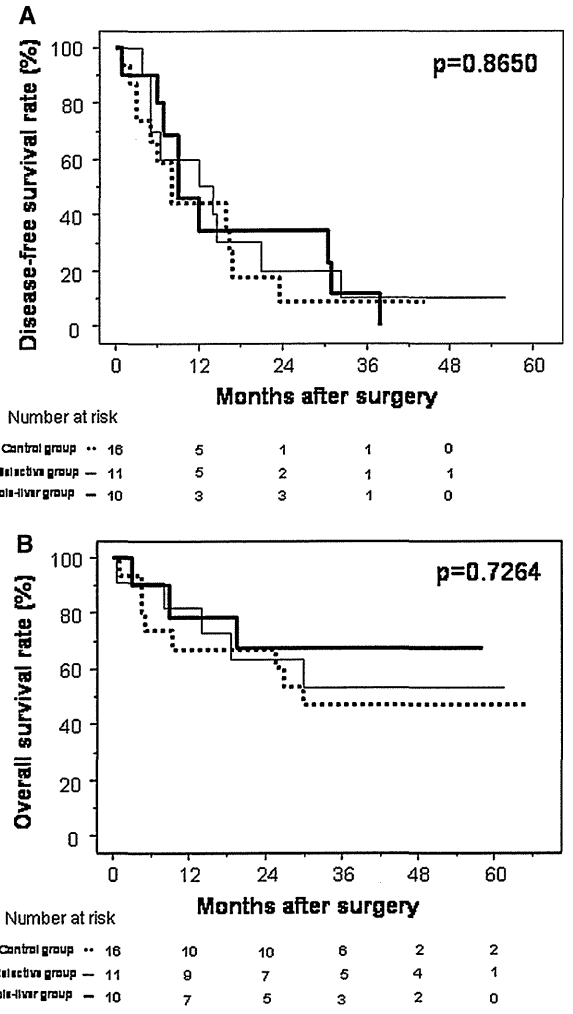

Fig. 4 **a** Comparison of disease-free survival after resection of a solitary HCC ≥5 cm in the greatest diameter among patients receiving preoperative selective TACE (selective group, $n = 11$, *thin solid line*), patients receiving preoperative TACE plus whole-liver chemolipiodolization (whole-liver group, $n = 10$, *thick solid line*), and patients without preoperative TACE (control group, $n = 16$, *dotted line*). There were no significant differences in disease-free survival among the three groups ($P = 0.8650$). **b** Comparison of overall survival after resection of a solitary HCC ≥5 cm in the greatest diameter among the selective group ($n = 11$, *thin solid line*), the whole-liver group ($n = 10$, *thick solid line*), and the control group ($n = 16$, *dotted line*). There were no significant differences in overall survival among the three groups ($P = 0.7264$)

2–8 cm in diameter. In contrast, it was reported that downstaging or total necrosis of the tumor was achieved by preoperative TACE in 62% of 103 HCC patients with cirrhosis, leading to an improvement of disease-free survival after liver resection and liver transplantation [13]. Thus, the value of preoperative TACE is still controversial.

A meta-analysis including seven randomized clinical trials was undertaken in the late 1990s to investigate the usefulness of TACE for treating unresectable HCC, which demonstrated an improvement in 2-year survival (odds ratio 0.53, $P = 0.017$) compared with control patients who were treated conservatively or received suboptimal management [27]. This established the role of TACE as the standard care for unresectable HCC, whether as palliative therapy or to improve resectability [27]. Subsequent investigations were directed towards the preoperative use of TACE as neoadjuvant therapy to prevent recurrence. To assess the clinical efficacy of preoperative TACE for resectable HCC, two randomized trials were conducted in 1995 and 1996 [15, 17] (Table 4). Both of these trials found no improvement in disease-free survival following neoadjuvant TACE, and Wu et al. [17] reported worse overall survival in the TACE group. In 2009, a randomized trial of neoadjuvant TACE for large resectable HCC was reported [18]. The results were similar, with no difference in disease-free survival or overall survival between the groups with or without TACE (Table 4). The present study is the fourth randomized trial to compare the long-term prognosis after the resection of HCC in patients with or without preoperative TACE. However, it is difficult to simply compare these trials. Zhou et al. [18] and Wu et al. [17] enrolled patients with large HCCs, whereas Yamasaki et al. [15] and the current trial enrolled patients with smaller HCCs. In the trial reported by Wu et al. [17], patients who received TACE underwent surgery a mean of 17.9 weeks after the detection of HCC, which was significantly longer than those not receiving TACE, who underwent resection 2.3 weeks after the detection of HCC ($P = 0.009$). In this study, patients in all groups underwent surgery in 20–23 days. Differences in the conclusions of the different trials could be attributed to the differences in the study designs or background characteristics.

We found no significant differences in disease-free survival or overall survival between the entire TACE group (selective and whole-liver groups) and the control group, or among the whole-liver, selective, and control groups, even among patients with tumor size >5 cm (Figs. 2, 3, and 4). The extrahepatic recurrence rate was significantly lower in the selective and whole-liver groups compared with the control group. However, even though preoperative TACE induced complete tumor necrosis, there were no significant differences in the pattern of intrahepatic recurrence or the time until recurrence among the three groups.

In conclusion, preoperative selective TACE or TACE plus whole-liver chemolipiodolization neither reduced the incidence of postoperative recurrence nor prolonged survival in patients with resectable HCC. Thus, despite its safety and feasibility, we cannot recommend preoperative TACE as a routine procedure before hepatectomy in patients with resectable HCC.

Table 4 Results of randomized controlled trials on neoadjuvant transarterial chemoembolization and non-transarterial chemoembolization before hepatectomy for resectable hepatocellular carcinoma (HCC)

Study	Year	Total patients (*n*)	(TACE/non-TACE) patients (*n*)	Percentage of HBV (TACE/non-TACE)	Percentage of HCV (TACE/non-TACE)	Percentage of Child–Pugh class A (TACE/non-TACE)
This study		124	81/43	12/26	73/53	88/91
Zhou et al. [18]	2009	108	52/56	98/98	0/0	84/89
Yamasaki et al. [15]	1996	97	50/47	NR	NR	NR
Wu et al. [17]	1995	52	24/28	75/68	NR	92/86

Study	Mean preoperative tumor size (cm) (TACE/non-TACE)	Cytotoxic agent	TACE sessions per patient (*n*)	Complete necrosis (%) (TACE/non-TACE)
This study	4.1/5.0	EPI	1	**21/0**
Zhou et al. [18]	9.0/9.5	5FU, CDDP	1.5	**15/0**
Yamasaki et al. [15]	3.1/3.3	DOX	1	**16/NR**
Wu et al. [17]	14.3/14.5	DOX	3	NR/NR

Study	Morbidity (%) (TACE/non-TACE)	Mortality (%) (TACE/non-TACE)	3-year disease-free survival (%)	3-year overall survival (%) (TACE/non-TACE)
This study	10/19	1/2	28/32	75/60
Zhou et al. [18]	**Adhesions and longer operating time in TACE group**	0/0	26/21	40/32
Yamasaki et al. [15]	NR	6/9	54/42	91/88
Wu et al. [17]	NR	4/7	40/50	**33/60**

Significant differences are shown in **bold**. The number of patients receiving TACE in this study was 81 (42 patients in the selective group and 39 patients in the whole-liver group)

TACE transcatheter arterial chemoembolization, *NR* not reported, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *EPI* epirubicin, *5FU* 5-fluorouracil, *CDDP* cisplatin, *DOX* doxorubicin

Conflict of interest None.

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ELSEVIER

Stimulation of Liver Regeneration After Hepatectomy in Mice by Injection of Bone Marrow Mesenchymal Stem Cells via the Portal Vein

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ABSTRACT

Aim. To investigate whether mouse bone marrow mesenchymal stem cells (BMC) stimulate liver regeneration after partial hepatectomy.

Methods. Isolated BMCs were purified by density gradient centrifugation. We performed a 70% hepatectomy in male BALB/c mice followed by injection of BMCs into the portal vein (PV-BMC group), or the tail vein (IV-BMC group), or of saline into the portal vein (control group).

Results. The wet weight of the liver remnant increased significantly in the PV-BMC group at 3 and 5 days after hepatectomy compared with the IV-BMC and control groups. The Ki-67 labeling index revealed that the increase to result from stimulation of DNA synthesis. The constitutive interleukin-6 and hepatocyte growth factor mRNAs in the remnant liver tended to increase in the PV-BMC group at 3 days after hepatectomy.

Conclusions. These results demonstrated that BMC injection into the portal vein enhanced liver growth after partial hepatectomy in mice.

AUTOLOGOUS BONE MARROW MESENCHYMAL STEM CELL (BMC) therapy has shown great promise to enhance tissue regeneration in a range of acute and chronic diseases.[1] Prospects for enhanced cardiac regeneration after myocardial infarction have received the greatest attention. The recent 5-year outcome data of BMC transplantation after myocardial infarction demonstrated long-standing improvement in cardiac performance and mortality.[2] BMC therapy has been shown to enhance hepatic regeneration in acute and chronic settings in both preclinical studies and pilot clinical investigations.[3,4] The administration of BMCs following liver resection has not been investigated as yet. Autologous BMC therapy may provide an effective treatment option to facilitate regeneration after liver resection. Administration of autologous BMCs offers a clear advantage over nonautologous cell therapies, as it avoids the requirement for immunosuppression and the risk of sensitization. In this study, we aimed to clarify the role of BMCs in liver regeneration after massive hepatectomy.

MATERIALS AND METHODS

Animals

C57BL/6 (B6) mice were purchased from Shimizu Laboratory Supplies (Shizuoka, Japan). All mice were kept in a pathogen-free room, and 8- to 10-week-old male mice were used in the present study. The university's Committee for Animal Research approved all experiments. BMCs were harvested from the femoral, tibial, and pelvic bones of the mice and suspended in phosphate-buffered saline (PBS). The BMCs were then filtered through a 70-μm nylon wool mesh (Bectone Dickinson Labware, Franklin Lakes, NJ, USA) and centrifuged at 1500 rpm for 7 minutes at 4°C. After centrifugation, the BMCs were suspended and adjusted to 3.0×10^9 cells/mL in PBS containing 2% fetal calf serum.

Seventy percent hepatectomy was performed as previously described by Higgins and Anderson.[5] Briefly, the left lateral, left median, and right median lobes were removed with a single ligature under pentobarbital anesthesia. The mice were divided into three groups: in one group BMCs (3.0×10^7) were injected into the portal vein immediately after hepatectomy (PV-BMC group); in one group BMCs (3.0×10^7) were injected into the tail vein

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immediately after hepatectomy (IV-BMC group); in one group saline was injected into the portal vein immediately after hepatectomy (control group). The livers were excised, weighed, and preserved at 1, 3, or 5 days after hepatectomy (n = 7–10 animals per group for each time point) for subsequent molecular and histological analysis.

Assessment of Liver Regeneration

The weight of regenerated liver was used to calculate the growth of the residual liver lobes, using the formula: weight of regenerated liver/preoperative liver weight × 100 (%). The preoperative liver weight was assumed to be the resected liver weight at hepatectomy × 100/70.

Histology of Liver Tissue

To assess the number of hepatocyte mitoses, liver sections were stained with hematoxylin-eosin, and the percentage of hepatocytes undergoing mitosis was calculated. Liver sections were also incubated with Ki-67 antibody (Novocastra Laboratories Ltd, UK) and the ratio of Ki-67 positive/total hepatocytes was calculated.

mRNA Analysis of Liver Tissue

Total RNA from 50 mg of liver tissue was isolated using TRIzol Reagent (Life Technologies, Rockville, Md, USA) according to the manufacturer's instructions. RNA concentration was determined spectrophotometrically. cDNA was prepared by reverse transcription of 1 mg of total RNA using oligo (dT)18 primer (Biolabs, Frankfurt am Main, Germany) and Superscript II RNaseH-Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). Mouse hepatocyte growth factor (HGF) and interleukin-6 (IL-6) were amplified by polymerase chain reaction (PCR) for 35 to 40 cycles using Taq polymerase (Perkin-Elmer, Rodgau-Jugesheim, Germany). In a comparable assay, RNA integrity and cDNA synthesis were tested using mouse elongation factor (EF)-1α as a housekeeping gene. PCR products were separated by electrophoresis on 2.0% agarose gels. Ethidium-bromide-stained bands were visualized by UV illumination and desitometrically quantified (TotalLab). The data represent expression of HGF and IL-6 gene product in relation to EF-1α.

Statistical Analysis

All data are expressed as the mean ± standard deviation of samples. Statistical analyses were carried out with one-way analysis of variance and significant data were examined by Bonferroni-Dunn multiple comparisons post hoc test. In all cases, a P value < .05 was considered significant.

RESULTS

Liver Regeneration and Ki-67 Labeling Index

Figure 1 shows liver regeneration, expressed as a percentage of the calculated original liver weight. Liver weight increased in the control and IV-BMC groups, reaching about 65% and 70% of the prehepatectomy weight on day 5, respectively. Portal vein injection of BMCs resulted in an approximately twofold increase in weight regeneration over the control and IV-BMC groups on days 3 and 5. The mitotic index was significantly higher in the PV-BMC group than in the control and IV-BMC groups on day 3 after

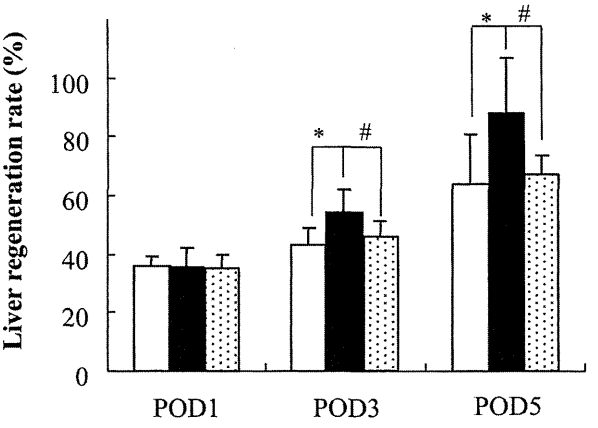

Fig 1. The effect of bone marrow mesenchymal stem cell (BMC) injection on liver regeneration. Liver regeneration in the control group (□), portal vein BMC group (■), and tail vein BMC group (▒) mice are shown as the mean ± standard deviation (n = 7–10). POD, postoperative day. *P < .05 versus control; #P < .05 vs tail vein BMC group.

hepatectomy (control, 1.9% ±1.9%; PV-BMC, 14.1% ± 5.8%; IV-BMC, 6.1% ± 3.0%; P = .0002 between the PV-BMC and control groups, P = .0072 between the PV-BMC and IV-BMC groups). The Ki-67 labeling index was also significantly higher in the PV-BMC group than in the control and IV-BMC groups. These results indicate that portal vein injection of BMCs accelerates liver regeneration in the early period after hepatectomy (control, 9.3% ± 4.7%; PV-BMC, 45.4% ± 17.9%; IV-BMC, 22.8% ± 18.2%; P = .0002 between PV-BMC and control groups, P = .0371 between PV-BMC and IV-BMC groups).

Growth Factors

We analyzed IL-6 and HGF expression by reverse transcriptase PCR in the three groups after hepatectomy. Expression of these growth factors was significantly up-regulated in the PV-BMC group on days 1 and 3 compared with the IV-BMC and control groups (data not shown).

DISCUSSION

The liver has a large capacity for regeneration after resection. However, below a critical level of remnant liver volume, partial hepatectomy is accompanied by a significant increase in postoperative liver failure.[6] Evidence that BMCs contribute to liver regeneration is accumulating.[7,8] Am Esch et al reported that portal vein administration of autologous CD133$^+$ BMCs accelerated liver regeneration after clinical portal vein embolization, providing a novel therapy to support hepatic regeneration.[7] Terai and Sakaida reported nine liver cirrhosis cases that underwent autologous bone marrow cell infusion (ABMI) via a peripheral vein and followed their progress for 24 weeks.[8] After ABMI therapy, liver function and Child-Pugh Score were significantly improved at 4 and 24 weeks. There are few

reports describing the administration of BMCs following liver resection. In this study, we evaluated whether injection of BMCs into the portal vein was effective in stimulating liver regeneration after 70% hepatectomy in mice. Our results showed that BMCs stimulated DNA synthesis in hepatocytes and increased the weight of the remnant liver after hepatectomy. We speculate that injection of BMCs into the portal vein accelerates the production of hepatopoietic factors such as IL-6 and HGF in the early period after hepatectomy. The mechanisms by which BMCs may repopulate the regenerating liver are still under discussion. Conversion to liver cells via cell fusion[9,10] or via transdifferentiation without fusion[11] may occur. BMCs may also be a potential source of intrahepatic oval cells, which support liver regeneration.[12] Oval cells are assumed to act as intrahepatic BMCs with the capacity to differentiate into both hepatocytes and bile duct cells.[13–15] In the near future, autologous BMCs may provide a targeted therapy to enhance hepatic regeneration following liver resection, potentially reducing the risks of the procedure.

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Clinical Science

Clinicopathologic characteristics of patients with non-B non-C hepatitis virus hepatocellular carcinoma after hepatectomy

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KEYWORDS:
Liver cancer;
Hepatectomy;
Non-B non-C hepatocellular carcinoma;
Des-gamma-carboxy prothrombin

Abstract

BACKGROUND: A substantial population of hepatocellular carcinoma (HCC) patients is negative for markers of hepatitis B virus and hepatitis C virus (HCV) infection (non-B non-C hepatitis virus [NBC]).

METHODS: Clinicopathologic data and outcomes were compared retrospectively for HCC patients with hepatitis B virus, HCV, and NBC who had undergone hepatectomy.

RESULTS: The TNM stage was significantly higher, and the prevalence of cirrhosis was significantly lower, in the NBC group compared with the HCV group. Among patients with a maximum tumor diameter of 5 cm or less, the survival rates were significantly higher in the NBC group than in the HCV group. Multivariate analysis revealed that preoperative serum des-gamma-carboxy prothrombin (DCP) level was a prognostic factor for survival in NBC–HCC patients. The DCP/tumor size ratio was significantly higher in NBC–HCC patients with normal liver histology than in patients with hepatitis or cirrhosis.

CONCLUSIONS: NBC–HCC patients had more advanced tumors compared with HCV–HCC patients, but significantly higher survival rates. Measurement of DCP potentially is significant for early diagnosis of NBC HCC, which may increase the chance of curative therapy without recurrence.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide.[1] Although most HCC cases are still found in Asia and Africa, recent studies have shown that the incidence of HCC, and mortality resulting from HCC, are increasing in North America and Europe.[2,3] Evidence shows that 50% of all cases of HCC worldwide are associated with hepatitis B virus (HBV) infection, with a further 25% associated with hepatitis C virus (HCV).[4–6] Some recent reports have shown that the primary risk factor for developing HCC is cirrhosis.[4–8] The annual incidence rates of HCC in patients with cirrhosis resulting from HBV and HCV are reported as 1% to 8% and 1% to 15%, respectively.[6–8] The oncogenic mechanisms and the clinicopathologic characteristics of HCC strongly are influenced by HBV or HCV infection.[9–11] For example, patients with HBV-related HCC have a shorter history of infection and better liver function reserve than those with HCV-related HCC. HCV antibody (HCVAb)-positive HCC accounts for more than 80% of all cases of HCC in Japan, and these patients are characteristically older and have more severe

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cirrhosis than those with hepatitis B surface antigen (HBsAg)-positive HCC. The prognosis of HCVAb-positive HCC is worse than that of HBsAg-positive HCC[9] because multicentric carcinogenesis is more common in patients with HCV infection than in patients with HBV infection,[10,12,13] and the outcome after resection differs between patients with HBV and HCV infection.[14–16]

Although most HCC is associated with viral infection, there is a substantial population of HCC patients (5%–15%) who are negative for markers of HBV and HCV infection (non-B non-C hepatitis virus [NBC]) in Japan and Taiwan.[10,11,17–22] Although several studies have compared the clinicopathologic features of patients with NBC HCC and viral-related HCC, controversy remains about the liver function of NBC-HCC patients, the biological behavior of their tumors, and the outcome after surgical treatment. In the present study, we retrospectively analyzed NBC-HCC patients who had undergone potentially curative resection to determine the risk factors for recurrence after hepatectomy and to help clarify the appropriate treatment for this type of HCC.

Materials and Methods

Subjects

Between February 1992 and January 2009, a total of 534 patients with HCC underwent curative resection (defined as macroscopic removal of all tumor) at our institution. Of these, 19 patients died before hospital discharge and the remaining 515 were followed up as outpatients. We excluded 7 patients with both HBsAg and HCVAb, 1 patient with autoimmune hepatitis, 1 patient with primary biliary cirrhosis, and 10 patients with alcoholic cirrhosis. The remaining 496 patients were divided into the following 3 groups: the HBV-HCC group (n = 85), which were positive for HBsAg and negative for HCVAb; the HCV-HCC group (n = 351), which were negative for HBsAg and positive for HCVAb; and the NBC-HCC group (n = 60), which were negative for both HBsAg and HCVAb.

Clinicopathologic variables and surgery

Before surgery, each patient underwent conventional liver function tests, measurement of the indocyanine green retention rate at 15 minutes (ICGR15), and technetium-99m-diethylenetriamine-pentaacetic acid-galactosyl-human serum albumin liver scintigraphy.[23] Hepatitis virus screening was performed by measurement of HBsAg and HCVAb. α-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) levels were measured in all patients. Surgical procedures were classified according to the Brisbane terminology proposed by Strasberg et al.[24] Anatomic resection was defined as resection of the tumor together with the related portal vein branches and corresponding hepatic territory, and was classified as hemihepatectomy (resection of half of the liver), extended hemihepatectomy (hemihepatectomy plus removal of additional contiguous segments), sectionectomy (resection of 2 Couinaud subsegments[25]), or segmentectomy (resection of 1 Couinaud subsegment). All other procedures were classified as limited resection, which frequently was performed for peripheral or central tumors. Peripheral tumors and those with extrahepatic growth were treated by partial hepatectomy because this procedure achieved a sufficient surgical margin. Central tumors located near the hepatic hilum or major vessels were treated by enucleation only because it was too difficult and/or dangerous to remove enough liver tissue to obtain an adequate margin. One senior pathologist reviewed all specimens for histologic confirmation of the diagnosis. The width of the surgical margin was measured as the distance from the tumor edge to the resection line.

Follow-up evaluation

Perioperative and postoperative complications and deaths were recorded to determine morbidity and mortality after hepatectomy.

All patients who survived were followed up at least every 3 months after discharge. Follow-up evaluation included physical examination, liver function tests, chest radiographs to check for pulmonary metastases, and ultrasonography, computed tomography, or magnetic resonance imaging to check for intrahepatic recurrence. Chest computed tomography was performed if the chest radiograph showed any abnormalities. Bone metastases were diagnosed by bone scintigraphy.

When recurrence of HCC was detected by changes in tumor markers or on imaging, recurrence limited to the remnant liver was treated by transarterial chemoembolization, lipiodolization, re-resection, or percutaneous local ablative therapy such as radiofrequency ablation. When extrahepatic metastases were detected, active treatment was undertaken in patients with good hepatic functional reserve (Child–Pugh class A or B) and good performance status (0 or 1), whereas other patients were given only radiation therapy to relieve symptoms of bone metastases. Surgical resection was undertaken in patients with a solitary extrahepatic metastasis and no intrahepatic recurrence.

Prognostic factors

We performed univariate and multivariate analyses of 30 clinicopathologic factors to identify independent variables related to postoperative disease-free survival and overall survival of NBC–HCC patients. The patient factors we investigated were sex, age, body mass index, alcohol abuse, the presence or absence of diabetes mellitus, and liver function (including albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, prothrombin time, cholinesterase, platelet count, alkaline phospha-

tase, ICGR15, maximal removal rate of GSA, and Child–Pugh class). The tumor factors investigated were AFP level, DCP level, histologic features (including tumor diameter, differentiation, microscopic capsule formation, surgical margin, and vascular invasion), the number of tumors, and stage according to the TNM classification.[26] The surgical factors investigated were surgical time, blood loss, perioperative blood transfusion, surgical procedure, and complications. All the variables that were significant according to univariate analysis then were examined with a Cox proportional hazards model to identify variables that had an independent influence on disease-free survival or overall survival.

Statistical analysis

Continuous variables are presented as mean ± standard deviation. The significance of differences among the 3 groups was assessed by the chi-square test, the Mann–Whitney *U* test, or the Kruskal–Wallis test as appropriate. Correlations between various tumor factors and the DCP/tumor size ratio were determined by the Pearson correlation coefficient analysis. The Kaplan–Meier method was used to calculate the disease-free survival rate and overall survival rate as of June 2009, and the significance of differences in survival rates was estimated with the generalized log-rank test. The Cox regression model (stepwise method) was used for multivariate analysis. In all analyses, a *P* value less than .05 was considered statistically significant.

Results

Preoperative characteristics

Table 1 summarizes the preoperative characteristics of the 3 groups of HCC patients. Patients in the NBC–HCC group had a significantly higher body mass index and a higher prevalence of diabetes mellitus than patients in the HBV–HCC and HCV–HCC groups. The mean age of the patients in the HBV–HCC group was significantly lower than that of the NBC–HCC and HCV–HCC groups. There were no significant differences in the prevalence of preoperative alcohol abuse among the 3 groups. The NBC–HCC and HBV–HCC groups had significantly better preoperative liver function (serum albumin, alanine aminotransferase, platelet count, and ICGR15) than the HCV–HCC group. The NBC–HCC and HBV–HCC groups had significantly higher AFP and DCP levels than the HCV–HCC group.

Perioperative parameters and pathologic findings

Table 2 shows that surgical time, blood loss, and blood transfusion did not differ significantly among the 3 groups.

Table 1 Preoperative clinical characteristics of the 3 groups of HCC patients[1]

	NBC–HCC group (n = 60)	HBV–HCC group (n = 85)	HCV–HCC group (n = 351)
Sex, male/female	52/8	68/17	272/79
Age, y	66.6 ± 13.3	59.3 ± 11.4*	66.5 ± 7.1
BMI, kg/m^2	24.2 ± 3.6†	22.8 ± 3.2	22.4 ± 2.8
Alcohol abuse, +/−	30/30	40/45	139/212
Diabetes, +/−	25/35‡	21/64	81/270
Child–Pugh class A/B	54/6	74/11	317/34
ICGR15, %	15.3 ± 10.9	14.6 ± 10.2	20.0 ± 9.9§
Platelet count, $10^4/\mu L$	18.5 ± 9.5	18.1 ± 7.2	13.5 ± 6.6‖
Total bilirubin level, mg/dL	.78 ± .31	.80 ± .63	.88 ± .33¶
Albumin level, g/dL	3.84 ± .53	3.81 ± .45	3.68 ± .43#
Prothrombin time, %	92 ± 14	89 ± 14	88 ± 13**
ALT level, U/L	33 ± 21	44 ± 36	59 ± 39††
AFP, ng/mL	2,246 ± 8,584	15,600 ± 55,009	454 ± 3,250‡‡
DCP, mAU/mL	3,755 ± 9,726	4,655 ± 12,379	1,527 ± 6,464§§

Data represent the mean ± standard deviation or the number of patients.
ALT = alanine aminotransferase; BMI = body mass index.
*P = .0006 and <.0001 versus NBC–HCC and HCV–HCC patients.
†P = .0108 and <.0001 versus HBV–HCC and HCV–HCC patients.
‡P = .0307 and .0024 versus HBV–HCC and HCV–HCC patients.
§P = .0388 and <.0001 versus NBC–HCC and HBV–HCC patients.
‖P < .0001 and <.0001 versus NBC–HCC and HBV–HCC patients.
¶P = .0480 versus NBC–HCC patients.
#P = .0126 and .0178 versus NBC–HCC and HBV–HCC patients
**P = .0325 versus NBC–HCC patients.
††P < .0001 and .0016 versus NBC–HCC and HBV–HCC patients.
‡‡P = .0045 and <.0001 versus NBC–HCC and HBV–HCC patients.
§§P = .0245 and .0014 versus NBC–HCC and HBV–HCC patients.

Table 2 Intraoperative and postoperative characteristics of the 3 groups of HCC patients

	NBC–HCC group (n = 60)	HBV–HCC group (n = 85)	HCV–HCC group (n = 351)
Surgical time, min	319 ± 119	305 ± 114	288 ± 111
Surgical blood loss, mL	1,498 ± 1,450	1,538 ± 2,410	1,465 ± 1,736
Blood transfusion, +	28 (47%)	37 (44%)	145 (41%)
Surgical procedure			
Limited resection	40 (67%)	47 (55%)	290 (83%)*
Anatomic resection	20 (33%)	38 (45%)	61 (17%)
Patients with complications, n	10 (17%)	7 (8%)	86 (25%)†
Tumor size, cm	5.57 ± 4.58	5.43 ± 4.03	3.55 ± 2.48‡
Tumor differentiation			
Well or moderate	56 (93%)	74 (87%)	304 (87%)
Poor or necrosis	4 (7%)	11 (13%)	47 (13%)
Microscopic surgical margin, +	3 (5%)	12 (14%)	38 (11%)
Microvascular invasion, +	26 (43%)	48 (56%)	154 (44%)
Tumors, n			
Single	50 (83%)	59 (69%)	262 (75%)
Multiple	10 (17%)	26 (31%)	89 (25%)
Underlying liver histology			
Normal	24 (40%)	11 (13%)	14 (4%)
Hepatitis	21 (35%)	51 (60%)	181 (52%)
Cirrhosis	15 (25%)	23 (27%)§	156 (44%)‖
Tumor stage (TNM)			
I or II	32 (53%)	43 (51%)	249 (71%)
III or IV	28 (47%)	42 (49%)	102 (29%)¶

Data represent the mean ± standard deviation or the number of patients.
NS = not significant.
*P = .0041 and <.0001 versus NBC–HCC and HBV–HCC patients.
†P = .001 versus HBV–HCC patients.
‡P < .0001 and <.0001 versus NBC–HCC and HBV–HCC patients.
§P = .0005 versus NBC–HCC patients.
‖P < .0001 and .0004 versus NBC–HCC and HBV–HCC patients.
¶P = .0067 and .0003 versus NBC–HCC and HBV–HCC patients.

The NBC–HCC and HBV–HCC groups had a lower percentage of limited resection cases than the HCV–HCC group. On pathologic examination, tumor size was significantly larger and TNM stage was significantly more advanced in the NBC–HCC and HBV–HCC groups than in the HCV–HCC group. Normal liver histology was significantly more common in the NBC–HCC group than in the other 2 groups.

Outcome

The disease-free survival and overall survival rates of all 496 patients were 35% and 73% at 3 years, 22% and 57% at 5 years, and 11% and 26% at 10 years, respectively (Fig. 1). There were significant differences in disease-free survival rates between the NBC–HCC or HBV–HCC groups and the HCV–HCC group (45% in NBC–, 41% in HBV–, and 33% in HCV–HCC patients at 3 years; 35%, 32%, and 17% at 5 years; and 30%, 29%, and 11% at 7 years, respectively; P = .0395), although there were no significant differences in overall survival rates among the groups (75% in NBC, 66% in HBV, and 74% in HCV–HCC patients at 3 years; 62%, 53%, and 57% at 5 years; and 58%, 37%, and 44% at 7 years, respectively; P = .2123). Among patients with a maximum tumor diameter of 5 cm or less, disease-free survival rates were significantly higher in the NBC–HCC and HBV–HCC groups than in the HCV–HCC group (P = .0003 and P = .0073, respectively) (Fig. 2A), and

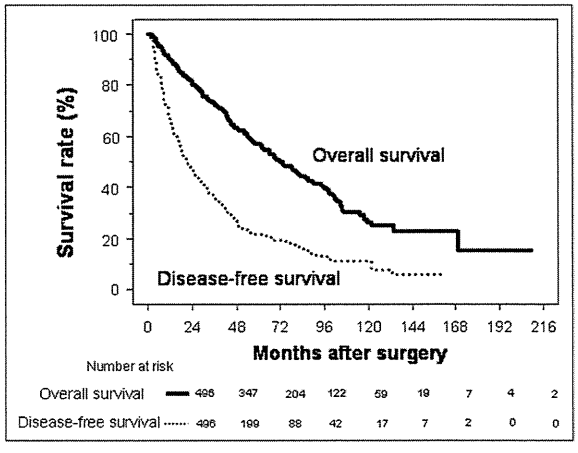

Figure 1 Disease-free and overall survival rates in all 496 patients after hepatectomy for HCC.

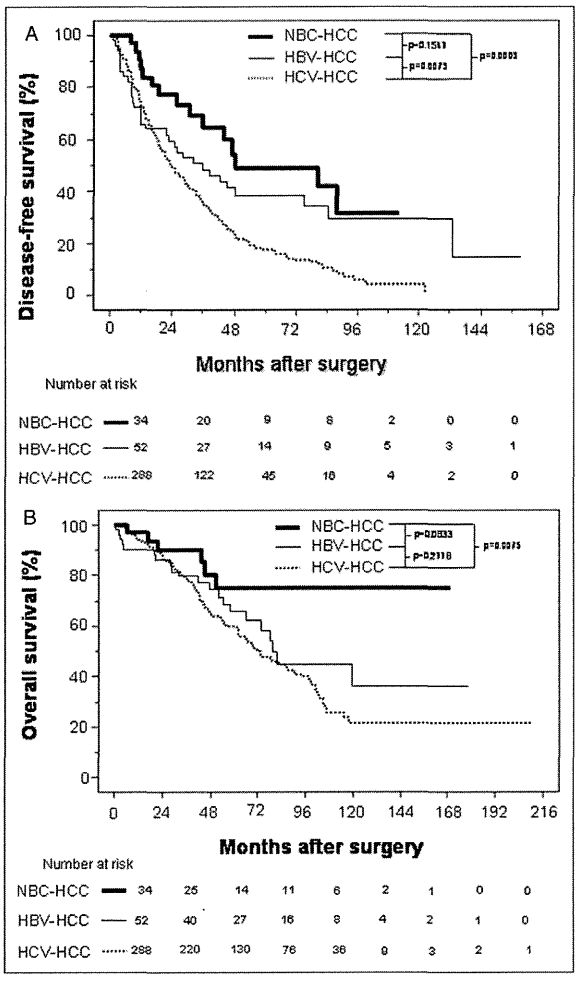

Figure 2 Comparisons of disease-free survival and overall survival rates after hepatectomy among patients in the HBV–HCC, HCV–HCC, and NBC–HCC groups with a maximum tumor diameter of 5 cm or less. (A) Disease-free survival. The survival rate of HCV–HCC patients (dotted line) was significantly lower than that of NBC–HCC (unbroken thick line, $P = .0003$) or HBV–HCC patients (unbroken thin line, $P = .0073$). (B) Overall survival. The survival rate of NBC–HCC patients (unbroken thick line) was significantly better than that of HCV–HCC patients (dotted line, $P = .0075$). The numbers of patients at risk are shown below each graph.

there was also a significant difference in overall survival rate between the NBC–HCC group and the HCV–HCC group ($P = .0075$) (Fig. 2B).

Factors affecting disease-free survival and overall survival rates

Univariate analysis showed that the factors associated with lower disease-free survival in the NBC–HCC group were sex, serum albumin level, DCP level, number of tumors, microscopic vascular invasion, and TNM stage, whereas the factors associated with lower overall survival were serum albumin level, total bilirubin level, prothrombin time, DCP level, and complications. Table 3 shows the results obtained by multivariate analysis (Cox proportional hazards model) of factors with an influence on disease-free survival or overall survival rates. A serum albumin level less than 3.9 g/dL, a DCP level of 300 mAU/mL or greater, and multiple tumors were identified as independent prognostic indicators of disease-free survival, whereas a serum albumin level less than 3.9 g/dL and a DCP level of 300 mAU/mL or greater had an independent influence on overall survival.

Correlations between tumor factors and DCP/tumor size ratio or underlying liver histology in NBC–HCC patients

There were significant correlations between tumor size and both AFP and DCP levels in our 496 patients (AFP: $r = .347$, $P < .0001$; DCP: $r = .562$, $P < .0001$). We divided the AFP and DCP levels by tumor diameter to calculate tumor marker/tumor size ratios. The DCP/tumor size ratio was correlated positively with tumor stage ($r = .34$; $P = .0091$), number of tumors ($r = .392$; $P = .0026$), tumor histology ($r = .42$; $P = .0018$), and microvascular invasion (MVI) ($r = .546$; $P < .0001$), whereas the AFP/tumor size ratio was not (data not shown).

Correlations between perioperative factors and underlying liver histology were analyzed in the NBC–HCC group (normal histology, n = 24; hepatitis, n = 21; or cirrhosis, n = 15). There were significant correlations between MVI and normal histology (MVI with normal histology, n = 17; hepatitis, n = 5; and cirrhosis, n = 4; $P = .0021$), and between anatomic resection and normal histology (anatomic resection with normal histology, n = 16; hepatitis, n = 4; and cirrhosis, n = 0; $P < .0001$). There were no significant correlations between histology and blood loss, blood transfusion, tumor stage, number of tumors, or tumor differentiation.

Correlations between tumor marker/tumor size ratios and underlying liver histology

The AFP/tumor size ratio in the NBC–HCC group was 316 ± 918 ng/mL/cm, 50 ± 101 ng/mL/cm, and 842 ± 581 ng/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. The AFP/tumor size ratio in the HBV–HCC group was 3,175 ± 3,241 ng/mL/cm, 1,774 ± 4,346 ng/mL/cm, and 394 ± 691 ng/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. The AFP/tumor size ratio in the HCV–HCC group was 43 ± 89 ng/mL/cm, 107 ± 537 ng/mL/cm, and 73 ± 160 ng/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. There were no significant correlations between the AFP/tumor size ratio and underlying histology in the NBC–HCC, HBV–HCC, or HCV–HCC groups.

Table 3 Prognostic factors for disease-free survival and overall survival identified by multivariate analysis in NBC–HCC patients

Variable	Coefficient	Standard error	Relative risk	*P* value
Disease-free survival				
Albumin level, <3.9 g/dL	1.110	.414	3.034	.0074
DCP, ≥300 mAU/mL	.923	.396	2.959	.0063
Multiple tumors	.749	.446	3.802	.0028
Overall survival				
Albumin level, <3.9 g/dL	1.560	.647	4.761	.0159
DCP, ≥300 mAU/mL	.813	.541	2.778	.0389

The DCP/tumor size ratio in the NBC–HCC group was 757 ± 1,142 mAU/mL/cm, 146 ± 270 mAU/mL/cm, and 132 ± 153 mAU/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. The DCP/tumor size ratio in the HBV–HCC group was 719 ± 867 mAU/mL/cm, 692 ± 1,734 mAU/mL/cm, and 489 ± 654 mAU/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. The DCP/tumor size ratio in the HCV–HCC group was 363 ± 464 mAU/mL/cm, 301 ± 934 mAU/mL/cm, and 135 ± 756 mAU/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. There were no significant correlations between the DCP/tumor size ratio and underlying histology in the HBV–HCC and HCV–HCC groups. In contrast, the DCP/tumor size ratio was significantly higher in NBC–HCC patients with normal histology than with hepatitis or cirrhosis ($P = .0211$ and $P = .0426$, respectively).

Comments

Although most HCC still occurs in patients with persistent HCV infection, the incidence of HCV HCC has been decreasing over the past years because of the promotion of anti-HCV therapy[27] and a decrease in the number of patients with chronic HCV infection.[28] In recent reports, the percentage of HCC patients with NBC HCC has reached as high as 20%, and the incidence is increasing.[10,11,17–22,29] These findings indicate that NBC HCC may become more important in the near future.

We found that NBC–HCC patients were significantly older than HBV–HCC patients, but not HCV–HCC patients, which is consistent with a previous report by Dohmen et al.[30] It seems that although most HBV–HCC cases result from vertical transmission of HBV in infancy, causing HCC at a young age, NBC HCC develops over a long time period later in life.[31] Abe et al reported that the most important etiologic factor for the development of NBC HCC is alcohol consumption, followed by nonalcoholic fatty liver disease.[29] However, we did not find a higher prevalence of alcohol abuse among NBC–HCC patients in the present study. Comparisons among the 3 groups found that preoperative liver function was best in the NBC–HCC group. The smaller tumors and lower levels of AFP and DCP in the HCV–HCC group may reflect periodic screening for HCC in these patients. Normal liver histology was significantly more common in the NBC–HCC group than in the HCV–HCC or HBV–HCC groups. Assessment of liver histology revealed a lower grade of inflammation and earlier stage of fibrosis in the NBC–HCC group. The increased frequency of anatomic resection in the NBC–HCC group may have been related to the better liver function compared with the HCV–HCC group.

Tumor size, multiple tumors, portal invasion, and curative resection with an adequate surgical margin have been reported as prognostic indicators in NBC–HCC patients undergoing resection.[32,33] In the present study, we found that a lower serum albumin level and a higher DCP level were independent predictors of disease-free survival and overall survival in the NBC–HCC group. There has been no previous report that the preoperative serum DCP level is a prognostic indicator for NBC HCC. According to Yamamoto et al,[34] although both AFP and DCP increase with tumor growth, DCP is a more accurate tumor marker. In most NBC–HCC patients, the tumor is discovered at an advanced stage[17–22] because these patients have milder hepatic dysfunction and fewer symptoms compared with HBV–HCC and HCV–HCC patients owing to the absence or mildness of underlying chronic liver disease. Because they have fewer symptoms, NBC–HCC patients visit the hospital less frequently than HBV–HCC or HCV–HCC patients, and thus undergo imaging studies less frequently. NBC HCC usually is not discovered until the tumor is large enough to cause abdominal pain and distension.

We found significant differences in disease-free survival rates, but not overall survival rates, among the 3 groups. However, both disease-free and overall survival rates of NBC–HCC patients after hepatic resection were significantly higher if the maximum tumor diameter was 5 cm or less. Several studies have indicated that large tumors, especially those greater than 5 cm in size, have a significantly higher risk of recurrence.[35–38] The influence of size is attributed to the increased invasiveness of larger tumors, as shown by a higher incidence of intrahepatic metastases and portal venous invasion.[39,40] Therefore, in HCC patients with larger tumors, survival depends on progression of the tumor itself (including its size and/or number of lesions) irrespective of the type of underlying hepatitis virus infection. Although NBC–HCC patients with normal liver histology often had advanced tumors with microvascular invasion, these patients showed higher survival rates owing to curative anatomic resection and good residual liver function. We

also found that measurement of DCP level in the perioperative and postoperative periods may be a useful prognostic indicator. If measurement of DCP level in NBC–HCC patients could lead to earlier diagnosis of the primary tumor or of recurrence after surgical resection, the use of DCP levels for screening may improve prognosis.

In conclusion, we found that the DCP/tumor size ratio of NBC–HCC patients was correlated positively with several tumor factors, including tumor stage, the number of tumors, tumor histology, and microvascular invasion. NBC–HCC patients had significantly higher survival rates after hepatic resection than HBV–HCC or HCV–HCC patients because they generally had milder hepatic dysfunction. The DCP/tumor size ratio was significantly higher in NBC–HCC patients with normal histology than those with hepatitis or cirrhosis. These findings should be considered when treating NBC–HCC patients. Further prospective studies are required to fully evaluate the significance of DCP level in NBC patients with potentially curable HCC.

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Clinicopathological Features of Recurrence in Patients After 10-year Disease-free Survival Following Curative Hepatic Resection of Hepatocellular Carcinoma

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Abstract

Background The present study aimed to clarify the clinicopathologic features of long-term disease-fee survival after resection of hepatocellular carcinoma (HCC).

Methods This retrospective study identified 940 patients who underwent curative resection of HCC between 1991 and 2000 at five university hospitals. Seventy-four patients with 10 years of recurrence-free survival were identified and followed up. They were divided into two groups, 60 recurrence-free and 14 with recurrence after a 10-year recurrence-free period.

Results Overall survival rates of recurrence and non-recurrence groups were 68 and 91 % at 16 years, and 34 and 91 % at 20 years ($p = 0.02$), respectively. There were five (36 %), and two deaths (3 %), respectively, after 10 recurrence-free years. A second resection for recurrence was performed in four patients (29 %), and mean survival was 15.3 years after the first hepatectomy. Although three patients in the non-recurrence group (5 %) developed esophageal and/or gastric varices, seven patients in the recurrence group (50 %) developed varices during 10 years ($p < 0.0001$). In multivariate analysis, preoperative and 10-year platelet count was identified as a favorable independent factor for maintained recurrence-free survival after a 10-year recurrence-free period following curative hepatic resection of HCC.

Conclusions Recurrence of HCC may occur even after a 10-year recurrence-free period. Long-term follow-up after resection of HCC is important, and should be life-long. Patients with higher preoperative and 10-year platelet counts are more likely to have long-term survival after resection. A low platelet count, related to the degree of liver fibrosis, is a risk factor for recurrence and survival of HCC after curative resection.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide [1]. Although the incidence of HCC is highest in Asia and Africa, recent studies have shown that the incidence and mortality rates of HCC are also rising in North America and Europe [2, 3]. There has been an increase in the number of reports of nonsurgical therapeutic options for small HCC, such as percutaneous ethanol injection therapy [4], microwave coagulation therapy [5], and percutaneous radiofrequency ablation (RFA) [6], but there is ongoing controversy regarding the best method to treat small tumors. In Japan, liver transplantation is not a practical option for most HCC patients, because the national health insurance scheme only covers transplantation for patients with decompensated cirrhosis whose

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tumors fit the Milan criteria. Resection is therefore generally the first-line treatment for patients with small tumors and underlying chronic liver disease, but the long-term survival rate after potentially curative resection of HCC is still unsatisfactory because of the high rate of recurrence [7]. To improve prognosis, it is important to prevent the recurrence of HCC after its initial resection, but standard therapy for intrahepatic metastasis has not yet been developed. Therefore, the number of reports on long-term survivors after the initial hepatectomy remains small, especially on the basis of long-term (>10 years) observation of patients, with or without recurrence [8–12]. Furthermore, there are few reports regarding the characteristics of patients who survive without recurrence for at least 10 years after curative liver resection. In the present study, we retrospectively analyzed patients with long-term survival and compared them with patients who had recurrence of HCC after a 10-year disease-free period, in order to gain insight into the demography and biological behavior of HCC, and to identify the prognostic factors associated with survival.

Materials and methods

Subjects

Between 1991 and 2000, a total of 940 patients with HCC underwent curative resection (defined as macroscopic removal of all tumors) at five participating university hospitals. Thirty two patients died in the hospital and the remaining 908 were followed up as outpatients. Seventy-four patients (8 %) with 10-year recurrence-free survival after initial hepatectomy were included in this study. The median and mean duration of follow-up for the maximum time or until death was 12.6 and 13.3 years (range: 10.0–21.0 years). The median age of the patients at the initial hepatectomy was 59 years (range: 26–77 years). The 74 patients were stratified into a recurrence group and a recurrence-free group.

Clinicopathologic variables and surgery

Before surgery, each patient underwent conventional liver function tests, measurement of the indocyanine green retention rate at 15 min (ICGR15), and measurement of serum type IV collagen 7S. Hepatitis virus screening was done by measurement of hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (HCVAb). The levels of alpha-fetoprotein (AFP) and protein induced by vitamin K absence/antagonism-II (PIVKA-II) were also measured in all patients. Surgical procedures were classified according to the Brisbane terminology proposed by Strasberg et al. [13]. Accordingly, anatomic resection was defined as resection of the tumor together with the related portal vein branches and the corresponding hepatic territory, and was classified as hemihepatectomy (resection of half of the liver), extended hemihepatectomy (hemihepatectomy plus removal of additional contiguous segments), sectionectomy (resection of two Couinaud subsegments [14]), or segmentectomy (resection of one Couinaud subsegment). All of the non-anatomically designated procedures were classified as limited resection, and were performed in patients with peripheral or central tumors and moderate liver dysfunction [15]. Patients with peripheral tumors and those with extrahepatic growths were treated by partial hepatectomy because this procedure achieved a sufficient surgical margin. In contrast, central tumors located near the hepatic hilum or major vessels were only treated by enucleation because it was too difficult and/or dangerous to remove enough liver tissue to obtain an adequate margin. A senior pathologist at each of the five participating university hospitals reviewed each specimen for histologic confirmation of the diagnosis. The width of the surgical margin was measured as the distance from the tumor edge to the resection line. The tumor stage was defined according to the TNM classification [16]. Histologic staging of fibrosis and grading of necroinflammation in the underlying liver was performed using the Knodell histologic activity index (HAI) [17] at the initial operation and at the second operation for those requiring repeat hepatectomy.

Follow-up

Perioperative/postoperative complications and deaths were recorded to determine the morbidity and mortality of hepatectomy. All surviving patients were followed up after discharge, with physical examination, liver function tests, ultrasonography, computed tomography (CT), or magnetic resonance imaging being performed at least every 3 months to check for intrahepatic recurrence, with chest radiographs obtained to detect pulmonary metastasis. A chest CT was performed if a chest radiograph showed any abnormalities. Bone metastases were diagnosed by bone scintigraphy.

When recurrence of HCC was detected from changes in tumor markers or from imaging findings, recurrence limited to the remnant liver was treated by transarterial chemoembolization (TACE), lipiodolization, second resection, or percutaneous local ablative therapy such as radiofrequency ablation (RFA). After detection of extrahepatic metastases, active treatment was performed in patients with a good hepatic functional reserve (Child-Pugh class A or B) and good performance status (0 or 1), while other patients were only given radiation therapy for bone metastases to relieve

symptoms. Surgical resection was performed in patients with a solitary extrahepatic metastasis and no intrahepatic recurrence. In addition, all patients underwent endoscopy to evaluate the severity of esophageal and/or gastric varices before surgery and at 10 years after surgery.

Prognostic factors

We performed univariate and multivariate analysis of 28 clinicopathologic operative factors to identify independent variables related to the postoperative recurrence of HCC in patients with 10-year recurrence-free survival after curative hepatic resection. The factors investigated were gender, presence or absence of hepatitis C virus (HCV), and liver function (including albumin, alanine aminotransferase [ALT], and platelet count). The tumor factors studied were alpha fetoprotein (AFP), vitamin K absence or antagonist II (PIVKA-II), and histologic features (including tumor diameter, differentiation, vascular invasion, and associated liver disease). The operative factors that we assessed were operative blood loss, perioperative blood transfusion, and surgical procedure. All of the variables that were significant according to univariate analysis were then examined with a Cox proportional hazards model to identify variables that were independently associated with recurrence.

We also performed univariate and multivariate analysis of six clinical factors to identify independent variables related to postoperative recurrence of HCC more than 10 years after curative hepatic resection. The factors investigated were 10-year liver function (including albumin, aspartate aminotransferase [AST], ALT, and platelet count). The tumor factors studied were AFP and PIVKA-II.

Statistical analysis

Continuous variables are presented as mean ± standard deviation. The significance of differences between the recurrence-free and recurrence groups was assessed by the Chi square test or the Mann–Whitney *U*-test, as appropriate. The Kaplan–Meier method was used to calculate the recurrence rate and overall survival rate as of December 2010, and the significance of differences in survival was estimated with the generalized log-rank test. The Cox regression model (stepwise method) was used for multivariate analysis. In all analyses, $p < 0.05$ was considered to indicate statistical significance.

Table 1 Preoperative and 10-year clinical characteristics of hepatocellular carcinoma (HCC) recurrence and non-recurrence groups

Variable	Non-recurrence group ($n = 60$)	Recurrence group ($n = 14$)	p value
Gender, male/female	47/13	13/1	0.21
Age, years	58.4 ± 9.8	58.9 ± 8.8	0.85
HBsAg (±)	16/44	3/11	0.69
HCVAb (±)	23/37	3/11	0.23
Child-Pugh class: A/B	57/3	13/1	0.75
Alcohol abuse (±)	19/41	5/9	0.77
Preoperative TACE (±)	36/24	9/5	0.77
ICGR15, %	11.9 ± 5.6	14.8 ± 5.9	0.16
Albumin, g/dL	4.1 ± 0.3	3.9 ± 0.3	0.08
Albumin (10 years), g/dL	4.1 ± 0.3	3.9 ± 0.4	0.10
Total bilirubin, mg/dL	0.79 ± 0.38	0.80 ± 0.24	0.91
Total bilirubin (10 years), mg/dL	0.75 ± 0.20	0.90 ± 0.60	0.81
Prothrombin time, %	100 ± 18	105 ± 28	0.54
Prothrombin time (10 years), %	102 ± 14	99 ± 19	0.90
Platelet count ($\times 10^4/\mu L$)	19.2 ± 7.3	13.3 ± 3.4	0.02
Platelet count (10 years) ($\times 10^4/\mu L$)	15.5 ± 4.1	12.1 ± 4.2	0.03
AST, IU/L	48 ± 44	38 ± 11	0.48
AST (10 years), IU/L	41 ± 26	55 ± 35	0.23
ALT, IU/L	57 ± 56	46 ± 26	0.56
ALT (10 years), IU/L	39 ± 27	63 ± 50	0.08
Type IV collagen 7S (10 years), ng/ml)[a]	4.93 ± 0.84	7.58 ± 0.79	0.002
AFP, ng/ml	311 ± 1198	918 ± 1646	0.12
AFP (10 years), ng/mL	9.3 ± 11.7	56.1 ± 104.1	0.04
PIVKA-II (mAU/mL)	601 ± 1626	38 ± 44	0.40
PIVKA-II (10 years), mAU/mL	25 ± 29	47 ± 61	0.21

Data presented as mean ± standard deviation (SD) or the number of patients. *HBsAg* hepatitis B surface antigen; *HCVAb* hepatitis C virus antibody; *TACE* transcatheter arterial chemoembolization; *ICGR15* indocyanine green retention rate at 15 min; *AST* aspartate aminotransferase; *ALT* alanine aminotransferase; *AFP* α-fetoprotein; *PIVKA-II* protein induced by vitamin K absence/antagonism-II

[a] Indicated data were not available for all patients. The number of patients with the measurement was 34 and 6 in the non-recurrence and recurrence groups, respectively

Results

Preoperative and postoperative characteristics

Table 1 summarizes the preoperative characteristics of recurrence-free and recurrence groups. No difference was detected between the two groups with respect to gender, age, HBsAg, HCVAb, Child-Pugh class, alcohol abuse, preoperative TACE, ICGR15, and serum albumin, total bilirubin, prothrombin time, AST, ALT, AFP, or PIVKA-II. Postoperative characteristics at 10 years after surgery for each group are listed in Table 1. No difference was

Table 2 Changes in gastric and/or esophageal varices after 10 years of recurrence-free survival following curative hepatic resection

	Recurrence group ($n = 14$)				Non-recurrence group ($n = 60$)			
10 years after surgery: Before surgery	F0	F1	F2	F3	F0	F1	F2	F3
F0	7 (50 %)	5 (36 %)	0	0	54 (90 %)	3 (5 %)	0	0
F1	0	0	1 (7 %)	0	0	3 (5 %)	0	0
F2	0	0	0	1 (7 %)	0	0	0	0

detected between the two groups with respect to serum albumin, total bilirubin, prothrombin time, AST, ALT, and PIVKA-II. Patients in the non-recurrence group had a significantly higher platelet count preoperatively and at 10 years after surgery compared with those in the recurrence group. Patients in the non-recurrence group had significantly lower levels of type IV collagen 7S at 10 years after surgery compared with those in the recurrence group. Patients in the recurrence group had higher AFP levels at 10 years after surgery compared with those in the non-recurrence group. Table 2 shows the changes in esophageal and/or gastric varices after 10 years of recurrence-free survival following hepatic resection. Seven patients in the recurrence group (50 %) developed varices during the 10 years, while only three patients in the non-recurrence group (5 %) developed varices ($p < 0.0001$).

Surgical results and pathologic classification

The operating time, blood loss, blood transfusion requirement, procedures, and complications attributable to surgery did not differ significantly between the two groups. The pathologic features of each group are presented in Table 3. The number of tumors, tumor diameter, histology, associated liver disease, incidence of positive microscopic capsule formation, surgical margins, vascular invasion, and TNM stage did not differ significantly between the two groups.

Factors that influence recurrence of HCC after curative hepatic resection

Table 4 shows univariate and multivariate analyses of potential operative risk factors associated with recurrence of HCC in patients after a 10-year recurrence-free period following curative resection. Variables significantly associated with recurrence in the univariate and multivariate analyses were preoperative albumin $\leq$4.0 g/dL (odds ratio = 17.86; 95 % confidence interval (CI) = 1.64–42.0; $p = 0.02$), and preoperative platelet count $\leq 15 \times 10^4$/mL (odds ratio = 37.48; 95 % CI = 2.25–52.0; $p = 0.01$).

Table 5 shows univariate and multivariate analyses of potential 10-year risk factors associated with recurrence of HCC in patients after 10 years of recurrence-free survival following curative hepatic resection. Univariate and multivariate analyses showed that only platelet count $\leq 15 \times 10^4$/mL at 10 years after surgery (odds ratio = 12.92;

Table 3 Intraoperative and postoperative characteristics of the two groups

Variable	Non-recurrence group ($n = 60$)	Recurrence group ($n = 14$)	p value
Operating time, min	261 ± 77	284 ± 87	0.38
Operative blood loss, mL	1,095 ± 1129	1,508 ± 1,545	0.26
Blood transfusion (±)	18/42	4/10	0.92
Surgical procedure (Anatomic/limited resection)	32/28	5/9	0.24
Number of tumors (single/multiple)	55/5	13/1	0.88
Tumor size, cm	4.48 ± 3.98	3.16 ± 1.65	0.23
Histology (good/moderate/poor/necrosis)	11/30/15/4	3/5/3/3	0.70
Microscopic capsule formation (±)	50/10	13/1	0.37
Surgical margin (±)	8/52	0/14	0.60
Microscopic vascular invasion (±)	16/44	1/13	0.15
Associated liver disease (normal/fibrosis or hepatitis/cirrhosis)	21/31/8	3/8/3	0.55
TNM stage (I or II/III or IV)	48/12	12/2	0.62
Morbidity (±)	8/52	2/12	0.93

Data presented as mean ± SD or the number of patients

Table 4 Preoperative and operative risk factors predicting HCC recurrence in patients after 10 years of recurrence-free survival following curative hepatic resection

Variable	Univariate analysis			Multivariate analysis		
	Odds ratio	95 % CI	*p* value	Odds ratio	95 % CI	*p* value
Male gender	3.60	0.43–20.1	0.24	–	–	–
HCV Ab	0.58	0.14–2.48	0.46	–	–	–
Albumin ≤4.0 g/dL	8.64	1.01–44.62	0.049	17.86	1.64–42.0	0.02
Platelet ≤15 × 10^4/mL	10.75	1.25–60.11	0.03	37.48	2.25–52.0	0.01
ALT >41 IU/L	2.50	0.55–11.11	0.24	–	–	–
AFP >10 ng/ml	1.33	0.41–4.31	0.63	–	–	–
PIVKA-II >40 mAU/mL	6.25	0.68–30.0	0.10	–	–	–
Operative blood loss >800 mL	3.85	0.15–14.67	0.26	–	–	–
Blood transfusion	3.90	0.85–17.82	0.08	9.56	0.68–33.61	0.09
Limited resection	2.51	0.74–8.48	0.14	–	–	–
Tumor size >3.4 cm	3.23	0.92–11.11	0.07	6.67	0.51–50.0	0.15
Vascular invasion	4.95	0.60–11.67	0.14	–	–	–
Poorly differentiated	0.90	0.22–3.71	0.88	–	–	–
Cirrhosis	0.67	0.13–3.39	0.63	–	–	–

CI confidence interval

Table 5 Risk factors at 10 years predicting HCC recurrence in patients after 10 years of recurrence-free survival following curative hepatic resection

Variable	Univariate analysis			Multivariate analysis		
	Odds ratio	95 % CI	*p* value	Odds ratio	95 % CI	*p* value
Albumin ≤4.0 g/dL	4.50	0.89–22.75	0.07	7.69	0.70–43.33	0.10
Platelet ≤15 × 10^4/mL	6.06	1.11–33.33	0.04	12.92	1.08–63.91	0.04
AST >46 IU/L	1.46	0.34–6.35	0.62	–	–	–
ALT >47 IU/L	1.79	0.40–7.91	0.45	–	–	–
AFP >23 ng/mL	8.57	0.76–26.54	0.08	15.22	0.49–68.99	0.12
PIVKA-II >48 mAU/mL	7.29	0.64–32.64	0.11	–	–	–

95 % CI = 1.08–63.91; $p = 0.04$) was an independent predictor of recurrence of HCC.

In the non-recurrence group, the platelet count decreased at 1 year after curative hepatic resection, but there was no further marked decrease thereafter (Fig. 1). On the other hand, the platelet count in the recurrence group gradually decreased at 5 years after surgery. Patients in the non-recurrence group had a significantly higher platelet count preoperatively and at 7 and 10 years after surgery compared with those in the recurrence group.

Recurrence and survival

Figure 2 shows the recurrence status and therapeutic modalities used for disease recurrence in the 14 patients who had recurrence after a 10-year disease-free period.

Intrahepatic disease recurrence with fewer than three nodules and intrahepatic disease recurrence with more than three nodules were observed in 11 patients (79 %) and three patients (21 %), respectively. None of the patients were found to have an extrahepatic recurrence as the primary recurrence. The median and mean disease-free survival in the 14 patients with HCC recurrence was 11.0 and 11.6 years (range, 10.2–15.7 years), respectively, after the first hepatectomy (Fig. 3). The therapeutic modalities used included repeat hepatectomy in four patients (29 %), RFA in two patients (14 %), TACE in seven patients (50 %), and best supportive care in one patient (7 %). The HAI score in the underlying liver of these four patients changed from 1.0 ± 1.2 at first hepatectomy to 7.8 ± 1.3 at second resection ($p = 0.02$).

In the recurrence group, there were five deaths (36 %), four of which could be attributed to recurrence of HCC.

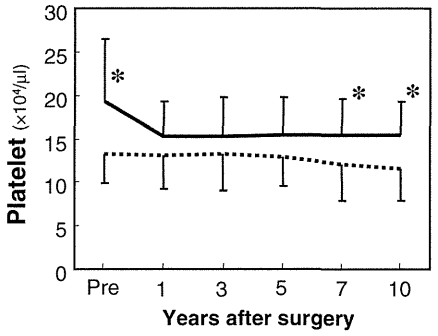

Fig. 1 Platelet count of recurrence and non-recurrence groups after hepatic resection. Data are shown as the mean ± SD. *$p < .05$ versus the recurrence group at the corresponding time

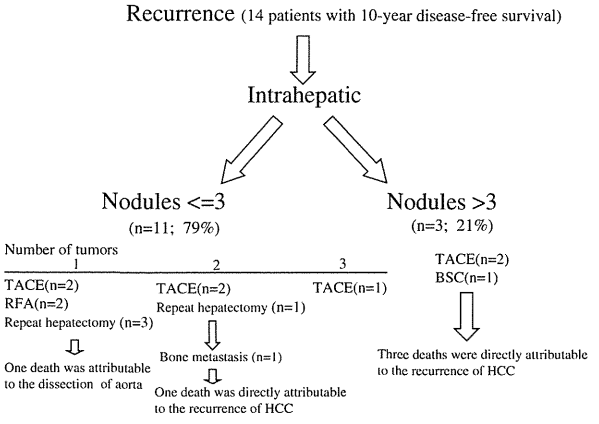

Fig. 2 Hepatocellular carcinoma (HCC) recurrence status and therapeutic modalities for treatment of recurrence in the 14 10-year disease-free survivors in whom late disease recurrence was detected. *RFA* percutaneous radiofrequency ablation; *BSC* best supportive care

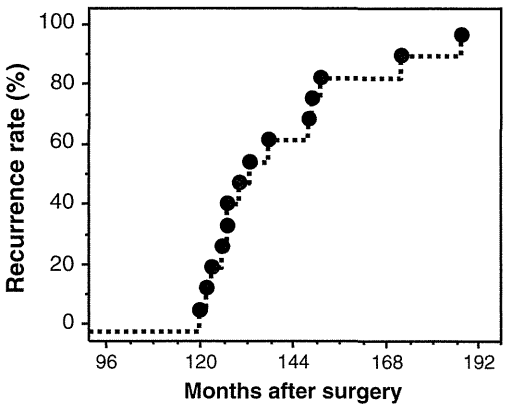

Fig. 3 Recurrence rate in patients with recurrence of HCC. The median and mean disease-free survival in the 14 patients with late disease recurrence was 11.0 and 11.6 years (range: 10.2–15.7 years) after the first hepatectomy

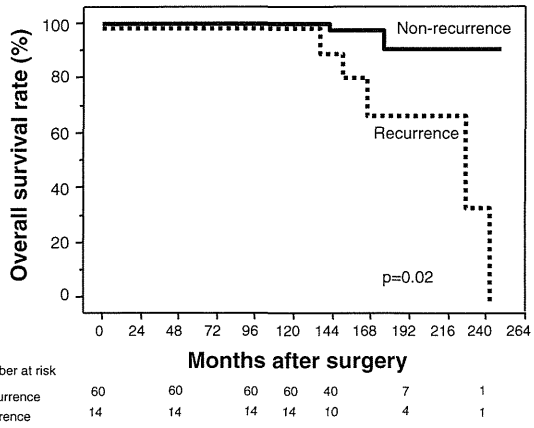

Fig. 4 Comparison of overall survival after hepatectomy in patients with or without recurrence of HCC. The survival rate of the recurrence group (*dotted line*) was significantly poorer than that of the non-recurrence group (*unbroken thin line*; $p = 0.02$). The number of patients at risk is shown below the graph

A second resection was performed in four patients, none of whom died, and median and mean survival was 15.0 and 15.3 years (range, 10.9–20.2 years), respectively, after the first hepatectomy. In the non-recurrence group, two of the 60 patients (3 %) died, with one death attributable to lung cancer at 11.9 years and the other to bile duct cancer at 14.7 years, respectively, after surgery. The overall survival rates of the recurrence and non-recurrence groups were 91 and 97 % at 12 years, 68 and 91 % at 16 years, and 34 and 91 % at 20 years, respectively (Fig. 4). There were significant differences ($p = 0.02$) in survival between the two groups.

Discussion

The recurrence rate of HCC after surgical resection is high, ranging from 50 to 100 % after 5 years in high-volume specialty centers worldwide [18–21]. Recently, close postoperative follow-up to detect recurrent HCC at an early stage, and various management strategies for recurrent HCC (including repeat resection, local ablative therapy, and/or TACE) have contributed to prolonged survival after initial hepatectomy [22–24]. Numerous studies to date have reported cumulative 5-year survival rates of approximately 40–50 %, based largely on relatively short-term follow-up [18, 22, 23, 25–27], and only a few reports of long-term follow-up of more than 10 years have been reported to date. Shimada et al. [11] reported 105 10-year survivors (21.8 %), including 42 disease-free survivors (8.7 %), among 578 patients. Fukuda et al. [12] reported that 29 of 250 patients survived for more than 10 years after initial hepatectomy, and 9 of those patients survived without