A retrospective study was carried out at our institution to investigate the outcome of patients older than 80 years who underwent hepatic resection for various indications. The outcome of these patients was compared to the outcome of patients less than 80 years of age undergoing hepatic resection during the same time.

Methods

All hepatic resections performed from April 2004 to October 2008 at the Department of Hepatogastroenterological Surgery at Aso Iizuka Hospital in Japan were included in this study. Data were retrieved from the prospective database of patients 80 years of age and older (group I, $n = 43$) and patients younger than 80 years of age (group II, $n = 307$) undergoing hepatectomy. The first author had conducted all surgical operations in this consecutive series.

Patients with World Health Organization (WHO) performance status score of 4—bedbound (completely disabled: cannot carry on any self-care: totally confined to bed or chair) were excluded. Liver function was evaluated preoperatively with the Child classification and indocyanine green retention test at 15 min (ICGR15) in patients with underlying liver disease, as reported elsewhere [13]. The operative procedure was selected accordingly to the following criteria: trisegmentectomy—ICGR15 below 15%; bisegmentectomy—ICGR15 of 25%; monosegmentectomy—ICGR15 of 35%; and subsegmentectomy—ICGR15 of 45% or less. The presence of ascites and an ICGR15 value greater than 45% were considered absolute contraindications for resection. Other organ failure, including ischemic heart disease, was examined, and the decision to operate or not was made based on consultation with to the attending physician and anesthesiologist. Exclusion criteria for liver resection were not based on age.

Hepatitis B surface antigen (HBsAg) and hepatitis C antibody (HCVAb) were routinely measured preoperatively. Ten patients in group II had obstructive jaundice and were managed with preoperative replacement of percutaneous transhepatic biliary drainage to lower the serum total bilirubin level below 5 mg/dl. When the estimated remnant liver volume was judged to be less than 250 ml/m^2 by computed tomographic volumetry, embolization of the right branch of the portal vein was performed 3–4 weeks before surgery [14]. This procedure was performed in two patients in group II diagnosed with hilar cholangiocarcinoma.

American Association of Anesthesiologists (ASA) grade was compared between the two groups. Cardiovascular assessment included electrocardiogram and chest radiograph. In cases of abnormal exercise electrocardiogram, cardiac ultrasonography and coronary angiography were carried out, and corrective intervention was used when appropriate. Six patients with chronic renal failure were on haemodialysis preoperatively, one patient in group I and five in group II.

Surgical technique and intraoperative care were standardized by the first author in this study. These included a J-shaped incision for routine abdominal access, a slow and gentle hepatic dissection with an ultrasonic dissector with coagulator (CUSA Excel, Integra Co., Plainsboro, NJ), with systematic ligation of all sizable vessels, and close ultrasonographic guidance along the transection line. Cholecystectomy was performed in all patients if the gallbladder was present. Intraoperative vascular control was achieved either by a hemihepatic vascular occlusion inflow control or by Pringle's maneuver. To prevent backflow bleeding, central venous pressure was decreased, to as low as 5 mmHg when possible, with careful circulating volume and respiratory assistance. When central venous pressure control was insufficient, outflow control was achieved, either by selective vascular exclusion [15] or by clamping of the infrahepatic inferior vena cava [16]. The liver hanging maneuver was used in right-sided major hepatic resection in cases without contraindication or technical difficulty [17].

The main endpoint of this study was the analysis and comparison of the preoperative clinicopathological and intraoperative data, operative mortality, defined as death within the first 30 postoperative days, in-hospital mortality, and postoperative complications in the two groups of patients. Preoperative variables included in the analysis included sex of the patient, indication for hepatectomy, hepatitis viral status, associated disease, estimated glomerular filtration rate (eGFR) [calculated by Modification of Diet in Renal Disease (MDRD) formulas, as follows: MDRD-GFR (ml/min/1.73 m^2) = 186 * (serum creatinine)$^{-1.154}$ * (age)$^{-0.203}$ * (0.742 if female)], incidence of preoperative chemotherapy, ASA grade, platelet count, levels of serum transaminase, albumin, total bilirubin, prothrombin time, and indocyanine green retention rates at 15 min (ICGR15) in patients with underlying liver disease, diabetes mellitus. and body mass index. The incidence of histological liver cirrhosis was compared between the two groups. The distance from the tumor edge to the surgical margin (SM) in patients with primary liver cancer and metastatic liver cancer was examined on the resected specimen.

Intraoperative variables included in the analysis were type of hepatectomy, combined resection, estimated blood loss, requirement of blood transfusion, and total operative time.

To clarify our rationale for selecting age 80 as the cut-off point, group I was divided into two groups. The median age in group I was 82 years, and this group was further

subdivided into two groups: group I-low ($n = 20$ patients 80–82 years of age), and group I-high ($n = 23$ patients over 82 years old). All clinicopathological data, intraoperative data, and postoperative outcome were compared between the two groups. The length of follow-up and oncologic outcome in patients with primary liver cancer, metastatic liver cancer, and bile duct cancer were also compared between groups.

Statistical methods

We analyzed associations between the continuous and categorical clinicopathologic variables with the Student *t*-test and chi-square test, respectively. Survival was generalized with the Kaplan–Meier method and then was compared with the log-rank test. A p value less than 0.05 was considered statistically significant.

Results

Clinicopathological factors

Comparison of preoperative clinicopathological factors is shown in Table 1. There was no significant difference between the two groups with regard to gender or indication for surgery. Twenty-three patients (53%) in group I and 216 patients (70%) in group II had primary liver cancer. Regarding extrahepatic biliary cancer, 5 patients had gallbladder cancer in group I and no patients had hilar cholangiocarcinoma. In group II, 12 patients had gallbladder cancer and 10 patients had hilar cholangiocellular carcinoma with obstructive jaundice.

The hepatitis viral status was significantly different between groups ($p = 0.002$). In group I, there were no patients with HBV, and 79% had neither HCV or HBV infection. In group II, 30% of patients had HBV, 37% had HCV, and 45% did not have viral hepatitis infection.

Regarding co-morbid disease, group I showed a higher prevalence of pulmonary disease than group II ($p = 0.03$) and there was a higher percentage of group I patients with cardiac disease compared with group II, although statistical significance was not reached ($p = 0.14$). There was no difference in the prevalence of renal failure between the two groups. There was no significant difference between the two groups regarding diabetes mellitus, body mass index, and distribution of ASA score. The eGFR in group I was significantly higher than in group II ($p = 0.0001$).

Table 1 Comparison of clinicopathological data of the groups

Characteristics	Group I Age ≥ 80 years ($n = 43$)	Group II Aged < 80 years ($n = 307$)	p Value
Gender			
Male	24 (56%)	204 (66%)	0.18
Female	19 (44%)	103 (34%)	
Viral status			
HBV	0 (0%)	51 (30%)	0.0002
HCV	9 (21%)	114 (37%)	
None	34 (79%)	137 (45%)	
HBV + HCV	0 (0%)	5 (2%)	
Indication for operation			0.09
Primary liver cancer	23 (53%)	216 (70%)	
Metastatic disease	13 (30%)	13 (4%)	
Extrahepatic biliary cancer	5 (12%)	22 (7%)	
Others	2 (5%)	18 (6%)	
Associated disease			
Pulmonary	6 (14%)	15 (5%)	0.03
Emphysema	1	6	
Asthma	3	4	
Others	2	5	
Cardiac	25 (58%)	138 (45%)	0.14
Hypertension	17	113	
Ischemia	5	9	
Valvulopathy	0	2	
Arrhythmia	1	12	
Other	2	2	
Renal failure	2 (5%)	12 (4%)	0.69
eGFR (ml/min/1.73 m^2)[a]	49.7 ± 18.0	63.7 ± 20.7	0.0001
Diabetes mellitus	12 (28%)	81 (26%)	0.83
Body mass index	22.0 ± 2.9	22.9 ± 3.6	0.13
ASA grade			
I:II:III	0:30:13	8:237:62	0.21
Preoperative chemotherapy	3 (7%)	16 (5.2%)	0.71
Mean ICGR15 (%)	16.1 ± 9.5	15.8 ± 11.6	0.88
Albumin (g/dl)	3.6 ± 0.5	3.8 ± 0.5	0.008
Total bilirubin (mg/dl)	0.7 ± 0.3	0.8 ± 0.6	0.36
Prothrombin time (%)	100 ± 16.6	100 ± 16.8	0.86
Liver cirrhosis (%)	9 (18.3)	89 (29.0)	0.36
Surgical margin (mm)	6.2 ± 8.0	6.4 ± 6.8	0.85

HBV hepatitis B antigen positive, *HCV* hepatitis C antibody positive, *ASA* American Society of Anesthesiologists

[a] eGFR (ml/min/1.73 m^2) = 186 * (serum creatinine)$^{-1.154}$ * (age)$^{-0.203}$ * (0.742 if female)

In the evaluation of preoperative liver function, serum albumin was significantly lower in group I than in group II ($p = 0.008$). There was no significant difference between groups in other parameters, including ICGR15, serum bilirubin levels, prothrombin time, histological evidence of cirrhosis, and surgical margin status.

Intraoperative data

Comparisons of intraoperative data between the two groups are shown in Table 2. There was no significant difference in type of hepatectomy or combined resection. Extrahepatic bile duct resection was performed in 16 patients in group II and none in group I. Operative time was significantly shorter in group I than in group II ($p = 0.048$). After eliminating 16 patients in group I who underwent bile duct resection and reconstruction, there was no significant difference between the two groups ($p = 0.19$). The estimated blood loss during the operation was 697 ± 1,522 g in group I and 827 ± 1,571 g in group II; there was no significant difference between groups ($p = 0.61$). The incidence of blood transfusion, including fresh frozen plasma was 19% in group I and 17% in group II; this difference was not statistically significant ($p = 0.83$).

Table 2 Comparison of intraoperative data of the two groups

Characteristics	Group I Age ≥ 80 years ($n = 43$)	Group II Age < 80 years ($n = 307$)	p Value
Type of hepatectomy			0.51
Non-anatomic	20 (47%)	162 (53%)	
Subsegmentectomy	5 (12%)	36 (12%)	
Segmentectomy	11(26%)	50 (16%)	
Two or more segments	7 (16%)	59 (19%)	
Combined resection	4(16%)	36 (12%)	0.80
Colon or rectum	2	4	
Stomach	1	3	
Bile duct	0	16	
HCC thrombus	0 6		
Inferior vena cava	1	1	
Diaphragm	0 2		
Spleen	0 2		
PD	0	2	
Vascular control	0.39		
Inflow only	9 (21%)	56 (18%)	
SHVE	22 (51%)	132 (43%)	
IVC clamp	12 (28%)	119 (39%)	
Operative time (min)	284 ± 117	329 ± 140	0.048
Estimated blood loss, g	697 ± 1,522	827 ± 1,571	0.61
Blood transfusion rate	8 (19%)	53 (17%)	0.83

HCC hepatocellular carcinoma, *PD* pancreaticoduodenectomy, *SHVE* selective hepatic vascular exclusion method, *IVC clamping* clamping of the inferior vena cava below the liver

Postoperative outcome

The comparison of postoperative outcome is shown in Table 3. Neither postoperative mortality nor in-hospital mortality occurred in group I; in group II, one postoperative death (0.3%) was recorded, as were two (0.6%) cases of in-hospital mortality. One patient died of liver failure secondary to portal vein thrombosis and another died of sepsis.

There was no difference in morbidity incidence between groups. The most common complication was bile leakage, and the second most common complication was wound infection. Reoperation was performed in one patient from each group: in one patient for drainage of an intra-abdominal abscess and in the other for homeostasis of postoperative bleeding. There was no significant difference in the length of postoperative hospital stay between groups ($p = 0.85$).

Table 3 Comparison of postoperative outcome of the two groups

Characteristics	Group I Age ≥ 80 years ($n = 43$)	Group II Age < 80 years ($n = 307$)	p Value
Postoperative mortality	0	1 (0.3%)	0.99
In-hospital mortality	0	2 (0.6%)	0.99
Postoperative complications	11 (26%)	67 (22%)	0.56
Liver dysfunction	0	2	
Liver failure	0	1	
Bile leakage	6	27	
Ascites	0	4	
Pneumonia	0	2	
Respiratory failure	0	1	
Peritonitis	1	1	
Sepsis	0	1	
Abdominal bleeding	0	1	
Wound infection	2	22	
Enteritis	0	2	
Brain stroke	0	2	
Depression	1	0	
Reoperations	1 (2%)	1 (0.3%)	0.99
Postoperative hospital stay (median)	14	14	0.85
Survival at 3 years (%)	65.4	71.5	0.76

Subclass analysis between group I-low and group I-high

All clinicopathological data, intraoperative data, and postoperative outcomes were compared between the two groups. Serum levels of albumin in group I-high tended to be lower than that in group I-low ($p = 0.08$), and the eGFR in group I-low tended to be higher than that in group I-high ($p = 0.06$). Neither of these differences reached statistical significance. All other results were not different between the two groups.

Length of follow-up and oncologic outcome

The mean length of follow-up in primary liver cancer was 3.2 and 3.2 years in groups I and II, respectively. The 2-year survival was 75.6% in group I and 84.4% in group II. There was no significant difference in survival rate between the two groups ($p = 0.56$). The mean length of follow-up in metastatic liver cancer was 2.1 and 2.3 years in groups I and II, respectively. The 2-year survival was 70.3% in group I and 63.3% in group II. There was no significant difference in survival rate between the two groups ($p = 0.89$). The mean length of follow-up in bile duct cancer was 2.6 and 2.1 in group I and II, respectively. The 2-year survival was 80% in group I and 66.9% in group II. There was no significant difference in survival rate between the two groups ($p = 0.25$).

Conclusions

The number of aged people is increasing in Japan and the United States. Based on the latest WHO report [18], the mean age of the general population in Japan was 83 years in 2006. In 2005 in the United States, the actual life expectancy of a person at 75 years of age was 12.0 years [19]. Furthermore, the number of patients 85 years of age or older is increasing in the United States. Additionally, the median age at diagnosis of a malignant hepatobiliary cancer was 70 years or older for HCC (female patients), gallbladder, and bile duct malignancies in United States. Petowsky et al. [12] showed that the median age of patients with colorectal liver metastases is over 70 years in Japan. As the population ages, the number of older patients undergoing hepatic resection is increasing to the point that the operation is becoming common. In this study, 43 of the 350 patients (12%) undergoing hepatic resection were over 80 years of age; the median age in group I was 82 years and the oldest patient was 91 years old. Nevertheless, reports regarding the outcome for patients 80 years or older with hepatobiliary cancer are limited: only one article by Wu et al. is present in the literature [3]. Therefore, the feasibility of hepatic resection in patients over 80 years of age is still unclear.

Function of most bodily organs usually deteriorates with age. In this study, the incidence of pulmonary dysfunction was significantly higher in group I than in group II, eGFR in group I was significantly higher than that in group II, and the incidence of cardiac dysfunction in group I showed a trend toward a higher rate than in group II. Regarding liver function, only preoperative serum albumin levels in group I were significantly lower than those in group II. This may reflect poorer nutritional status in group I than in group II, because there were no significant differences between two groups with regard to all other parameters reflecting liver function. Furthermore, because there was no significant difference between the two groups in ASA score, the severity of associated diseases might be comparable between groups.

Indication for surgery was not significantly different between groups. Metastatic disease tended to be more common and benign disease less common in group I. Also, there was no significant differences in type of hepatectomy between groups. Also, there were no patients in group I who underwent combined resection of extrahepatic bile ducts and reconstruction, so we cannot speculate on the feasibility of this type of surgery, which is usually performed for the treatment of hilar cholangiocarcinoma in this age group.

The viral status between groups was shown to be significantly different, a finding that may be secondary to the viral status of the patients with primary liver cancer. In group I, HCC patients without HCV or HBV infection were more common, and no patients with HBV were identified. Recent studies from Japan [20] revealed that the age of HCC patients without HCV or HBV infection had been increasing. These aged patients had better liver function and larger tumors than younger patients. Therefore, these patients may be good candidates for hepatic resections.

In previous studies, the reports regarding mortality rates in patients over 80 years of age are limited. In HCC, Wu et al. [3] reported zero mortality in 21 patients who underwent hepatic resection. In colorectal metastases and extrahepatic bile duct cancer, including gallbladder cancer, no previous reports exist. Other publications have defined elderly patients as those over 70 years and have reported mortality rates of 3%–43% for elderly patients with HCC [1–6]. The mortality rate of hepatic resection for colorectal liver metastases has been reported to be from 3.9% to 7.3% in elderly patients [7–12]. These data underscore the fact that hepatic resections can be safely performed in elderly patients. Nevertheless, previous studies found that mortality was more than 3%, and a zero mortality rate in elderly patients has not been reported, except by Wu et al. [3] Operative blood loss during hepatectomy is a factor that seriously affects postoperative outcomes [15, 21, 22]. In previous articles, intraoperative blood loss exceeded

1,000 ml [2, 3, 5, 6, 10]. In the present study, mean blood loss was 697 ml in elderly patients and 827 ml in younger patients. This reduction of blood loss during hepatic resection may be one of the most important explanations for the zero mortality rate achieved in elderly patients in this study. In 80% of the patients in this series, we reduced the blood loss not only by clamping of hepatic inflow but also by outflow control by means of selective hepatic vascular exclusion methods [15] and clamping the IVC below the liver [16]. These procedures were effective for control of backflow from the hepatic vein. Menon et al. [23] have demonstrated that blood transfusion >3 U was an independent predictor of poor long-term prognosis in elderly patients with colorectal liver metastases after hepatic resection. Therefore, reduction of blood loss during hepatic resection may be important not only for early outcome but also for late outcome in elderly patients.

There was no significant difference in the incidence of postoperative complications between the two groups, and there were no age-specific complication in group I. In both groups, the first and second most common complications were bile leakage and wound infection.

Regarding prognosis, our data are preliminary. The follow-up period in the present study is too short to allow a final conclusion concerning prognostic data. Nevertheless, the survival rates in each group were the same during our follow-up period. Further examination is necessary but, it seems important that we consider treatment with curative intent in elderly patients, and this research supports that philosophy.

In conclusion, hepatic resection is a feasible procedure yielding 0% operative mortality in specialized tertiary centers in patients older than 80 years of age who have preserved liver function and controllable medical conditions.

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Characterization of Hepatocellular Carcinoma Developed After Achieving Sustained Virological Response to Interferon Therapy for Hepatitis C

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Background: Interferon (IFN) reduces the risk of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C (CHC). However, HCC develops in the some patients who have achieved a sustained virological response (SVR). The aim of this study was to clarify the features and prognosis of SVR patients who developed HCC.
Materials and Methods: Twenty-six patients who underwent curative hepatectomy for initial HCC after IFN therapy were closely investigated. Twenty patients who were seropositive for HCV-RNA (non-SVR), and a further 6 patients who achieved SVRs (SVR) were included. We analyzed the clinicopathological features, immunological expression levels of p53 and whether HCV-RNA is present in the excised liver.
Results: The liver functions of the SVR group were almost better than those of the non-SVR group. However, there was no significant difference in pathological features, surgical factors and prognosis between the groups. In one case with SVR out of eight specimens tested was HCV-RNA detected in the non-cancerous tissue. Immunohistochemistry revealed overexpression of p53 in eight HCCs (100%) from SVR patients.
Conclusion: Recurrent HCC still developed after the curative hepatectomy, even if viral elimination had been successful. And molecular alterations in hepatocarcinogenesis of SVR patients might be different from those of CHC patients.
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KEY WORDS: HCC; SVR; IFN; hepatoma; HCV

INTRODUCTION

Hepatits C virus (HCV) infection is a common cause of chronic hepatitis and hepatocarcinogenesis worldwide [1–5]. Interferon (IFN) has been widely used for the treatment of chronic hepatitis C (CHC) patients [6,7]. Many investigators have reported that IFN-based therapy improves hepatic inflammation, fibrosis, and serum alanine aminotransferase (ALT) levels, and reduces circulating HCV-RNA levels [8–13]. Therefore, IFN-based therapy was believed to reduce the risk of hepatocellular carcinoma (HCC). Early viral kinetics after the initiation of the therapy is a useful predictor of the sustained virological response (SVR), which is formally determined at 24 weeks after the completion of therapy. Regardless of the achievement of SVR, HCC developed in some patients [14–17]. However, there was little information on the clinicopathological and biological findings of HCCs that developed in patients who achieved SVRs. The aim of this study was to clarify the features and prognosis of SVR patients who develop HCC by analyzing the clinicopathological findings and immunological expression levels of the biological marker p53, and to determine whether HCV-RNA is present in the excised liver.

MATERIALS AND METHODS

Patients

From January 2000 to December 2006, a total of 126 consecutive curative hepatectomies were performed for primary HCC without preoperative treatment at the Department of Surgery and Science, Kyushu University Hospital, Fukuoka, Japan. Among them, 71 patients who were seropositive for anti-hepatitis C virus antibody (HCV-Ab) and seronegative for hepatitis B surface antigen (HBs-Ag) were included in this study; 45 of these (77.5%) who had no history of IFN therapy and were seropositive for HCV-RNA were excluded. Twenty patients who had received IFN therapy but did not respond to it and were seropositive for HCV-RNA comprised the non-SVR group, while six patients who achieved SVRs to preoperative IFN therapy and were seronegative for HCV-RNA comprised the SVR group. This investigation was performed only in cases in which written informed consent was obtained prior to the operation, and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Specimens

All tumors were defined as hepatocellular carcinoma (HCC) according to the General Rules for the Clinical and Pathological

Abbreviations: IFN, interferon; HCC, hepatocellular carcinoma; CHC, chronic hepatitis C; SVR, sustained virological response; HCV, hepatitis C virus; ALT, alanine aminotransferase; HBs-Ag, hepatitis B surface antigen; HCV-Ab, anti-hepatitis C antibody; Plt, platelet; Alb, albumin; PIVKA-II, protein induced by vitamin K absence or antagonist; T-bil, total bilirubin; PT, prothrombin time; ICG R15, indocyanine green 15-min retention test; AFP, α-fetoprotein; DM, diabetes mellitus; HT, hypertension; vp, pathological portal venous invasion; vv, pathological hepatic venous invasion; im, pathological intrahepatic metastasis; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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Study of Primary Liver Cancer of the Liver Cancer Study Group of Japan [18]. The fibrous stage and inflammatory grade in the non-cancerous tissues was determined according to the new Inuyama classification [19]. Macrovesicular fatty deposits in the non-cancerous tissue were also examined. Frozen samples of the cancerous and the non-cancerous tissues were immediately obtained after surgical resection and stored at −80°C.

RNA Extraction and RT-PCR

Total RNA was isolated from frozen tissue using ISOGEN RNA extraction kits (Nippon Gene, Inc., Tokyo, Japan) according to the manufacturer's instructions. RT-PCR for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed to confirm whether RNA was suitable for use as an internal control. RT-PCR reactions were performed using Transcriptor First Strand cDNA Synthesis Kits (Roche, Tokyo, Japan) and Takara Taq™ (Takara Bio Inc., Shiga, Japan), according to the manufacturer's instructions. PCR rounds consisted of 25 cycles of 30 sec at 94°C, 30 sec at 56°C and 30 sec at 72°C. The product size was 540 bp. The primer sequences are as follows: 5′-GTCACCGGATTTGGTCGTATT-3′ (forward primer), 5′-AGTCTTCTGGGTGGCAGTGAT-3′ (reverse primer).

Nested RT-PCR

PCR reactions were performed using Takara Ex taq® Hot start polymerase (Takara Bio Inc., Shiga, Japan), according to the manufacturer's instructions. Briefly, both rounds consisted of 35 cycles of PCR with 45 sec at 94°C, 45 sec at 55°C, and 90 sec at 72°C. Four microliters of cDNA was added in the first round and amplified with the outer primer set. One microliter of the first-round products was used in a second round of PCR with the inner primer set. The product size was 278 bp. The primer sequences are as follows: 5′-ACTGCCTGATAGGGTGCTTGCGAG-3′ (outer sense primer), 5′-ATGTACCCCATGAGGTCGGC-3′ (outer antisense primer), 5′-CAGATCGTTGGYGGAGT-3′ (inner sense primer), 5′-CAYGTRAGGGTATCGATGAC-3′ (inner antisense primer).

Strand-Specific RT-PCR

Strand-specific RT-PCR reactions were performed according to the manufacturer's instructions and as previously described [20], using the GeneAmp® Thermostable rTth reverse transcriptase RNA PCR kit (Applied Biosystems, Ltd., Tokyo, Japan) and Takara Ex taq® Hot start polymerase. After performing strand-specific RT reactions with specific tagged primers, 4 μl of cDNA was added in the first round and amplified with the specific primers in combination with a tag-only primer. One microliter of the first-round product was used in a second round of PCR with the inner primer set. Both rounds consisted of 35 cycles of PCR with 45 sec at 94°C, 45 sec at 55°C, and 90 sec at 72°C. The product size was 278 bp. The primer sequences were as follows: 5′-TCATGGTGGCGAATAAATGTACCCCATGAGGTCGG-C-3′ (outer antisense tagged primer), 5′-TCATGGTGGCGAATAACGCCCACAGGACGTYAAGTT-3′ (outer sense tagged primer), 5′-TCATGGTGGCGAATAA-3′ (tag-only primer). The underlined sequence represents a 16-nucleotide tag sequence unrelated to HCV. After 2% agarose gel electrophoresis, each PCR product was visualized with ethidium bromide staining on a UV transilluminator. Liver tissue of CHC was used as a positive control for both strand-specific RT-PCR. Serum of CHC was used as a positive control for the positive strand-specific RT-PCR, or as a negative control for the negative strand-specific RT-PCR.

Immunohistochemical Staining

Immunohistochemical observations were performed on adjacent deparaffinized sections using a DAKO EnVision™ + System, HRP (DAKO Inc. Tokyo, Japan). The primary antibody used in this study was against p53 (mouse monoclonal antibody clone DO-7; diluted 1:100; DAKO Inc.). Immunohistochemically stained sections were examined by two pathologists. Cases with nuclear staining in >10% of the cells were considered positive, according to the previously described method [21].

Statistical Analysis

A survival analysis was performed using the Kaplan–Meier method, and groups were compared statistically by the log-rank test. Continuous variables were compared with independent samples using the Kruskal–Wallis test and Mann–Whitney *U*-test. Categorical data were compared using the Fisher test and Chi-squared test. *P* values of less than 0.05 were considered significant. All statistical analyses were performed using Stat View 5.0 software for Macintosh (Abacus Concepts Inc., Berkeley, CA).

RESULTS

Comparison of Clinicopathological Characteristics

The clinical features of the SVR group and those of the non-SVR group are listed in Table I. The SVR group had lower ALT levels ($P = 0.04$) and higher Plt ($P = 0.02$), Alb ($P = 0.01$) and PIVKA-II ($P = 0.02$) levels than the non-SVR group. The pathological and surgical features of the SVR group and those of the non-SVR group are listed in Table II. Those pathological and surgical features of the SVR patients did not significantly differ from those of the non-SVR patients. The SVR patients had better liver functions than the non-SVR patients. However, the SVR patients had the same pathological and surgical characteristics as the non-SVR patients.

Clinical Course of SVR Patients

Figure 1 summarizes the clinical courses of the six SVR patients. The intervals between the achievement of SVR and the detection of the

TABLE I. Comparison of the Clinical Features Between SVR and Non-SVR Patients

Variables	SVR (n = 6)	Non-SVR (n = 20)	*P* value
Age (years)[a]	69.5 ± 2.7	67.1 ± 6.5	NS
Gender (male/female)	5/1	12/8	NS
DM (+/−)	2/4	7/13	NS
HT (+/−)	2/4	9/11	NS
Alcohol (+/−)	1/5	4/16	NS
Age at start of IFN therapy (years)[a]	63.3 ± 8.4	58.4 ± 7.6	NS
Time after IFN to operation (years)[a]	6.2 ± 4.1	8.7 ± 3.9	NS
Child-Pugh classification (A/B/C)	6/0/0	16/4/0	NS
ALT (U/L)[a]	33.3 ± 20.7	61.9 ± 42.3	<0.05
Plt ($\times 10^4$/μl)[a]	17.6 ± 5.0	12.7 ± 6.3	<0.05
Alb (g/dl)[a]	4.43 ± 0.27	3.87 ± 0.47	<0.05
T-bil (mg/dl)[a]	1.0 ± 0.4	0.9 ± 0.3	NS
PT (%)[a]	86.0 ± 8.4	81.3 ± 12.1	NS
ICG R15 (%)[a]	14.0 ± 6.6	16.7 ± 8.4	NS
AFP (ng/ml)[a]	23 ± 43	550 ± 2311	NS
PIVKA-II (mAU/ml)[a]	2980 ± 6167	159 ± 227	<0.05

SVR, sustained virological response; non-SVR, non-sustained virological response; IFN, interferon; DM, diabetes mellitus; HT, hypertension; ALT, alanine aminotransferase; Plt, platelet; Alb, albumin; T-bil, total bilirubin; PT, prothrombin time; ICG R15, indocyanine green 15-min retention test; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist; NS, not significant.
[a]Mean ± standard deviation.

TABLE II. Comparison of the Pathological and Surgical Features Between SVR and Non-SVR Patients

Variables	SVR (n = 6)	Non-SVR (n = 20)	*P* value
Maximal tumor size (cm)[a]	3.6 ± 3.4	3.0 ± 1.7	NS
Tumor number (single/multiple)	6/0	16/4	NS
Stage (I/II/III/IV)	2/4/0/0	7/11/2/0	NS
Surgical procedures (lobectomy/segmentectomy/partial)	1/2/3	2/4/14	NS
Histological differentiation (well/mod/poor)	0/5/1	1/15/4	NS
vp (+/−)	2/4	3/17	NS
vv (+/−)	1/5	3/17	NS
im (+/−)	0/6	0/20	NS
Fibrous stage (F0,1,2/F3,4)	4/2	7/13	NS
Inflammatorygrade (A0,1/A2,3)	4/2	10/10	NS
Macrovesicular fatty deposit (+/−)	4/2	8/12	NS

SVR, sustained virological response; non-SVR, non-sustained virological response; vp, pathological portal venous invasion; vv, pathological hepatic venous invasion; im, pathological intrahepatic metastasis; NS, not significant.
[a]Mean ± standard deviation.

initial HCC ranged from 13 to 152 months. The mean interval until detection of the initial HCC after achievement of SVR was 74 months (6.2 years). After a mean follow-up period of 45 months, 3 patients developed recurrent HCC after initial hepatectomy (Case #1, #3, and #4). Cases #2, #5, and #6 survived without recurrence. The intervals between the initial hepatectomy and the detection of recurrent HCC ranged from 18 to 32 months (the mean interval was 23 months). Two patients had extrahepatic recurrences and the other had a recurrent tumor only in the liver. Case #1 had tumors detectable in the para-aortic lymph node, bone and liver. Case #3 had tumors detectable in the lung. Case #4 had a tumor only in the liver; this case developed recurrent HCC in the liver twice after initial hepatectomy, at 32 and 70 months. The recurrent HCCs from case #4 were excised and diagnosed as

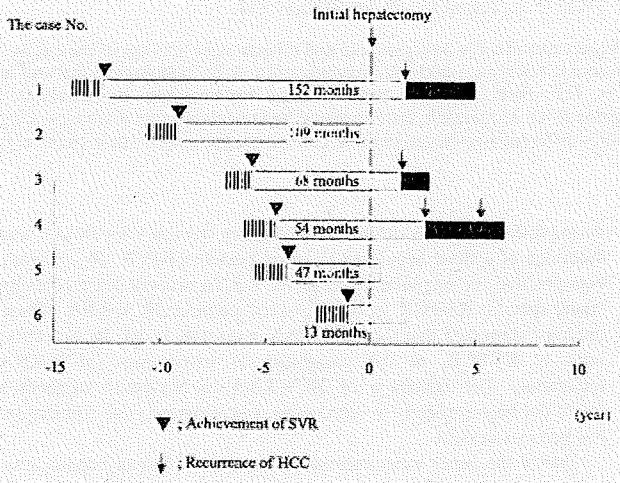

Fig. 1. A summary of the clinical histories of the six cases showing SVRs is shown. Zero was the initial hepatectomy point. The vertical stripes represent the term of IFN therapy. The white bar shows the term of the sustained virological response. The gray bar shows the disease-free survival term. The black bar shows the term of survival after recurrent HCC. Case #1 had a recurrent para-aortic LN metastasis, bone metastasis and multiple liver tumors. Case #3 had multiple lung metastases during recurrence. Case #4 had a recurrent intrahepatic lesion. Cases #2, #5, and #6 survived without recurrence.

multi-centric occurrences, on the basis of pathological features (Fig. 2).

Survival of Patients

Figure 3 shows the cumulative disease-free survival rates after initial hepatectomy. The 1- and 3-year disease-free survival rates were 79% and 57%, respectively, in the non-SVR group, and were 100% and 25%, respectively, in the SVR group. There was no significant difference in disease-free survival between the two groups. Thirteen patients (65%) from the non-SVR group received postoperative IFN therapy. There was no significant difference in cumulative disease-free survival rates between patients who received postoperative IFN therapy and those who did not (data not shown).

Detection of HCV-RNA

Both cancerous and non-cancerous tissue samples from the 6 SVR patients were tested for HCV-RNA using nested RT-PCR. The results are shown in Figure 4. Seronegative HCV-RNA patients were negative for HCV-RNA in both the cancerous and the non-cancerous tissue of the initial hepatectomy. However, only one specimen that was non-cancerous tissue obtained from case #1 after the second recurrence, was positive for HCV-RNA by nested RT-PCR. Although HCV-RNA was detected using nested RT-PCR, it was not detectable using strand-specific RT-PCR. All of those SVR patients had not been recurrent serological infection of HCV during follow-up.

Immunostaining for p53

Immunostaining was performed using an anti-p53 monoclonal antibody for expression analysis of p53. p53 was detected in all eight cancerous lesions (six of the eight were initial HCCs, and two were recurrent HCCs), but no non-cancerous tissues. These findings are shown in Figure 5.

DISCUSSION

In the current study, the characteristics of HCV patients who achieved SVRs were compared with those of non-SVR patients. The clinical and pathological findings of HCC did not differ, in spite of the achievement of SVR. The recurrence rates of HCC after resection did not differ between the two groups. HCV-RNA was not detected in the liver tissue of initial hepatectomy, except in one case of non-cancerous tissue of the second recurrent HCC in SVR patients, by nested RT-PCR. Furthermore the immunological expression levels of p53 of HCCs from SVR patients were high.

Although there have been several studies showing that post-operative IFN treatment improves the prognosis of CHC patients in relation to HCC, and IFN treatment prevented CHC patients from developing initial HCC [13,22–24], there has been little information about the prognosis after initial treatment for HCC after the achievement of SVRs, the characteristics of HCC from SVR patients, and investigations using excised liver tissue. Therefore, 26 CHC patients who had received only curative hepatectomy were selected in our study. All of them were positive for serum anti HCV-Ab and negative for serum HBs-Ag prior to the initial surgery.

Several recent studies have reported that pathological features such as tumor size, tumor number, intrahepatic metastasis, capsular invasion, portal venous invasion and hepatic venous invasion are prognostic factors of HCC after hepatectomy [25–27]. IFN-based therapy has been reported to improve ALT levels, hepatic inflammation and fibrosis [9,11–13,28]. It was also reported that the advanced hepatic fibrous stage prior to IFN-based therapy is a predictive factor for the development of initial HCC after an SVR [14,16]. In this

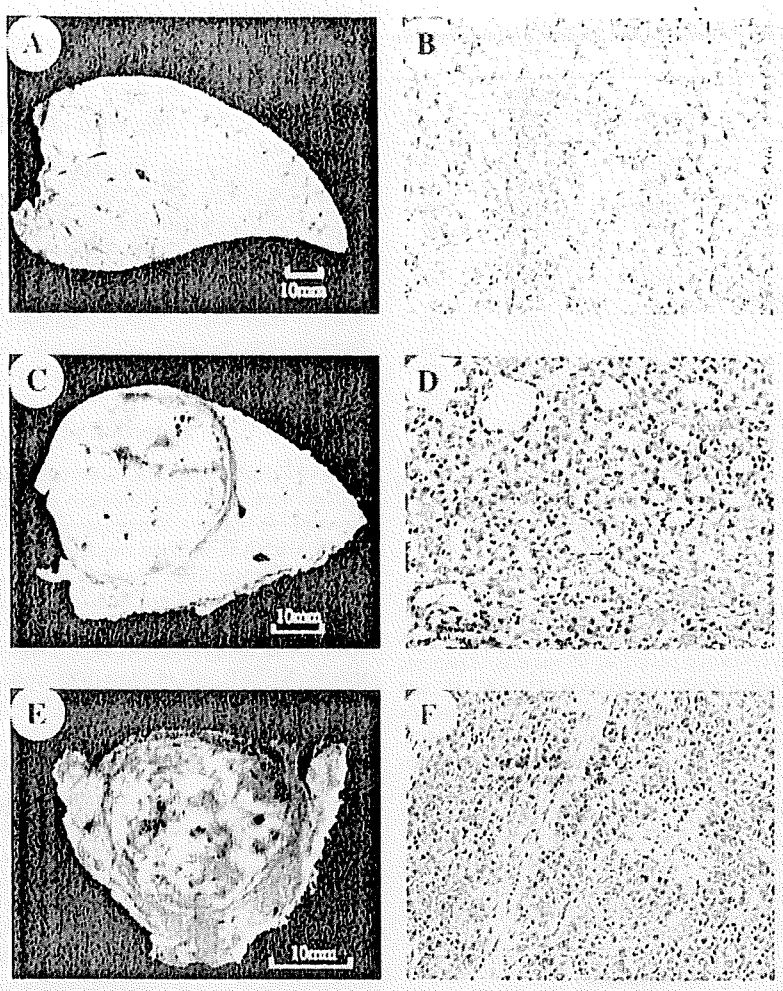

Fig. 2. The HCC from case #4, as a representative of the cases developing recurrent HCCs. Macroscopic and microscopic findings of the excised HCC from case #4 are shown. **A,C,E**: Macroscopic findings. **B,D,F**: Microscopic findings at 400-fold magnification. A,B: Pathological features at initial hepatectomy. C,D: Pathological features at second hepatectomy. E,F: Pathological features at third hepatectomy. B: Moderately differentiated hepatocellular carcinoma growing in a trabecular pattern, grade II. D: Moderately differentiated hepatocellular carcinoma growing in pseudoglandular and trabecular patterns, grade II. F: Moderately to poorly differentiated hepatocellular carcinoma growing in thick trabecular and compact patterns, grades II + III.

study, there were no significant differences in pathological features or surgical procedures between the two groups. Based on these results, the pathological and surgical backgrounds of the patients selected in this study were similar. Although liver function is one of the predictive factors for recurrent HCC after hepatectomy [26,27], the prognosis of the SVR patients with HCC was the same as those of non-SVR group. Furthermore the fibrous stage and inflammatory grade of the non-cancerous tissue were also examined according to the new Inuyama classification [19]. Those of the six SVR patients were various, and there were no significant differences between the two groups.

Previous studies showed that HCV-RNA was persistent in the peripheral blood mononuclear cells and the liver tissues, even if HCV-RNA was eliminated from serum [28,29]. Moreover, it was even reported that, in 2% of patients who achieved SVRs, viral sequences could be detected in the liver, and some of these patients ultimately experienced recurrent infection [28]. Therefore, we determined if viral sequences could be detected or not in excised specimens. HCV-RNA was not detected in any specimens of initial hepatectomy from patients with SVR. Although SVR patients had been seronegative HCV-RNA during follow-up, HCV-RNA was detected in one non-cancerous tissue specimen of a third hepatectomy using nested RT-PCR. By contrast, both positive and negative strand of HCV-RNA was not detected using strand-specific RT-PCR in the tissue of positive HCV-RNA. These findings suggested that the HCV-RNA level was under the detection limit of RT-PCR, or the possibility of extrahepatic HCV-RNA replication. The influence of HCV-RNA to hepatocarcinogeneses might not be completely avoided even if serological viral elimination has been successful.

One of the genes most extensively studied in human hepatocarcinogenesis is the p53. As in many cancers, abnormalities in the p53 gene occur during carcinogenesis [30–33]. The relationship between patient prognosis and abnormal expression of p53 of HCC has been reported previously [21]. Finally, immunostaining was performed using an anti-p53 monoclonal antibody to characterize the HCCs from SVR patients. p53 was previously reported to be overexpressed in 31.5% of randomly selected HCC cases by Hsu et al. [21], 53% of both HCV-related and HBV-related cases by Koskinas et al.[34], and 13% of HCV-related cases by Caruso and Valentini [35] In our center,

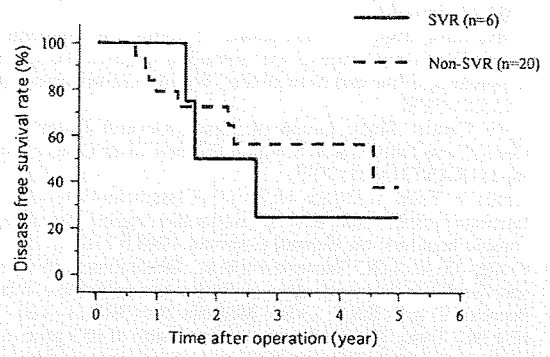

Fig. 3. Cumulative disease-free survival rates in the SVR group and non-SVR group. The 1- and 3-year disease-free survival rates were 79% and 57%, respectively, in the non-SVR group, and were 100% and 25%, respectively, in the SVR group. There were no significant differences between the SVR and non-SVR groups ($P = 0.66$).

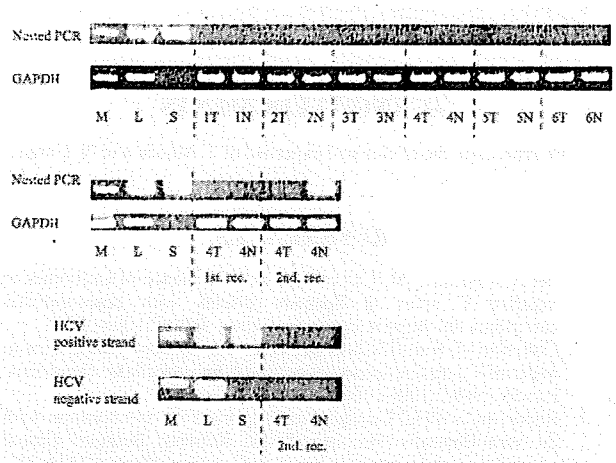

Fig. 4. Electrophoresis analyses of PCR products of HCV-RNA and GAPDH as an internal control were shown. The upper rows show the PCR products of initial hepatectomy. The middle rows show the RT-PCR products of second and third hepatectomy of Case #4. The lower rows show strand-specific RT-PCR products of second and third hepatectomy of Case #4. Lane numbers represent case numbers. Lanes containing products from cancerous tissues are labeled 1T, 2T, 3T, 4T, 5T, and 6T. Lanes containing products from the non-cancerous tissues are labeled 1N, 2N, 3N, 4N, 5N, and 6N. M, marker; L, liver tissue of CHC as a positive control for both strands; S, serum of CHC as a positive control for the positive strand, and as a negative control for the negative strand.

overexpression of p53 was detected in 58.8% of HCV-related HCCs (non published data). The expression levels of p53 in the cancerous lesions of the SVR patients were higher than those in CHC patients. Furthermore, recurrent HCC after hepatic resection often occurs as an intrahepatic lesion [36], but two of the three SVR patients had recurrent extrahepatic lesions. These findings indicated that molecular alternations of hepatocarcinogenesis of the SVR patients might be different from those of CHC patients.

In conclusion, HCC still developed after the achievement of SVR, furthermore, HCC still relapsed even if patients with SVR received curative hepatectomy. And the malignant phenotypes of HCC from the SVR patients might be different from those of patients seropositive for

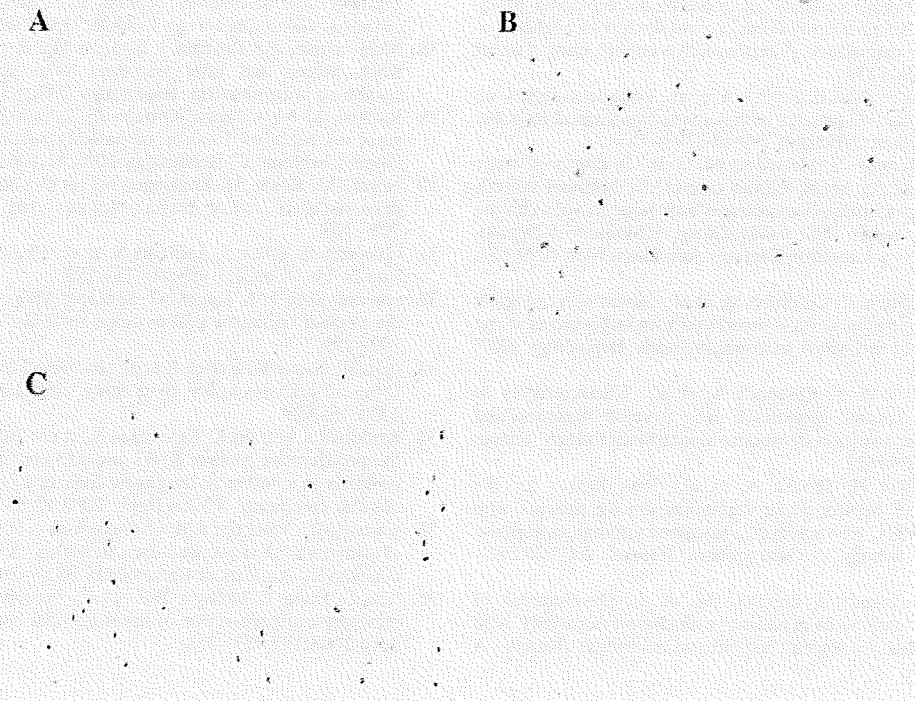

Fig. 5. Immunostaining with DO-7 for the expression analysis of nuclear p53 protein was performed. Positive case at 40-fold magnification (**A**). The cancerous lesion of a positive case at 400-fold magnification (**B**). The non-cancerous tissue at 400-fold magnification (**C**).

HCV-RNA. Thus, patients should be more closely followed up, even if viral elimination has been successful.

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Fascin Expression in Progression and Prognosis of Hepatocellular Carcinoma

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Background and Objectives: Fascin is an actin-bundling protein and induces membrane protrusions and cell motility after the formation of lamellipodia or filopodia. Fascin expression has been reported to be associated with progression or prognosis in various neoplasms, but the role of fascin in hepatocellular carcinoma (HCC) remains unknown. The aim of this study was to investigate the clinicopathological and prognostic relevance of fascin by immunohistochemistry.

Methods: A total of 137 patients with HCC were stained with anti-fascin antibody. The tumor cells having unequivocal cytoplasmic and/or membranous fascin immunoreactivity were defined as fascin-positive.

Results: Immunohistochemically, 23 (16.8%) HCCs having unequivocal fascin immunoreactivity were found. Tumors showing fascin expression were larger and less differentiated than those showing no fascin expression ($P = 0.0239$ and 0.0018, respectively). Portal venous invasion, bile duct invasion, and intrahepatic metastasis were detected significantly more frequently in fascin-positive group ($P = 0.0029$, 0.0333, and 0.0403, respectively). In addition, high alpha-fetoprotein (AFP) levels were significantly associated with the fascin expression in HCC ($P = 0.0116$). Fascin-positive group had significantly poorer outcomes than fascin-negative group and was an independent prognostic factor for disease-free survival.

Conclusions: Fascin might become a novel marker of progression in HCC and a significant indicator of a poor prognosis for patients with HCC. *J. Surg. Oncol. 2009;100:575–579.* © 2009 Wiley-Liss, Inc.

KEY WORDS: **immunohistochemistry; hepatoma; AFP; filopodia: lamellipodia**

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in the world [1–3]. Although the diagnostic and surgical approaches have made great progress in recent years, patient survival remains unsatisfactory because of a high incidence of recurrence after hepatic resection or other types of loco-regional therapy [4]. However, the mechanisms underlying the development of HCC remain unclear.

Fascin is a globular actin-bundling protein encoded by a gene located on chromosome 7p22 [5–7]. Its major function is forming the parallel actin bundles that support lamellipodial and filopodial cell protrusions [5–8]. Recently, the clinicopathological role of fascin expression has been associated with progression or prognosis in various neoplasms [9–20]. However, the clinicopathological relevance of fascin in HCC remains unknown.

The aim of this study was to examine the expression of fascin in HCC and to investigate its prognostic relevance by immunohistochemical analyses.

MATERIALS AND METHODS

Patients and Tissue Specimens

Between January 1997 and December 2001, 137 patients seen in our institute were found to have HCC and were selected consecutively by reviewing their pathologic diagnosis. Any patients undergoing previous therapy or non-curative surgery were excluded. The mean number of follow-up days after the initial hepatic resection was 2.52 ± 0.86 years, maximum 3.0 years, for the purpose of evaluating early recurrence from the primary HCCs. A monthly measurement of alpha-fetoprotein (AFP) was performed. In addition, ultrasonography and dynamic computed tomography were also performed every 3 months. Any postoperative survival or recurrence was entered into the database immediately when a patient died due to HCC or if a recurrence was strongly suspected on the diagnostic imaging, such as the computed tomography or magnetic resonance imaging system. Informed consent was obtained from each patient included in the study. The clinicopathological variables were defined according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer of the Liver Cancer Study Group of Japan [21]. The clinicopathological findings of the 137 patients are summarized in Table I. All specimens were fixed in 10% formalin, embedded in paraffin, and cut into 3-μm serial sections for immunohistochemical staining, in addition to the usual hematoxylin–eosin staining.

Immunohistochemical Staining and Evaluation

Immunohistochemical staining was carried out using the primary antibody to human fascin (monoclonal, 55K-2; Dako, Carpinteria, California; 1:50 dilution). The sections were dewaxed through xylene and ethanol. After blocking of endogenous peroxidase and antigen retrieval (microwave heating in citrate buffer for 40 min), the sections were exposed to the primary antibody at 4°C overnight and stained with a streptavidin–biotin–peroxidase kit (Nichirei, Tokyo, Japan). Finally, the labeled antigen was visualized by 3,3′-diaminobenzidine tetrahydrochloride, followed by counterstaining with hematoxylin.

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TABLE I. Clinicopathological Findings of the Resected Specimens of HCC

Factors	Number (n = 137)
Clinical factors	
Age (years)	65 (60, 69)
Sex (male/female)	107/30
Viral infection	
HBs-Ag (+) (%)	17.5
HCV-Ab (+) (%)	64.2
Albumin (g/dl)	3.9 (0.4)[c]
Total bilirubin (mg/dl)	0.8 (0.6, 1.1)[d]
AST (IU/L)	53 (34, 78)[d]
ALT (IU/L)	48 (28, 83)[d]
Platelet ($\times 10^4$/μl)	12.3 (10.2, 17.7)[d]
Prothrombin time (%)	85 (76, 95)[d]
ICG_{15} (%)	16.2 (10.4, 24.9)[d]
AFP (ng/ml)	19.3 (7.2, 176.2)[d]
Surgical factors	
Operative procedures, major[a] (%)	32.4
Operation time (min)	315 (262, 355)[d]
Blood loss (g)	780 (496, 1,238)[d]
Surgical margin (>5 mm/<5 mm)	83/53
Pathological data	
Tumor size (cm)	3.0 (2.1, 4.4)[d]
Histological differentiation (well/mode/poor)	10/85/42
Portal venous invasion (%)	45.3
Hepatic venous invasion (%)	21.2
Bile duct invasion (%)	3.7
Intrahepatic metastasis (%)	28.5
Non-cancerous liver (non-cirrhosis/cirrhosis)	88/49
Results	
Recurrence from cancer (n, the median of interval)[b]	73, 0.96 year
Death from cancer (n, the median of interval)[b]	20, 1.47 years

HBs-Ag, hepatitis B virus antigen; HCV-Ab, hepatitis C virus antibody; AST, asparate aminotransferase; ALT, alanine aminotransferase; ICG_{15}, indocyanine green retention rate at 15 min; AFP, alpha-fetoprotein.
[a]Segmentectomy, lobectomy, or extended lobectomy.
[b]The mean number of follow-up days after the initial hepatic resection was 2.52 ± 0.86 years, maximum 3.0 years.
[c]The median (standard deviation).
[d]Median (25th and 75th percentile).

Interlobular bile duct was considered to be an internal positive control [20].

Each section undergoing immunohistochemical staining for fascin was evaluated within the tumor by two pathologists (T.I., S.A.) who had no knowledge of the clinicopathological findings. The tumor cells having unequivocal cytoplasmic and/or membranous fascin immunoreactivity regardless of the proportion or intensity were defined as fascin-positive.

Statistical Analysis

A comparison between the expression of fascin and the clinicopathological findings was evaluated using the chi-square test, the Student's *t*-test, and the Mann–Whitney's *U*-test. Akaike's information criterion (AIC) was used to evaluate the fit of the logistic regression models, predicting whether fascin expression was defined from all clinicopathological parameters. The patient survival analysis was calculated by the Kaplan–Meier method, and the differences were evaluated by the log-rank test. The factors of the metric variables suspected the assumption of the linear risk were divided by the median points, and a Cox proportional hazards model was used in the multivariate survival analysis. These results were analyzed using the StatView-J 5.0 software program (SAS Institute, Inc., Cary, NC, 1992–1998). *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

Immunohistochemical Examination of Fascin

Fascin expression was absent or sporadic in normal epithelium, including hepatocytes, whereas biliary epithelium and vascular endothelial cells were positive (Fig. 1A). The fascin-positive cells showed unequivocal cytoplasmic and/or membranous staining patterns. Of the 137 patients with HCC, only 23 (16.8%) were evaluated as belonging to the fascin-positive group. The distribution of tumor cells with fascin immunoreactivity occurred diffusely or focally. Representative images of a positive case (Fig. 1B) and a negative case (Fig. 1C) in HCC are shown. In addition, tumor cells with portal venous invasion also showed fascin expression (Fig. 1D).

Comparison of Fascin Status and Clinicopathological Findings

We compared the clinicopathological findings of the fascin-positive and fascin-negative groups (Table II). Whereas no significant differences were noted with respect to sex, age, the parameter of preoperative liver function, and surgical factors, the fascin-positive tumors had higher AFP values and were larger and less differentiated than the fascin-negative tumors ($P = 0.0021$, 0.0239, and 0.0018, respectively). All tumors showing well-differentiated HCC had no fascin immunoreactivity. The portal venous invasion, bile duct invasion, and intrahepatic metastasis were detected significantly more frequently in the fascin-positive group ($P = 0.0029$, 0.0333, and 0.0403, respectively). In addition, of all the clinicopathological factors, only high AFP levels were significantly associated with the fascin expression in HCC, according to a logistic regression analysis ($P = 0.0102$; data not shown).

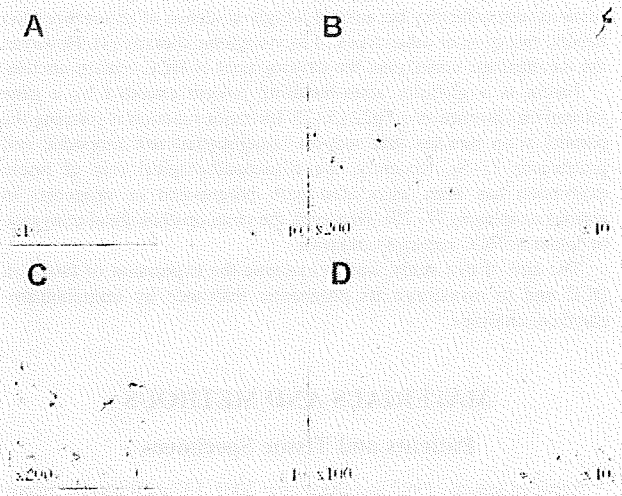

Fig. 1. Immunohistochemical staining of fascin in the non-cancerous liver (**A**), hepatocellular carcinoma (HCC) (**B**,**C**), and portal venous invasion (**D**). Fascin was expressed in cytoplasm and/or on cell membrane. A: Fascin expression was absent or sporadic in normal epithelium including hepatocytes, whereas biliary epithelium and vascular endothelial cells were positive. B: Representative fascin-positive case of HCC. C: Representative fascin-negative case of ICC. D: Portal venous invasion having fascin immunoreactivity.

TABLE II. Comparative Analysis of the Clinicopathological Findings Between Fascin-Negative Group and Fascin-Positive Group

Factors	Fascin-negative group (n = 114)	Fascin-positive group (n = 23)	*P*-value
Clinical factors			
Age (years)	66 (60, 69)[b]	64 (60, 69)[b]	NS
Sex (male/female)	89/25	18/5	NS
Viral infection			
HBs-Ag (+) (%)	16.67	21.74	NS
HCV-Ab (+) (%)	64.91	60.87	NS
Albumin (g/dl)	3.9 (0.4)[c]	3.9 (0.4)[c]	NS
Total bilirubin (mg/dl)	0.9 (0.7, 1.1)[b]	0.8 (0.6, 1.1)[b]	NS
AST (IU/L)	53 (32, 74)[b]	59 (37, 80)[b]	NS
ALT (IU/L)	48 (27, 84)[b]	38 (31, 78)[b]	NS
Platelet ($\times 10^4/\mu l$)	12.3 (10.3, 17.1)[b]	13.7 (9.1, 18.9)[b]	NS
Prothrombin time (%)	85 (77, 95)[b]	86 (74, 97)[b]	NS
ICG_{15} (%)	16.1 (10.7, 24.8)[b]	17.3 (10.0, 24.6)[b]	NS
AFP (ng/ml)	15.7 (5.8, 86.5)[b]	362.5 (11.8, 8,018.4)[b]	0.0021
Surgical factors			
Operative procedures, major[a] (%)	30.70	40.91	NS
Operation time (min)	315 (260, 355)[b]	315 (280, 355)[b]	NS
Blood loss (g)	765 (473, 1,225)[b]	800 (525, 1,635)[b]	NS
Surgical margin (>5 mm/<5 mm)	69/44	14/9	NS
Pathological data			
Tumor size (cm)	2.9 (2.1, 4.2)[b]	4.2 (2.3, 8.0)[b]	0.0239
Histological differentiation (well/mode/poor)	10/76/28	0/9/14	0.0018
Portal venous invasion (%)	39.5	73.9	0.0029
Hepatic venous invasion (%)	18.4	34.8	NS
Bile duct invasion (%)	1.75	13.0	0.0333
Intrahepatic metastasis (%)	24.6	47.8	0.0403
Non-cancerous liver (non-cirrhosis/cirrhosis)	75/39	13/10	NS

HBs-Ag, hepatitis B virus antigen; HCV-Ab, hepatitis C virus antibody; AST, asparate aminotransferase; ALT, alanine aminotransferase; ICG_{15}, indocyanine green retention rate at 15 min; AFP, alpha-fetoprotein.
[a]Segmentectomy, lobectomy, or extended lobectomy.
[b]Median (25th and 75th percentile).
[c]The median (standard deviation).

Outcomes After Surgery

The disease-free survival rates for the fascin-negative group at 1 and 3 years were 73.2% and 42.4% and those for the fascin-positive group type at 1 and 3 years were 43.7% and 26.2%, respectively (Fig. 2A). The analysis of the disease-free survival revealed that patients in the fascin-positive group had significantly poorer outcomes than those in the fascin-negative group ($P = 0.0221$). In addition, the overall survival rates for the fascin-negative group at 1 and 3 years were 97.3% and 87.1% and those for the fascin-positive group at 1 and 3 years were 77.3% and 67.3%, respectively (Fig. 2B). The analysis of the overall survival also revealed that the patients in the fascin-positive group had significantly poorer outcomes than those in the fascin-negative group ($P = 0.0076$). Furthermore, a multivariate proportional hazard model revealed that belonging to the fascin-positive group ($P = 0.0066$), intrahepatic metastasis ($P < 0.0001$), asparate aminotransferase (AST) >53 IU/L ($P = 0.0013$), indocyanine green retention rate at 15 min (ICG_{15}) >16.2% ($P = 0.0026$), blood loss <780 g ($P = 0.0066$), and a cirrhotic liver ($P = 0.0257$) were independent prognostic factors for disease-free survival (Table III), while belonging to the fascin-positive group was not an independent factor for overall survival (data not shown).

DISCUSSION

Fascin expression in normal epithelium is absent or sporadic, but in various neoplasms, fascin expression is dramatically altered. How fascin is up-regulated during malignant transformation and progression has been examined, and tissue-specific mechanisms have been suggested [5]. In our study, only 23 of HCC samples, 16.8%, showed fascin expression and early HCC, indicating that well-differentiated HCC has no fascin immunoreactivity. A high proportion of fascin is already present in the premalignant stage [10,12,18,20]. Therefore, independent mechanisms of fascin up-regulation seem to be involved in HCC.

Fascin has been reported to have various functions such as involvement in cell migration induced by the formation of lamellipodial and filopodial cell protrusions [5–7] as well as differentiation [22,23] and proliferation [9,24]. In our study, portal venous invasion, bile duct invasion, and intrahepatic metastasis were found significantly more frequently in the fascin-positive group and tumors showing fascin expression were larger and less differentiated than those showing no fascin expression. Using RNA interference, down-regulation of fascin has been shown to inhibit cell invasiveness as well as proliferation in vitro [9,24]. In vitro, fascin overexpression also decreased the capacity for cell adherence [22,23]. In addition, the clinicopathological relevance of fascin has been associated with tumor invasion or metastasis in carcinomas of the esophagus [9,10], stomach [11], and lung [14], as well as extrahepatic cholangiocarcinoma [17] and oral squamous carcinoma [19]. Fascin expression in HCC might also alter the motility, adherence, and proliferation of carcinoma cells.

Interestingly, in our study, AFP values were higher in the fascin-positive group than in the fascin-negative group. When AFP is monitored as a tumor marker, aberrant reactivation occurs in up to 85% of all HCC cases [25] and is considered to be a predictive marker for tumor invasiveness and recurrence of HCC [26,27]. Deregulated beta-

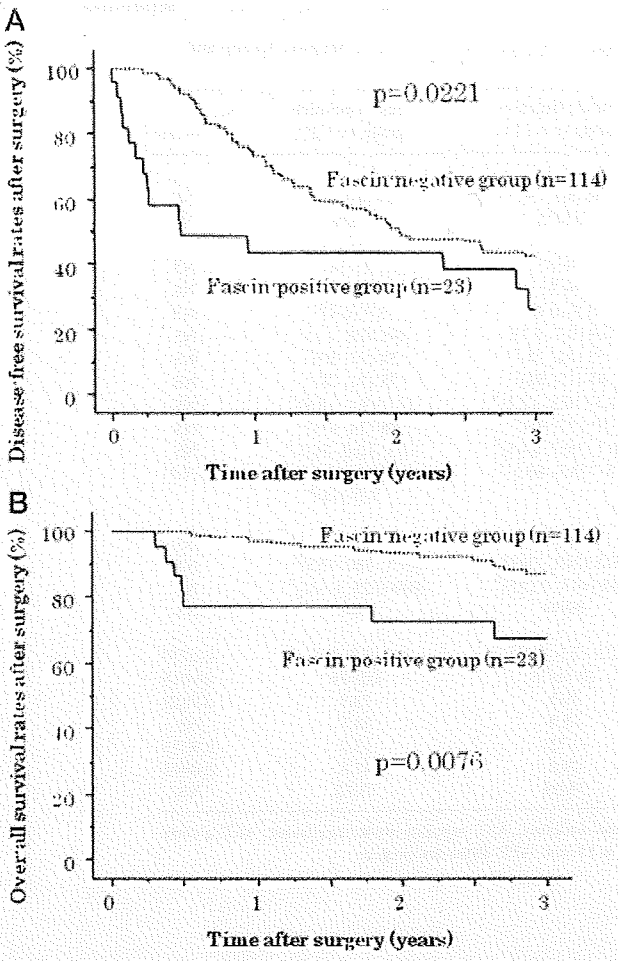

Fig. 2. Disease-free survival (**A**) and overall survival (**B**) curve for fascin-positive (solid line) and fascin-negative (dotted line) groups in patients with hepatocellular carcinoma ($P=0.0021$ and 0.0076, respectively).

catenin can induce p53 expression via the induction of ARF [28]. In addition, p53 suppresses AFP transcription through sequence-specific binding within the AFP developmental repressor domain [29,30]. Nuclear translocation and accumulation of beta-catenin can also activate the transcription of fascin through TCF-binding sites [31]. Fascin might regulate the AFP through a complex interaction with this pathway, but it is necessary to further examine how fascin affects AFP values.

TABLE III. Multivariate Analysis of the Disease-Free Survival for HCC

Variables	Hazard ratio	95% confidence interval	*P*-value
Intrahepatic metastasis	3.568	2.029–6.274	<0.0001
AST >53 (IU/L)	2.465	1.424–4.267	0.0013
ICG_{15} >16.2 (%)	2.484	1.374–4.490	0.0026
Blood loss ≤780 (g)	2.171	1.276–3.692	0.0042
Fascin-positive group	2.542	1.295–4.989	0.0066
Liver cirrhosis	1.868	1.079–3.234	0.0257

AST, asparate aminotransferase; ICG_{15}, indocyanine green retention rate at 15 min.

The recurrence of HCC after resection is generally considered to originate from either intrahepatic metastasis or multicentric occurrence. Early recurrence is associated only with tumor factors but not host status, while late recurrence is associated with non-cancerous liver status [32]. Namely, the aggressive behavior of the HCC itself is shown by early recurrence after initial hepatic resection. Hence, we have focused on a time interval of less than 3 years after hepatic resection.

Recently, the prognostic relevance of fascin expression has been reported in various neoplasms, esophageal squamous cell carcinoma [9], gastric carcinoma [11], breast cancer [16], and intrahepatic cholangiocarcinoma [20]. Patients with fascin overexpression have been reported to have shorter survival. In our study, fascin expression was involved in the prognosis of HCC and was found to be an independent prognostic factor. Therefore, fascin expression in HCC might also be a significant indicator of a poor prognosis. However, it remains to be seen whether fascin expression represents merely a surrogate marker for prognosis or whether it plays a pathogenic role in carcinogenesis and tumor progression.

In summary, we have for the first time demonstrated fascin expression in HCC clinical samples. Fascin overexpression was found to be associated with aggressive behavior and poor prognosis in HCC. Our results suggest that fascin may become a novel marker of progression and prognosis in HCC.

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BRIEF ARTICLES

Prognosis of hepatocellular carcinoma accompanied by microscopic portal vein invasion

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Abstract

AIM: To investigate the prognostic factors in patients with hepatocellular carcinoma (HCC) accompanied by microscopic portal vein invasion (PVI).

METHODS: Of the 267 patients with HCC undergoing hepatic resection at Aso Iizuka Hospital, 71 had PVI. After excluding 16 patients with HCC that invaded the main trunk and the first and second branches of the portal vein, 55 patients with microscopic PVI were enrolled.

RESULTS: The patients with HCC accompanied by microscopic invasion were divided into two groups: solitary PVI (PVI-S: n = 44), and multiple PVIs (PVI-M: n = 11). The number of portal vein branches invaded by tumor thrombi was 5.4 ± 3.8 (2-16) in patients with PVI-M. In cumulative survival, PVI-M was found to be a significantly poor prognostic factor (P = 0.0019); while PVI-M and non-anatomical resection were significantly poor prognostic factors in disease-free survival (P = 0.0213, and 0.0115, respectively). In patients with PVI-M, multiple intrahepatic recurrence was more common than in the patients with PVI-S (P = 0.0049). In patients with PVI-S, non-anatomical resection was a significantly poor prognostic factor in disease-free survival (P = 0.0370). Operative procedure was not a significant prognostic factor in patients with PVI-M.

CONCLUSION: The presence of PVI-M was a poor prognostic factor in patients with HCC, accompanied by microscopic PVI. Anatomical resection is recommended in these patients with HCC. Patients with HCC and PVI-M may also be good candidates for adjuvant chemotherapy.

Key words: Hepatocellular carcinoma; Microscopic portal vein invasion; Hepatectomy; Prognosis; Recurrence

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant tumor with periportal venous metastasis. Vascular invasion, especially portal vein invasion (PVI), is a major determinant of outcome after hepatic resection in patients with HCC[1-7].

Magnetic resonance imaging (MRI) and ultrasonography can detect tumor invasion of the major branches of the portal or hepatic veins, 81%-95% of the time[8-10]. However, the presence of microscopic PVI, limited to the subsegmental portal vein branches, cannot be diagnosed before hepatic resection. Recently reported predictors of microscopic PVI include size, number, and histological grade of tumors, as well as serum

level of des-γ-carboxy prothrombin (DCP)[11-13]. Miyata *et al*[14] have demonstrated that tumorous arterioportal (A-P) shunt formation on computed tomography (CT) during hepatic arteriography is an important predictive value for PVI. A recent study by Nishie *et al*[15] has shown that an area exhibiting low attenuation on CT during arterio-portography, and high attenuation on CT during hepatic arteriography around the tumor, is a good predictor of PVI. Thus, recent studies have suggested that the presence of microscopic PVI can be predicted.

Nevertheless, the prognostic factors of HCC with microscopic PVI have remained elusive and the operative procedures for this type of HCC have not been determined. In the present study, we evaluated the prognostic factors in cumulative and disease-free survival in patients with HCC, accompanied by microscopic PVI.

MATERIALS AND METHODS

Patients

From April 1992 to December 2005, 267 patients underwent their first liver resection for HCC at the Department of Hepatogastro-enterological Surgery at Aso Iizuka Hospital in Japan. From a retrospective database, 55 patients were enrolled in this study, according to the following criteria: (1) an absence of HCC invading the main trunk and the first and second branches of the portal vein, upon preoperative radiological evidence and intraoperative findings; (2) no remnant cancer after surgery, as confirmed by ultrasonography, CT and/or MRI; and (3) the presence of microscopic PVI upon histological examination.

There were 41 male and 14 female patients with an average age of 64 years (median: 66 years). Among these patients, 35 (64 %) were infected with hepatitis C virus, which leads to chronic liver disease. The indocyanine green retention test at 15 min was 15.5% ± 10.2%. On pathological examination, the tumor size was 5.0 ± 1.1 cm and the main grade of cancer cell was moderately differentiated in 38 patients (69%) and poorly differentiated in 17 (31%). Microscopic intrahepatic metastasis was found in 24 patients (44%).

Methods

The prognostic factors were examined in cumulative and disease-free survival, using the following variables: age (older or younger than 67 years); gender (male *versus* female); platelet numbers (greater than *versus* less than or equal to 150000/mm^3); serum albumin levels (greater than *versus* less than or equal to 3.8 g/dL); tumor size (greater than *versus* less than or equal to 4.2 cm); serum levels of alpha-fetoprotein (AFP) (greater than *versus* less than or equal to 28 ng/mL); DCP (greater than *versus* less than or equal to 300 mAU/mL); operative procedures (anatomical *versus* non-anatomical resection); histological grading of cancer cell differentiation (moderate *versus* poor); presence of intrahepatic metastases (negative *versus* positive); and microscopic PVI (solitary or multiple). The measurement of serum DCP has been described previously[13]. The measurement of serum DCP was started at our hospital in 1999 and was therefore only available in the latest 36 patients.

Figure 1 Multiple microscopic PVI surrounding a tumor (arrow heads). HE staining (Original magnification, × 20).

Anatomical resection included hemi-hepatectomy, segmentectomy, and subsegmentectomy, based on Couinaud's classification. Non-anatomical resection was partial hepatectomy, including the tumor.

Histological study

All of the resected specimens were cut into serial 5-10-mm thick slices and fixed in 10% formalin. After macroscopic examination, the slice with the greatest dimension was trimmed for paraffin blocks and cut into 4-μm microscopic sections. The slices were then stained with hematoxylin and eosin (HE). When clusters of cancer cells were present in the extra tumoral portal vein, accompanied with bile duct and hepatic artery, it was defined as positive for extra tumoral PVI. When more than two clusters of cancer cells were present in different portal vein branches, it was defined as multiple PVI (PVI-M) (Figure 1). When only one cluster was present in a single portal vein branch, it was defined as solitary PVI (PVI-S).

Follow-up strategy and recurrence pattern

After discharge, all patients were examined for recurrence by ultrasonography and tumor markers, such as AFP and DCP every month, and by CT every 6 mo. When recurrence was suspected, additional examinations such as hepatic angiography were performed. The recurrence pattern was determined by ultrasonography, CT, MRI and hepatic angiography and was defined as previously reported[7]. Briefly, none was the absence of HCC recurrence, nodular recurrence was fewer than four recurrent nodules, and multiple recurrence was four or more recurrent nodules.

Impact of operative procedures in patients with PVI-S and PVI-M

In patients with PVI-S and PVI-M, the impact of operative procedures (non-anatomical *versus* anatomical resection) was compared on cumulative and disease-free survival.

Factors	Survival rate (%)			P value
	1 yr	3 yr	5 yr	
Age (yr)				
< 67 (n = 27)	80.6	60.4	42.1	0.9546
67 (n = 28)	96.6	75.7	50.0	
Gender				
Male (n = 41)	92.6	72.3	45.2	0.7614
Female (n = 14)	78.6	50.0	42.9	
Platelets (10^4/mm^3)				
< 1.5 (n = 27)	92.6	61.7	46.6	0.7341
≥ 1.5 (n = 28)	85.7	67.5	46.0	
Albumin (g/dL)				
< 3.9 (n = 27)	100	76.9	47.2	0.6123
3.9 (n = 28)	79.0	56.8	47.5	
Tumor size (cm)				
< 4.3 (n = 27)	92.4	72.8	35.9	0.8752
≥ 4.3 (n = 28)	85.7	60.7	52.0	
AFP (ng/mL)				
0-27 (n = 28)	96.4	71.3	55.4	0.1001
> 27 (n = 27)	81.2	61.6	35.8	
DCP (mAU/mL)				
0-300 (n = 18)	94.1	75.1	75.1	0.1834
300 (n = 18)	83.3	55.6	55.6	
Operative procedure				
Anatomical resection (n = 32)	90.5	74.2	51.4	0.0620
Non-anatomic resection (n = 23)	87.0	55.9	39.5	
Tumor grade of differentiation				
Moderate (n = 38)	97.3	70.0	54.9	0.1076
Poor (n = 17)	70.6	58.8	30.3	
IM (-) (n = 31)	96.8	66.7	56.5	0.2625
IM (+) (n = 24)	79.2	66.7	32.8	
PVI-S (n = 44)	97.7	76.5	51.2	0.0019
PVI-M (n = 11)	54.5	27.3	27.3	

IM: Intrahepatic metastasis.

Statistical analysis

All data were expressed as mean ± SD. The χ^2 test of independence was used with categorical variables. The continuous variables were divided by their median values. The survival and disease-free survival curves were generalized using the Kaplan-Meier method and then compared using the log-rank test. The Stat view software (Version 4.11; Abacus Concepts Inc., Berkeley, CA, USA) was used for the analysis on a Macintosh computer. $P < 0.05$ was considered to be statistically significant.

RESULTS

Histological examination of PVI-S and PVI-M

Of the 267 patients, 55 (21%) had microscopic PVI. The overall incidence of PVI-S was 16% (44 patients) and that of PVI-M was 4% (11 patients). Of the 55 patients with PVI, the 11 patients with PVI-M represented 20%. The number of portal vein branches invaded by tumor thrombi was 5.4 ± 3.8 (2-16) on the slices, stained with HE in patients with PVI-M.

Significant prognostic factors in cumulative survival

The overall survival after hepatectomy in 55 patients with microscopic PVI was 89.0% at 1 year, 66.6% at 3 years, 46.0% at 5 years, and 36.1% at 10 years (Figure 2).

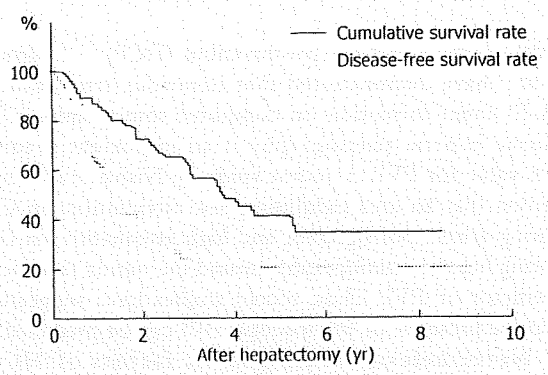

Figure 2 Cumulative and disease-free survival rate in patients with HCC, accompanied by microscopic PVI. The overall cumulative and disease-free survival after hepatectomy in 55 patients was 89.0% and 68.7% at 1 year, 66.6% and 27.3% at 3 years, 46.0% and 22.7% at 5 years.

The median survival was 4.6 ± 0.6 years. Among 11 clinicopathological factors, only the extent of PVI was significant (Table 1). Thus, the cumulative survival curve for patients with PVI-M was significantly worse than that of those with PVI-S (P = 0.0019). The median survival of patients with PVI-S and PVI-M was 5.4 ± 1.8 and 1.3 ± 0.7 years, respectively. In operative procedures, the survival rate for anatomical resection tended to be better than that for non-anatomical resection, although the difference was not significant (P = 0.0620).

Significant prognostic factors in disease-free survival

The disease-free survival in 55 patients was 68.7% at 1 year, 27.3 % at 3 years, 22.7 % at 5 years, and 22.7 % at 10 years (Figure 2). Among 11 clinicopathological factors, the extent of PVI and operative procedures were significant (Table 2). The disease-free survival curve for patients with PVI-M was significantly worse than that for patients with PVI-S (P = 0.0072). The median disease-free survival in PVI-S and PVI-M was 1.5 ± 0.8 and 0.4 ± 0.2 years, respectively. In operative procedures, the disease-free survival rate for anatomical resection was significantly better than that for non-anatomical resection (P = 0.0074). The median disease-free survival for anatomical and non-anatomical resection was 2.0 ± 0.2 and 0.8 ± 0.1 years, respectively.

Comparison of PVI-S and PVI-M recurrence patterns

A comparison of the recurrence pattern is shown in Table 3. The incidence of multiple recurrence in patients with PVI-M was 82% and 30% in patients with PVI-S (P = 0.0049).

Impact of operative procedures in patients with PVI-S and PVI-M

In patients with PVI-S, non-anatomical resection tended to be a poor prognostic factor for cumulative survival (P = 0.0782), while non-anatomical resection was significantly poor prognostic factor in disease-free survival (P = 0.0370) (Table 4). In patients with PVI-M, operative procedures were not significant in disease-free survival (Table 5).

Factors	Survival rates (%)			*P* value
	1 yr	3 yr	5 yr	
Age (yr)				
< 67 (*n* = 27)	54.7	31.9	31.9	0.7438
≥ 67 (*n* = 28)	72.5	21.3		
Gender				
Male (*n* = 41)	68.7	27.3	22.7	0.2716
Female (*n* = 14)	48.6	19.4	19.4	
Platelets (10^4/mm^3)				
< 1.5 (*n* = 27)	52.8	7.9	7.9	0.1703
≥ 1.5 (*n* = 28)	74.2	33.7	33.7	
Albumin (g/dL)				
< 3.9 (*n* = 27)	61.5	22.6	16.9	0.8555
≥ 3.9 (*n* = 28)	65.8	29.4	29.4	
Tumor size (cm)				
< 4.3 (*n* = 27)	62.9	35.0	35.0	0.4474
≥ 4.3 (*n* = 28)	64.8	17.0	11.3	
AFP (ng/mL)				
0-27 (*n* = 28)	59.1	39.4	39.4	0.1692
> 27 (*n* = 27)	68.0	14.6	9.7	
DCP (mAU/mL)				
< 300 (*n* = 18)	72.5	43.5	43.5	0.2995
≥ 300 (*n* = 18)	63.0	21.0	14.0	
Operative procedure				
Anatomical resection (*n* = 32)	82.3	33.0	33.0	0.0074
Non-anatomical resection (*n* = 23)	40.9	17.0	11.4	
Tumor grade of differentiation				
Moderate (*n* = 38)	69.3	36.7	31.4	0.0661
Poor (*n* = 17)	52.9	7.4	7.4	
IM (-) (*n* = 31)	67.1	28.2	23.5	0.4761
IM (+) (*n* = 24)	59.8	22.1	22.1	
PVI-S (*n* = 44)	74.6	27.4	23.5	0.0072
PVI-M (*n* = 11)	20.0	20.0		

Recurrence pattern	None	Nodular	Multiple
PVI-S (*n* = 44)	16 (36)	15 (34)	13 (30)
PVI-M (*n* = 11)	2 (18)	0	9 (82)

The incidence of multiple recurrence in PVI-M was significantly higher than that in PVI-S ($P = 0.0049$).

Factors	Survival rates (%)			*P* value
	1 yr	3 yr	5 yr	
Cumulative survival				
Anatomical resection (*n* = 26)	96.2	83.9	55.0	0.0782
Non-anatomical resection (*n* = 18)	100	65.8	46.1	
Disease-free survival				
Anatomical resection (*n* = 26)	90.9	32.2	32.2	0.0370
Non-anatomical resection (*n* = 18)	52.9	22.1	14.7	

Factors	Survival rates (%)			*P* value
	1 yr	3 yr	5 yr	
Cumulative survival				
Anatomical resection (*n* = 6)	66.7	33.3	33.3	0.4197
Non-anatomical resection (*n* = 5)	40.0	20.0	20.0	
Disease-free survival				
Anatomical resection (*n* = 6)	40.0	20.0	20.0	0.2651
Non-anatomical resection (*n* = 5)	0			

DISCUSSION

The incidence of microscopic PVI has been reported to be more than 20% in resected HCC[1,2]. Even in small HCCs, up to 2 cm in diameter, the incidence of PVI is 15%[3]. In this study, the incidence of PVI was found in 55 of 267 (20%) patients.

Vascular invasion, especially PVI, is a major determinant of the outcome after hepatic resection in patients with HCC[1-7]. In the present study, the survival rate was poorer for HCC patients with PVI than those without (data not shown). Nevertheless, the prognosis of patients with PVI varied. With regard to recurrence patterns, 18 (33%) of the 55 patients with HCC accompanied by PVI had no recurrence, and 22 patients (40%) had multiple recurrence. Clearly, the outcome in patients with no recurrence was better than that of patients with multiple recurrence.

Determination of the prognostic factors in patients with PVI is important for postoperative therapeutic strategy. There has been no study of prognostic factors in patients with HCC accompanied by PVI. In the present study, detailed histological examination of resected specimens revealed that PVI-M was a significantly poor prognostic factor after hepatectomy for cumulative and disease-free survival. With regard to operative procedures, anatomical resection tended to improve survival rates and significantly improve disease-free rates.

Histologically, the number of portal vein branches invaded by tumor thrombi was 5.4 ± 3.7 (2-16) in patients with PVI-M. Although PVI was limited to the subsegment of the liver, multiple portal vein branches that surrounded the tumor were invaded. In these patients, the biological behavior of HCC with PVI-M may be similar to that of HCC, with invasion to the first branches or main trunks of the portal vein. The survival rate in patients with PVI-M was only 54.5% at 1 year after hepatectomy. The mean survival and disease-free survival after hepatectomy in patients with PVI-M was 1.3 and 0.4 years, respectively. Multiple recurrence was more common in patients with PVI-M than those with PVI-S. This clinical outcome was similar to that previously shown for patients with HCC, accompanied by portal vein thrombi of the first branches or main trunks[16,17].

Anatomical resection of the liver significantly improved the disease-free rates for HCC with PVI in the present study. Hasegawa *et al*[18] have reported that anatomical resection, such as segmentectomy and subsegmentectomy for HCC, is a reasonable treatment option and yields more favorable results than non-anatomical resection. A recent comparison of the