introduction

Liver resection represents the mainstay of curative treatment for hepatocellular carcinoma (HCC) and provides the only consistent long-term survival [1]. Technical advances in liver surgery have expanded surgical indications towards advanced cases [2]. Solitary HCC is generally thought to have a good prognosis after resection. It might be generally believed that patients with large tumors have a worse prognosis than those with small tumors. As there are several pathologic factors, such as vascular invasion, satellite nodules, high differentiation grade, or underlying liver disease, that may predict poor outcome after hepatic resection for HCC [3–6], tumor size would also be an important prognostic factor, and has been adopted in recent staging systems [7, 8]. One of the cut-off values for HCC is defined as 2 cm in diameter [9].

Despite the known correlation between tumor size and vascular invasion, excellent long-term survival rates in patients with solitary large HCC have been reported in several studies [6, 10–12]. However, the significance of other pathologic risk factors, such as satellite nodules, histologic grade, or underlying liver disease, in solitary HCC, in combination with the tumor size, remains ill defined. Therefore, the aims of the present study were to report long-term outcomes and to identify predictors of survival and recurrence after liver resection in a single-center-based Eastern cohort of patients with solitary HCC, and to assess the relationship between tumor size and the other prognostic factors.

Methods

All patients who underwent curative hepatic resection for primary and solitary HCC at Tokyo University Hospital, Tokyo, Japan, between November 1994 and December 2010 were retrospectively studied. In this study, solitary HCC was defined as any single HCC of any size, with no satellite nodules and/or vascular invasion at the time of treatment indication, and corresponding to Barcelona Clinic Liver Cancer (BCLC) 'A', according to the BCLC guidelines and the European Association for the Study of the Liver–American Association for the Study of Liver Diseases (EASL–AASLD) recommendations [13, 14]. Patients with multiple lesions and/or vascular invasion on imaging or patients who underwent repeat hepatectomy for single lesion were excluded.

Surgical strategy

The indications and extent of hepatectomy were based on an algorithm including the presence of ascites, the serum bilirubin level, and the indocyanine green retention rate at 15 min (ICG-R15), as previously described [15]. The operative procedure was chosen according to the location of the tumor and evaluation of functional hepatic reserve. Our policy was to perform anatomical resection whenever possible, irrespective of tumor size. Anatomical resection was defined as any type of systematic resection of the portal area based on Couinaud liver segmentation. Technically, the procedure for anatomical resection included the following four steps: (1) confirmation and marking of the border of segments and sectors to be resected, using a combination of external anatomic landmarks, segmental staining method, and selective devascularization using clamping of the segmental inflow; [16, 17] (2) parenchymal transection from the segmental border to the landmark veins under ultrasonography guidance; [16] (3) full exposure of the landmark veins on the cut surface of the liver; and (4) ligation of the segmental or sectoral portal pedicle near the root of the segment or sector. Otherwise, wedge resection or enucleation was defined as non-anatomic resection. When a major right-sided hepatectomy (resection of four or more Couinaud's segments [18]) was required for the treatment of such large tumors, the indication for portal vein embolization (PVE) was determined based on the ICG-R15 and the volume of the remnant left liver [19].

When performing a right hepatectomy, we routinely used the conventional approach [20]. Briefly, a thoracophrenolaparotomy was performed to provide a good view around the vena cava. The right adrenal gland was carefully dissected from the liver, and dissection of the inferior vena cava ligament allowed the vena cava to protrude to the right, making it possible to control the right hepatic vein extrahepatically.

Indications for transcatheter arterial chemoembolization (TACE) before hepatectomy were as follows: (1) in case of ruptured HCC; [21] and (2) in some cases, before a right hepatectomy in association with PVE in order to improve the rate of hypertrophy of the left remnant liver [22].

Histopathology

The size of the tumor and width of the surgical margin were recorded before the specimen was fixed. Background liver status, grade of tumor cell differentiation, presence/absence of microvascular invasion, and satellite nodules were detected on microscopic evaluation. Microvascular invasion was defined as gross or microscopic invasion of the portal vein, hepatic vein, and inferior vena cava. Satellite nodules were defined as the presence of intrahepatic metastases to the segment in which the principal tumor was located.

Follow-up

All patients were regularly followed-up at an outpatient clinic and monitored for recurrence by serum alfa-feto-protein (AFP) and des-carboxy prothrombin (DCP) every 1–2 months, ultrasonography every 2 months, and dynamic computed tomography every 4 months, as previously reported [22]. Recurrence was defined as the appearance of a new lesion with radiological features compatible with HCC, as confirmed using at least two imaging modalities. When a recurrence was detected, the patient received further treatment by repeat hepatectomy, locoregional ablation treatments, including radiofrequency ablation, TACE, administration of sorafenib, or other treatment options. The disease-free survival period was defined as the interval between the operation and the date of the diagnosis of the first recurrence (either intrahepatic or extrahepatic). The remaining cases were censored at the date of the last follow-up assessment.

Statistical analysis

Continuous data were presented as median with range and were compared using the Student's t test or Mann–Whitney Wilcoxon test. Categorical data were analyzed by Pearson's χ^2 or Fischer's exact test. Time-to-endpoint analyses were performed using the Kaplan–Meier method. Overall survival was measured from date of resection to last living visit or loss to follow-up. Recurrence-free survival was measured from date of resection to recurrence or death. All variables were evaluated by the univariate log-rank test. Variables achieving a p value <0.1 were entered into a multivariate cox regression analysis. A p value of <0.05 was considered significant. Analyses were carried out using Statview software (version 5, 1992–1998, SAS Institute Inc., Cary, NC, USA).

Results

Clinical and histopathological characteristics

Our selection criteria identified 616 patients with resected solitary HCC. Overall, these patients had a median age of 66 years (range 13–85) (Table 1). The majority of patients were male (77 %). Among them, 292 (47 %) patients had cirrhosis, 530 patients (86 %) were classified as Child–Pugh A, and 86 (14 %) were Child–Pugh B. Of the 616 patients, 360 (58 %) patients were positive for hepatitis C and 138 (22 %) were positive for hepatitis B. The median tumor size was 35 mm (range 8–230).

In this study, four patients underwent TACE followed by liver resection for tumor rupture, and one patient underwent preoperative PVE following selective TACE for planned major hepatic resections.

When stratified according to tumor size, 403 (65 %) patients had tumors smaller than 5 cm, 172 (28 %) had tumors measuring between 5 and 10 cm, and 41 (7 %) had tumors larger than 10 cm (Table 2). Patients with larger tumors were less likely to be associated with hepatitis C

Table 1 Patient, operative, and pathologic characteristics

Characteristics	Overall ($n = 616$)
Patient	
Age (years)	66 (13–85)
Sex ratio (M/F)	476 (77)/140 (23)
Underlying liver disease	
HBV	138 (22)
HCV	360 (58)
Non-B non-C	130 (21)
Child-pugh grade	
A	530 (86)
B	86 (14)
Background liver	
Normal	38 (6)
Chronic hepatitis or fibrosis	286 (46)
Cirrhosis	292 (47)
HCC rupture	4 (0.6)
Portal vein embolization	21 (3.4)
ICG-R15	8.7 (2.2–72.30)
ICG-R15 >10	419 (68)
AFP (ng/ml)	19.20 (0–436,000)
AFP >200	169 (27)
DCP (mAu/ml)	62.5 (0–200,135)
DCP >100	265 (43)
Operative	
Major hepatectomy ≥4 segments	86 (14)
Anatomic resection	426 (69)
Pathologic	
Tumor size (mm)	35 (8–230)
Grade	
Well differentiated	97 (16)
Moderately differentiated	427 (69)
Poorly differentiated	71 (11.5)
Combined	6 (1)
Necrosis	15 (2)
Microvascular invasion	191 (31)
Bile duct invasion	27 (4)
Satellite nodules	80 (13)
Positive surgical margins	20 (3)

Data are presented as n (%) or median (range)

AFP alfa-fetoprotein, *DCP* des-γ-carboxyprothrombin, *F* female, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *ICG-R15* indocyanine green retention rate at 15 min, *M* male

Table 2 Clinicopathologic characteristics and operative details according to tumor size

	<5 cm $n = 403$ (65 %)	5–10 cm $n = 172$ (28 %)	>10 cm $n = 41$ (7 %)	p value
Clinical factors				
Age (years)	66 (13–85)	67 (22–85)	62 (21–81)	0.0251
Sex ratio (M/F)	306/97	136/36	34 (83)	0.4781
Underlying liver disease				
HBV	83 (21)	43 (25)	12 (29)	0.2814
HCV	260 (65)	87 (51)	13 (32)	<0.0001
Non-B non-C	67 (17)	47 (27)	16 (39)	0.0002
Child-pugh grade				0.1146
A	352 (87)	147 (85)	31 (76)	
B	51 (13)	25 (14.5)	10 (24)	
Background liver				<0.0001
Normal	15 (4)	16 (9)	7 (17)	
Chronic hepatitis or fibrosis	169 (42)	90 (52)	27 (66)	
Cirrhosis	219 (54)	66 (38)	7 (17)	
HCC rupture	0	2 (1)	2 (5)	0.0006
Portal vein embolization	5 (1)	9 (5)	7 (17)	<0.0001
ICG-R15	14.2 (2.5–72.3)	12.80 (2.5–48.9)	10.10 (2.2–34)	0.0042
ICG-R15 >10	282 (70)	115 (67)	22 (54)	0.0953
AFP (ng/ml)	16 (0–49,124)	25 (2–69,000)	1,314 (2–436,000)	<0.0001
AFP >200	86 (21)	59 (34)	24 (59)	<0.0001
DCP (mAu/ml)	51 (0–37,545)	243.5 (0–77,520)	14,730 (38–200,135)	<0.0001
DCP >100	124 (31)	104 (60)	37 (90)	<0.0001
Operative factors				
Major hepatectomy ≥4 segments	27 (7)	33 (19)	26 (63)	<0.0001
Anatomic resection	268 (66.5)	121 (70)	37 (90)	0.0068
Pathological factors				
Tumor size (mm)	28 (8–49)	65 (50–100)	130 (105–230)	<0.0001
Grade				0.0003
Well differentiated	82 (20)	14 (8)	1 (2)	
Moderately differentiated	273 (68)	125 (73)	29 (71)	
Poorly differentiated	36 (9)	25 (14.5)	10 (24)	
Combined	3 (1)	2 (1)	1 (2)	
Necrosis	9 (2)	6 (3.5)	0	
Microvascular invasion	83 (21)	80 (47)	28 (68)	<0.0001
Bile duct invasion	14 (3)	10 (6)	3 (7)	0.2898
Satellite nodules	30 (7)	39 (23)	11 (27)	<0.0001
Positive surgical margins	10 (2.5)	6 (3.5)	4 (10)	0.0426

Data are presented as n (%) or median (range)

AFP alfa-fetoprotein, *DCP* des-γ-carboxyprothrombin, *F* female, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *ICG-R15* indocyanine green retention rate at 15 min, *M* male

virus; more likely to be associated with non-B non-C hepatitis; more likely to have normal underlying liver parenchyma (17 % in the group with tumors >10 cm, 9 % in tumors measuring 5–10 cm, and 4 % in the group with tumors <5 cm; $p < 0.0001$), elevated tumor markers, including AFP ($p < 0.0001$) and DCP ($p < 0.0001$), and a better ICG-R15 value ($p = 0.0042$). Histopathologically, microvascular invasion (68 % in tumors >10 cm, 47 % in tumors 5–10 cm, and 21 % in tumors <5 cm; $p < 0.0001$), satellite nodules, and a less differentiated tumor were more prevalent in patients with larger tumors. The rate of macroscopic vascular invasion was 4.7 % (29 patients, including 16 patients with macroscopic portal vein invasion, 12 patients with hepatic vein invasion and one patient with both macroscopic portal and hepatic vein invasion). Of these 29 patients, three had tumors <5 cm, 16 had

tumors measuring 5–10 cm, and ten patients had tumors >10 cm.

Survival and recurrence of the entire cohort

The median follow-up period was 53.4 months (range 1.2–193.2). Seven (1 %) patients were lost to follow-up during the study period. In-hospital or 90-day mortality occurred in two patients (0.3 %). At the time of last follow-up, 274 (44 %) patients had died of recurrent disease progression, and 335 (54 %) patients were alive, 154 (25 %) of whom were disease free. For the entire cohort of 616 patients, overall median survival was 86 months, and 5- and 10-year overall survival rates were 65 and 42 %, respectively (Fig. 1). The median time to recurrence was 28 months, and disease-free survival rates after 3 and 5 years were 42 and 30 %, respectively.

Survival and recurrence according to tumor size

Patients with larger tumors were more likely to have a worse overall and disease-free survival (Fig. 2). The 5-year overall survival was better in patients with tumors <5 cm than in those with tumors 5–10 cm (69.6 % for tumors <5 cm vs. 58 % for tumors 5–10 cm; $p = 0.009$) and those with tumors >10 cm (69.6 % for tumors <5 cm vs. 53 %; $p = 0.0136$; Fig. 2a). The 5-year recurrence-free survival was also significantly better for patients with tumors <5 cm (32 %) than for those with tumors 5–10 cm (26 %, $p = 0.0092$) and tumors >10 cm (24 %, $p = 0.0090$; Fig. 2b). However, there was no significant difference in overall and recurrence-free survival between patients with tumors 5–10 cm and those with tumors >10 cm ($p = 0.6804$ and 0.4037, respectively).

Long-term survival according to current staging systems

Disease was stratified on the basis of HCC size and presence of macro- and microvascular invasion according to the current tumor/node/metastasis (TNM) staging systems: the fifth edition of the Liver Cancer Study Group of Japan (LCSGJ) classification [7] and American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) classification [8]. Within each staging system, the overall survival rates of patients with different stages of diseases were compared. The patients were well stratified by both staging systems (Fig. 3).

Prognostic factors of survival and recurrence of solitary HCC

We assessed the prognostic significance of tumor size in HCC by evaluating different cut-off points (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 cm). We adopted the cut-off value that was defined by the minimum p value to predict overall survival after surgical resection. The p value was lowest at 5 cm ($p = 0.0063$).

Univariate and multivariate analysis of potential prognostic factors within the total patient cohort identified five variables associated with worse overall survival: cirrhosis (hazard ratio (HR) 1.35; $p = 0.0307$), Child–Pugh B (HR 1.46; $p = 0.0159$), ICG-R15 > 10 (HR 1.6; $p = 0.0071$), microvascular invasion (HR 1.94; $p < 0.0001$), and satellite nodules (HR 1.7; $p = 0.0009$) (Table 3). Tumor size >5 cm was not an independent variable on multivariate analysis. In the 389 (63 %) solitary HCC patients without microvascular invasion and satellite nodules, tumor size had no impact on 5-year overall survival (74 %; $p = 0.61$).

As for recurrence-free survival, five variables were identified on univariate and multivariate analysis: cirrhosis (HR 1.4; $p = 0.0013$), ICG-R15 >10 (HR 1.286; $p = 0.0385$), microvascular invasion (HR 1.442; $p < 0.001$), and satellite nodules (HR 1.997; $p < 0.0001$). On the other hand, anatomic resection was associated with good recurrence-free survival (HR 0.795; $p = 0.0364$).

Prognostic factors of survival by tumor size

Among patients with tumors <5 cm, four factors were significant predictors of worse overall survival in both univariate and multivariate analysis: Child–Pugh B (HR 1.703, $p = 0.0120$), ICG-R15 >10 (HR 1.579; $p = 0.0486$), presence of microvascular invasion (HR 1.928; $p = 0.0006$), and satellite nodules (HR 1.911; $p = 00054$).

Among patients with tumors 5–10 cm, both microvascular invasion (HR 1.772; $p = 0.0113$) and satellite nodules (HR 1.930; $p = 0.0054$) were identified as significant predictors of worse overall survival. Among patients with tumors >10 cm, none of the studied factors were predictors of overall survival.

Discussion

In the present study, we retrospectively analyzed data on a cohort of patients from a single center with histologically confirmed solitary HCC. We found that the median survival in this entire cohort was 86 months, and overall 5-year survival rate was 65 %. The prognosis of patients with HCC <5 cm was significantly better than those with HCC >5 cm. Despite this, 5-year survival rates in patients with large tumors of diameter >10 cm were 58 % and comparable to that of patients with tumors 5–10 cm (53 %), which was acceptable. The frequency of microvascular invasion, satellite nodules, and advanced tumor

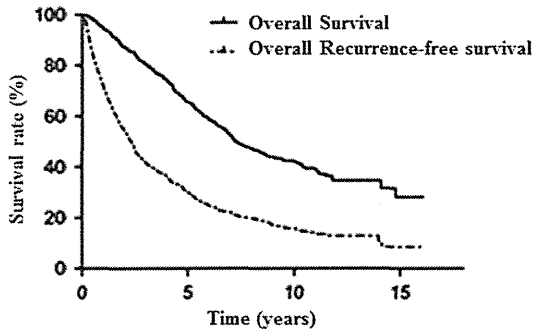

Fig. 1 Overall and recurrence-free survival curves

grade increases with tumor size; however, the influence of tumor size on the survival of patients decreased proportionally with the increase in size. This paradoxical phenomenon might be because, first, tumor size itself would not independently influence the survival of patients with solitary HCC, but size is associated with microvascular invasion and tumor aggressiveness. As previously reported, 2 cm [9] or 5 cm would be the threshold for microvascular invasion and satellite lesions that would rapidly increase with tumor size. Size and other important factors should be confounding factors. Second, most cirrhotic patients with large tumors or patients with multiple and/or bilateral tumors were not included in this study. Cirrhosis has been shown to influence survival and recurrence after resection of HCC [23]. In the current study, cirrhosis was present in more than half (54 %) of patients with tumors <5 cm, but in only 17 % of those with tumors >10 cm. This may be expected, as most patients with larger tumors require major hepatectomy, which was not possible in the presence of cirrhosis.

The present study revealed interesting data on the prevalence of various pathologic risk factors in solitary

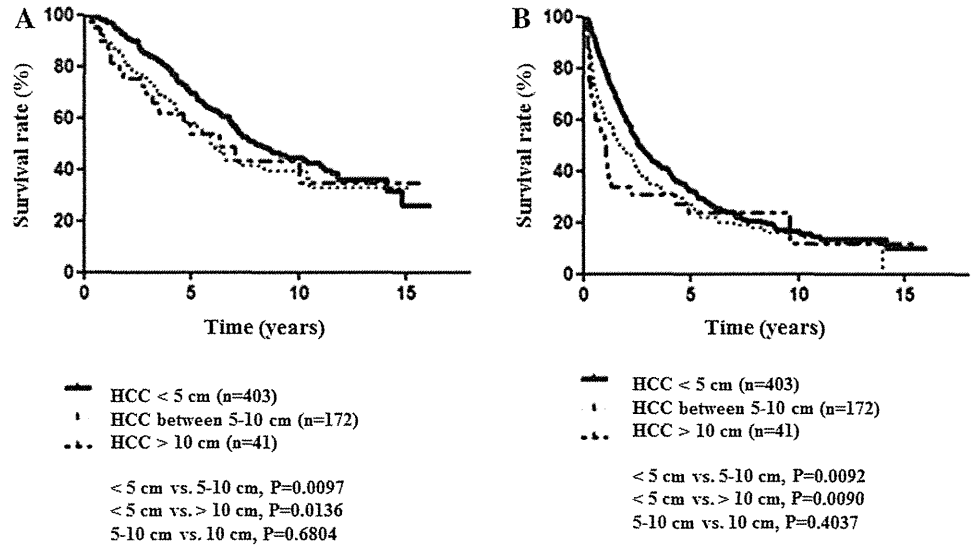

Fig. 2 Overall (**a**) and recurrence-free (**b**) survival curves stratified according to tumor size

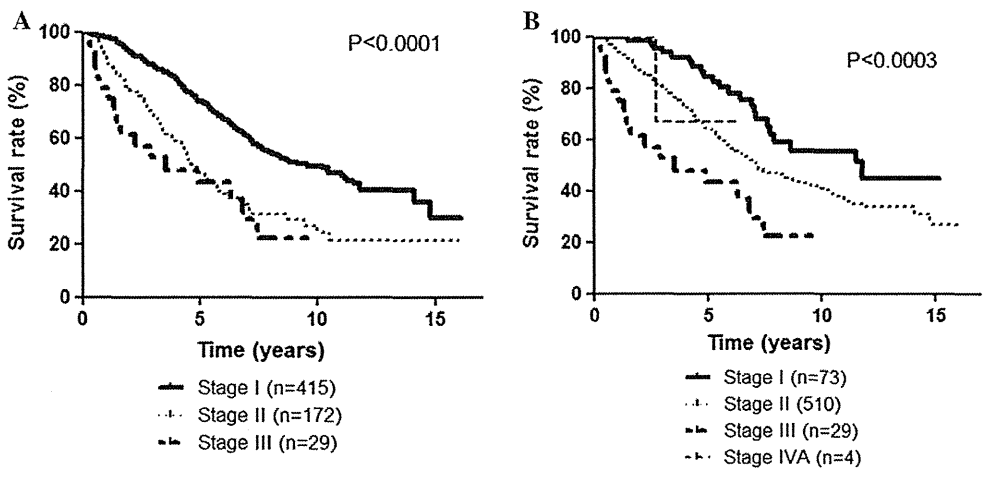

Fig. 3 Survival of patients with solitary hepatocellular carcinoma according to current staging systems.**a** American Joint Committee on Cancer, 7th edition. **b** Liver Cancer Study Group of Japan, 5th edition

Table 3 Prognostic factors of overall survival and recurrence-free survival in the entire population cohort with solitary hepatocellular carcinoma ($n = 616$)

Variable		Overall survival				Recurrence-free survival			
		Univariate	Multivariate analysis			Univariate	Multivariate analysis		
		p value	*p* value	HR	95 % CI	*p* value	*p* value	HR	95 % CI
Age ≥65 (years)	Yes	0.0171	NS			0.8448			
	No								
Male (vs. female)	Yes	0.1953				0.5474			
	No								
HBV	Yes	<0.0001	NS			0.1202			
	No								
HCV	Yes	<0.0001	NS			0.0039	NS		
	No								
Non-B non-C	Yes	0.2213				0.1283			
	No								
Child-Pugh B	Yes	0.0001	0.0159	1.464	1.074–1.995	0.0003	NS		
	No								
HCC rupture	Yes	0.9733				0.8990			
	No								
Portal vein embolization	Yes	0.4943				0.3483			
	No								
ICG-R15	<10	<0.0001	0.0071	1.602	1.137–2.256	<0.0001	0.0385	1.286	1.013–1.631
	≥10								
AFP (ng/ml)	<200	0.7214				0.1467			
	≥200								
DCP	<100	0.1058				0.0140	NS		
	≥100								
Anatomic resection	Yes	0.0203	NS			0.0009	0.0364	0.795	0.642–986
	No								
Tumor >5 cm	Yes	0.0063	NS			0.0019	NS		
	No								
Microvascular invasion	Yes	<0.0001	<0.0001	1.940	1.467–2.564	<0.0001	0.001	1.442	1.159–1.795
	No								
Bile duct invasion	Yes	0.0490	NS			0.2988			
	No								
Poorly differentiated	Yes	0.3658				0.9419			
	No								
Satellite nodules	Present	<0.0001	0.0009	1.706	1.246–2.2336	<0.0001	<0.0001	1.997	1.527–2.612
	Absent								
Cirrhosis	Present	0.0025	0.0307	1.351	1.028–1.775	<0.0001	0.0013	1.404	1.141–1.727
	Absent								
Major hepatectomy	Yes	0.5851				0.4433			
	No								
Surgical margins	Positive	0.1982				0.0910	NS		
	Negative								

AFP alfa-fetoprotein, *CI* confidence interval, *DCP* des-γ-carboxyprothrombin, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *HR* hazard ratio, *ICG-R15* indocyanine green retention rate at 15 min, *NS* non significant

HCC. Specifically, first, up to 80–90 % of all HCC in this series appeared in patients with underlying liver disease and chronic viral hepatitis. Second, most tumors (65 %) were smaller than 5 cm (vs. 7 % for tumors >10 cm), which suggests that a large proportion of HCC is being detected with increasing frequency due to routine screening

of patients with the hepatitis virus in the Japanese screening system. Third, we found that the incidence of microvascular invasion, satellite nodules, and advanced differentiation grade is associated with increased tumor size. In this series, microvascular invasion, which was found in 31 % of the entire cohort, significantly increased with tumor size (21 % in tumors <5 cm, 47 % in tumors 5–10 cm, and 68 % in tumors >10 cm, $p < 0.0001$). Similarly, 9 % of tumors <5 cm were high-grade differentiated, compared with 14.5 % of tumors 5–10 cm and 24 % of tumors >10 cm ($p = 0.003$). Further, 7 % of tumors <5 cm were high-grade differentiated, compared with 23 % of tumors 5–10 cm and 27 % of tumors >10 cm ($p < 0.0001$). These findings are consistent with previous results from one multicenter study that showed that 36 % of tumors <5 cm were high grade compared with 54 % of tumors sized 5.1–6.5 cm [24], and 55 % of tumors sized 5.1–6.5 cm were associated with microvascular invasion compared with 31 % of tumors sized <5 cm.

The reported 5-year survival rates for surgical resection of large HCC >10 cm varies widely in the literature, ranging from 19 to 54 % [10, 11, 25–32]. Heterogeneity in patients (cirrhosis) and tumor characteristics (vascular invasion vs. no vascular invasion, single vs. multiple lesions) may be one of the reasons for different outcomes following resection of large HCC. In our series, the 5-year overall and recurrence-free survival rates after resection of solitary HCC were comparable between tumors sized 5–10 cm and large tumors >10 cm. Therefore, patients with large solitary HCC >10 cm should always be considered for liver resection, as this treatment offers acceptable overall survival exceeding 50 %. Even in cases of recurrence, surgical resection should always be considered as long as R0 resection can be achieved, and clinical and pathological factors should not be used to exclude these patients from repeat hepatectomies because they do not reliably predict outcomes.

In our study, we identified five adverse predictors of survival: cirrhosis, Child–Pugh class B, ICG-R15 >10, microvascular invasion, and satellite nodules on histology. Only factors related to liver function were available at the time of surgery. Assessment of the other factors was based on the examination of the resected tumors, and this is information that is not available at the time of treatment indication. Thus, the results of our study show that patients with a single HCC of any size (including patients with a solitary HCC >10 cm), with evidence of portal hypertension or poor liver function at the time of treatment indication, should not be offered an operation. An interesting result of this study was the absence of the negative impact of R1 resection for overall and recurrence-free survival in the entire series, even in patients with tumors <5 cm. This is line with our previous report, which showed no correlation between tumor exposure and risks of tumor recurrence in patients with HCC <5 cm [33]. It is likely that the tumor exposure would impact less on prognosis in huge tumors because the frequency of micrometastases and vascular invasion increases with tumor size.

This study has several limitations. A strong selection bias exists in this work, as in any retrospective study. One limitation of this study might be the relatively few preoperative variables collected and analyzed, such as diabetes or quality of underlying liver disease. However, we included the most important variables in this study. Another limitation includes that the results of combination therapies, such as TACE and/or PVE plus liver resection, in instances of tumor rupture or for planned major hepatic resection, could not be assessed in this present series. The heterogeneity of both tumor and patient characteristics, combined with the sample size, preclude any relevant comment on this topic.

Conclusion

The present study showed that surgical resection for solitary HCC is associated with a good prognosis. Solitary large HCC >10 cm would be a good candidate for hepatectomy, as would solitary HCC between 5 and 10 cm. Size alone is not a contra-indication, but the presence of adverse predictors in some patients preclude good outcome.

Conflict of interest None.

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Involvement of Hepatitis C Virus NS5A Hyperphosphorylation Mediated by Casein Kinase I-α in Infectious Virus Production

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ABSTRACT

Nonstructural protein 5A (NS5A) of hepatitis C virus (HCV) possesses multiple functions in the viral life cycle. NS5A is a phosphoprotein that exists in hyperphosphorylated and basally phosphorylated forms. Although the phosphorylation status of NS5A is considered to have a significant impact on its function, the mechanistic details regulating NS5A phosphorylation, as well as its exact roles in the HCV life cycle, are still poorly understood. In this study, we screened 404 human protein kinases via *in vitro* binding and phosphorylation assays, followed by RNA interference-mediated gene silencing in an HCV cell culture system. Casein kinase I-α (CKI-α) was identified as an NS5A-associated kinase involved in NS5A hyperphosphorylation and infectious virus production. Subcellular fractionation and immunofluorescence confocal microscopy analyses showed that CKI-α-mediated hyperphosphorylation of NS5A contributes to the recruitment of NS5A to low-density membrane structures around lipid droplets (LDs) and facilitates its interaction with core protein and the viral assembly. Phospho-proteomic analysis of NS5A with or without CKI-α depletion identified peptide fragments that corresponded to the region located within the low-complexity sequence I, which is important for CKI-α-mediated NS5A hyperphosphorylation. This region contains eight serine residues that are highly conserved among HCV isolates, and subsequent mutagenesis analysis demonstrated that serine residues at amino acids 225 and 232 in NS5A (genotype 2a) may be involved in NS5A hyperphosphorylation and hyperphosphorylation-dependent regulation of virion production. These findings provide insight concerning the functional role of NS5A phosphorylation as a regulatory switch that modulates its multiple functions in the HCV life cycle.

IMPORTANCE

Mechanisms regulating NS5A phosphorylation and its exact function in the HCV life cycle have not been clearly defined. By using a high-throughput screening system targeting host protein kinases, we identified CKI-α as an NS5A-associated kinase involved in NS5A hyperphosphorylation and the production of infectious virus. Our results suggest that the impact of CKI-α in the HCV life cycle is more profound on virion assembly than viral replication via mediation of NS5A hyperphosphorylation. CKI-α-dependent hyperphosphorylation of NS5A plays a role in recruiting NS5A to low-density membrane structures around LDs and facilitating its interaction with the core for new virus particle formation. By using proteomic approach, we identified the region within the low-complexity sequence I of NS5A that is involved in NS5A hyperphosphorylation and hyperphosphorylation-dependent regulation of infectious virus production. These findings will provide novel mechanistic insights into the roles of NS5A-associated kinases and NS5A phosphorylation in the HCV life cycle.

Hepatitis C virus (HCV) is a major causative agent of liver-related morbidity and mortality worldwide and represents a global public health problem (1). An estimated 130 million individuals are chronically infected with HCV worldwide, and the treatment of HCV infection imposes a large economic and societal burden (2). HCV is an enveloped virus with a positive-sense, single-stranded RNA genome in the *Hepacivirus* genus within the *Flaviviridae* family (3). The approximately 9.6-kb genome is translated into a single polypeptide of approximately 3,000 amino acids (aa), which is cleaved by cellular and viral proteases to produce the structural proteins (core, E1, E2, and p7) and nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (4). NS3 to NS5B are sufficient for RNA replication in cell culture (5). NS5B is an RNA-dependent RNA polymerase (RdRp), and NS3 functions as both an RNA helicase and a serine protease (4). NS4A is the cofactor of the NS3 protease, and the NS3-NS4A complex is required for viral precursor processing (4). NS4B induces the formation of a specialized membrane compartment, a sort of membranous web where viral RNA replication may take

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