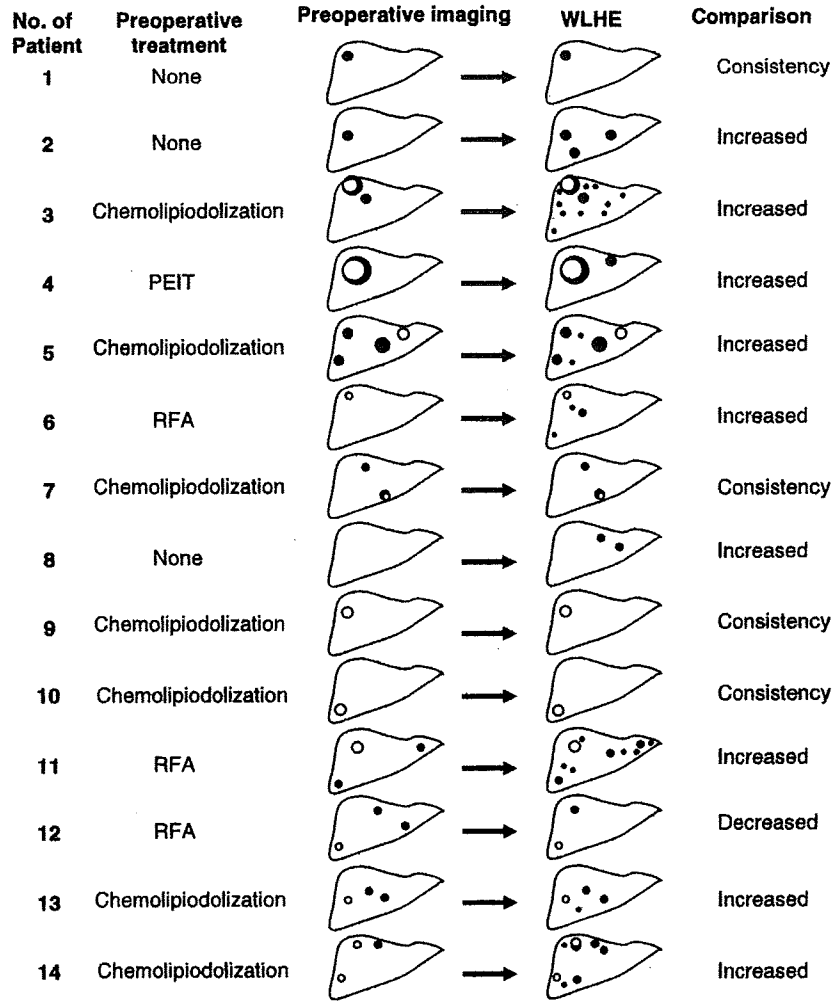

Figure 1 Detection of hepatocellular carcinoma using preoperative imaging and postoperative whole liver histological examination.

● Viable hepatocellular carcinoma (HCC).
○ Preoperative treatment with no viable HCC.
PEIT, percutaneous ethanol injection therapy; RFA, radiofrequency ablation; WLHE, whole liver histological exmination.

detected by the current imaging modalities were identified only by pathological examination. One patient (7.1%) (case 12) had a decreased number of HCCs compared to the number determined from preoperative imaging. The distribution of preoperatively undetectable nodules, which was based on segmental anatomy of the liver, was as follows: four nodules in segment 2, four nodules in segment 3, seven nodules in segment 4, one nodule in segment 5, six nodules in segment 6, three nodules in segment 7 and nine nodules in segment 8 (Fig. 1). After WLHE, five out of the 14 patients (35.7%) were beyond the M-C. When we compared the results for the number and largest size of HCCs between imaging and WLHE, the largest size of HCCs was not altered by WLHE, but the number of HCCs was increased by WLHE (Fig. 2). This was because small HCCs that could not be preoperatively detected by imaging were found in the cirrhotic liver by whole liver investigation.

Characteristics of preoperatively detectable HCCs

A total of 49 nodules were found by WLHE (Table 2). Fifteen nodules were found through preoperative images taken after histological examination, but two

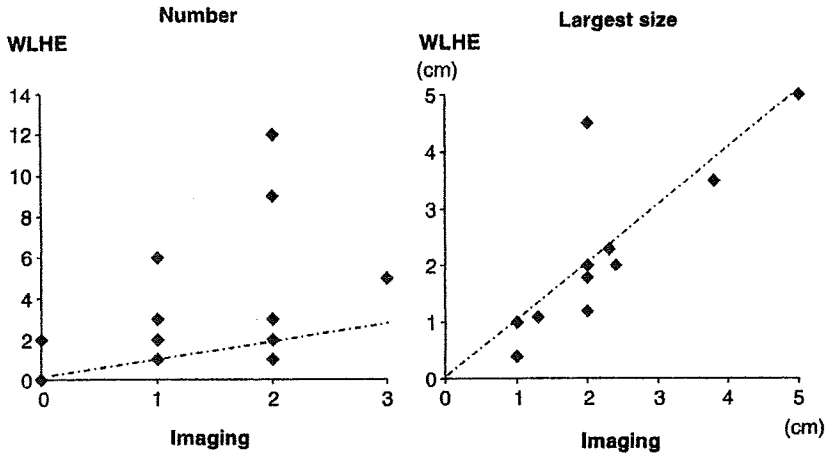

Figure 2 The relationship of size and number between imaging and whole liver histological examination.

nodules detected preoperatively were not found in the explanted liver after WLHE. The median diameter of the 15 preoperatively detectable HCCs was 18 mm (range, 10–50 mm). There were seven well-differentiated HCCs (46.7%) and eight moderately differentiated HCCs (53.3%). The preoperatively detectable nodules showed expansive growth (11/15; 73.3%) with fibrous capsules (60%) (Table 2). Only two nodules (13.3%) showed microscopic vascular invasion surrounding the main tumor in the detectable HCCs.

Characteristics of preoperatively undetectable HCCs

Thirty-four HCCs that were undetectable preoperatively were found by WLHE (Table 2). The median diameter of the nodules was 6 mm (range, 2–15 mm). The undetectable nodules consisted of 25 well-differentiated HCCs (73.5%), nine moderately differentiated HCCs (26.5%) and no poorly differentiated HCCs. The characteristic features of the preoperatively undetectable nodules included infiltrative growth (29/34; 85.3%), absence of fibrous capsules (32/34; 94.1%) and absence of microscopic vascular invasion (32/34; 94.1%).

Pathological comparison of preoperatively undetectable and detectable HCCs

By comparing the pathological features of undetectable and detectable HCCs based on the preoperative imaging results, we examined the relationship between tumor size and each of tumor differentiation, tumor growth and the presence of fibrous capsules (Figs 3–5). No significant difference in differentiation or growth was found between the preoperatively detectable and undetectable tumor size. However, we found significant differences in the presence of fibrous capsules between the undetectable and detectable HCCs (Fig. 4).

Table 2 The characteristics of hepatocellular carcinoma on preoperative imaging study and postoperative histological study

	HCC on imaging ($n = 15$)	HCC only on WLHE ($n = 34$)	
Diameter (median, range) (mm)	18 (10–50)	6 (2–15)	$P = 0.0000006$
Differentiation			
Well	7 (46.7%)	25 (73.5%)	NS
Moderate	8 (53.3%)	9 (26.5%)	NS
Poor	0	0	
Fibrous capsule	9 (60.0%)	2 (5.9%)	$P = 0.000028$
Growth			
Expansive growth	11 (73.3%)	5 (14.7%)	NS
Infiltrative growth	4 (26.7%)	29 (85.3%)	NS
Microvascular invasion	2 (13.3%)	2 (5.9%)	NS

HCC, hepatocellular carcinoma; NS, not significant; WLHE, whole liver histological examination.

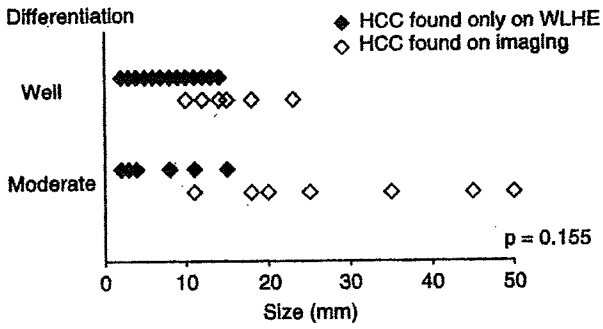

Figure 3 The relationship between differentiation and size between imaging and whole liver histological examination.

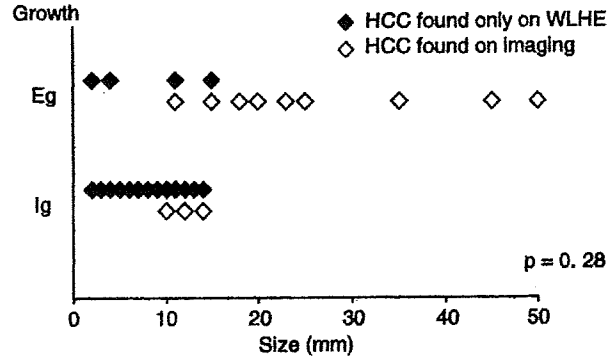

Figure 5 The relationship between growth and size between imaging and whole liver histological examination.
Eg, expansive growth; Ig, infiltrative growth.

Recurrence of HCCs

In the follow-up period (median follow-up, 30.1 months), there was no recurrence in any patient after LDLT.

DISCUSSION

IN THIS STUDY, we have demonstrated a discrepancy in the number of HCCs determined from preoperative imaging studies and the number determined from histological measurements using WLHE. It is especially important to note that multicentric HCCs were diffusely present in the end-stage cirrhotic liver, which is clinically significant since local therapy for only those HCCs that are detectable might not be a practical curative procedure in such cirrhotic livers. In addition, this study demonstrated that even HCCs within the M-C, which are usually regarded as early HCCs, can coincide with much earlier HCCs in the severely cirrhotic liver. This finding has not been demonstrated in autopsied livers from patients who died of advanced HCC.

In the present study, the accuracy of preoperative diagnosis for HCC was 28.6%, which is rather low compared to other reports.[9–16] However, other studies used histological examination only for those nodules that were suspicious based on imaging findings, and not for the whole liver. Moreover, MDCT tends to overdiagnose HCC, with a false-positive diagnosis rate as high as 8–11.8%.[11,12] In addition, in a previous study MDCT could detect only 61% of lesions smaller than 2 cm and 93.6% of lesions 2 cm or larger.[12] Since our study demonstrated that the median diameter of preoperatively undetectable nodules was 6 mm (range, 2–15 mm), the overlooked lesions in previous studies were definitely below the detectable range of CT.

Of the 14 patients preoperatively within the M-C, undetectable HCCs were found in nine patients (64.3%), with the result that five patients (35.7%) exceeded the M-C after WLHE, mainly due to the increased number of HCCs. The diameters of the largest HCCs determined from WLHE were identical to preoperative measurements. Therefore, current imaging modalities, such as MDCT, are good at evaluating the size of viable large HCCs, but not for determining the number of small HCCs. The reason the small HCCs were not detected by the current imaging modalities might be that they were characterized by an absence of fibrous capsules and an unclear border between the cancerous lesions and the normal liver tissue. Another possible reason for the failure of the imaging methods to detect small HCC might be that the blood supply to the early HCC came more from the portal vein than the hepatic artery, as in the case of advanced HCC. The

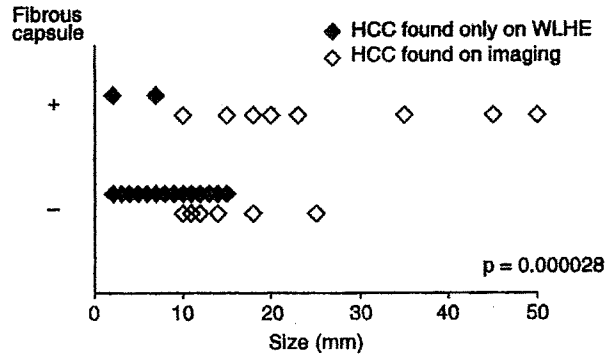

Figure 4 The relationship between fibrous capsule and size between imaging and whole liver histological examination.

distribution of undetectable HCCs did not show any special features; that is, undetectable HCCs could be found in any segment in the cirrhotic liver, implying that MDCT did not have blind areas.

In this study, there was no recurrence of HCCs after LT during the median follow-up period of 30.1 months, although two patients showed vascular invasion in the explanted livers by microscopic examination. Thus, HCCs in cases of advanced cirrhotic liver could be a multicentric phenomenon, and undetectable HCCs might not affect the survival rate after LT. Therefore, in the era of MDCT, the M-C would seem to be too strict a set of criteria for determining whether or not LT is indicated.[17–20] In other words, if we do follow the M-C as an indication of LT for HCC, the recurrence rate of HCC should be minimal.

In conclusion, HCCs in the severely cirrhotic liver might be characterized by multicentric occurrence. In this study, there were no recurrent HCCs after LDLT. Thus, preoperatively undetectable HCCs might not be associated with recurrence of HCC after LDLT. However, a high rate of recurrence can be expected when local therapy is performed for HCC, even with early-stage HCC, as determined by the M-C.

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CASE REPORT

Acute Deterioration of Idiopathic Portal Hypertension Requiring Living Donor Liver Transplantation: A Case Report

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Abstract Case reports of severe idiopathic portal hypertension (IPH) requiring liver transplantation are very rare. We report the case of a 65-year-old woman who was diagnosed as having IPH. At the age of 60 years, her initial symptom was hematemesis, due to ruptured esophageal varices. Computed tomography of the abdomen showed splenomegaly and a small amount of ascites, without liver cirrhosis. She was diagnosed as having IPH and followed-up as an outpatient. Five years later, she developed symptoms of a common cold and rapidly progressive abdominal distension. She was found to have severe liver atrophy, liver dysfunction, and massive ascites. Living donor liver transplantation was then performed, and her postoperative course was uneventful. Histopathological findings of the explanted liver showed collapse and stenosis of the peripheral portal vein. The areas of liver parenchyma were narrow, while the portal tracts and central veins were approximate one another, leading to a diagnosis of IPH. There was no liver cirrhosis. The natural history of refractory IPH could be observed in this case. Patients with end-stage liver failure due to severe IPH can be treated by liver transplantation.

Keywords Idiopathic portal hypertension · Liver transplantation

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Introduction

Idiopathic portal hypertension (IPH) is a relatively rare disease, and it has been reported mostly in patients from Japan [1]). Most patients with IPH have a good prognosis with treatment for their esophagogastric varices, but some develop end-stage liver failure despite various medical treatments [2–11]. Such end-stage liver failure is an indication for liver transplantation, but details of such cases have not been fully reported in the literature. We treated a patient with severe IPH who required living donor liver transplantation (LDLT); this case allows one to observe the natural history of severe refractory IPH.

Case Report

A 65-year-old woman who had been followed-up for IPH at our hospital developed abdominal distension. At the age of 60 years, the patient presented with sudden hematemesis and was taken to a nearby hospital. On emergent upper gastrointestinal endoscopy, the ruptured esophageal varices were successfully ligated. Subsequently, the patient was transferred to our hospital.

On her admission, her vital signs (heart rate, blood pressure, respiratory rate, and body temperature) were stable. On physical examination, her abdomen was soft, flat, and not tender. Her spleen was palpable, but her liver was not. She did not have encephalopathy. The patient denied any history of blood transplantation, alcohol abuse, or medications. Laboratory data showed pancytopenia (hemoglobin 9.4 g/dl; platelet count $4.5 \times 10^4/\mu l$, and white blood cell count 1,300 /μl). Results of liver and renal function tests and electrolytes were normal. Her prothrombin time was slightly prolonged (73%). Hepatitis B

Fig. 1 (**a**) Computed tomography at the time of the first admission shows splenomegaly and incomplete extrahepatic portal vein thrombus (*arrow*). No liver cirrhosis or liver tumor is seen. (**b**) Computed tomography 5 years after the first admission shows massive ascites and a very atrophic liver. (**c**) Histopathological findings show collapse of the peripheral portal vein. Inflammatory cells and dilated abnormal vessels are seen near the portal tract. Hematoxylin and eosin

surface antigen, hepatitis C virus antibody, and anti-mitochondria antibody were all negative.

Computed tomography of the abdomen showed splenomegaly, incomplete extrahepatic portal vein thrombus, and a slight volume of free peritoneal fluid, but no hepatomegaly, liver cirrhosis, or liver tumors (Fig. 1a). The gastrointestinal tract, gallbladder, pancreas, kidneys, and genital organs were unremarkable. There was no obstruction of the extrahepatic portal vein or the inferior vena cava. Upper gastrointestinal endoscopy showed persistent esophageal varices.

The patient had portal hypertension that consisted of esophageal varices, splenomegaly, and pancytopenia. Laboratory data showed that liver function was completely normal, and liver cirrhosis was not found on imaging, though liver biopsy was not performed. Obstruction of the extrahepatic portal vein and the inferior vena cava, hematological malignancies, and other known diseases were ruled out. IPH was diagnosed, and the patient was followed routinely at our hospital as an outpatient, during which time she was prescribed propranolol and warfarin. Her condition remained quite stable and uneventful for 5 years.

At the age of 65 years, she developed symptoms of the common cold and rapidly progressive abdominal distension. At that time, laboratory data showed anemia (hemoglobin 7.5 g/dl), but normal platelet and white cell counts. Serum aspartate aminotransferase, alanine aminotransferase, and total bilirubin levels were normal. Her serum albumin level had decreased to 2.6 g/dl. Her prothrombin time was 44%, which was drastically prolonged, despite vitamin K administration. She also had acute renal failure (blood urea nitrogen 61 mg/dl; serum creatinine 3.8 mg/dl). Computed tomography showed massive ascites, and volumetry showed that her liver was atrophic, with only 44% of the volume of that 5 years earlier (Fig. 1b). Extrahepatic portal vein thrombus, which had been found 5 years earlier, had disappeared. The Child–Pugh score was 11 points, and the model of end-stage liver disease (MELD) score was 23 points. She was diagnosed as having non-reversible, end-stage liver failure, and she underwent liver transplantation.

At laparotomy, she had 15 l of free intraperitoneal fluid. The liver was extremely atrophic; the resected whole liver weight was only 620 g. An extended left lobe liver graft, weighting 400 g, was transplanted from her husband. The graft weight standard liver volume rate was 36.8%. A total splenectomy was also performed. The patient's postoperative course was uneventful, and she was discharged 50 days after surgery. Five months after transplantation, the patient developed enterocolitis caused by cytomegalovirus infection; she died from peritonitis following perforation of the small intestine.

The explanted liver at the time of the LDLT was atrophic, with a wavy surface. On histopathology, there was collapse and stenosis of the peripheral portal vein (Fig. 1c). The areas of liver parenchyma had become narrow, so that the portal tracts and central veins were approximate to one another. The entire specimen showed narrowing of the liver parenchyma, which was especially severe near the capsule. Inflammatory cells and dilated abnormal vessels were seen around the portal tracts. There were no pseudolobules or bridging folds. The histopathology findings were compatible with IPH. There was no liver cirrhosis or evidence of malignancy.

Discussion

Portal hypertension is usually caused by liver cirrhosis, but some cases of portal hypertension occur without cirrhosis. Sinusoidal portal hypertension of unknown etiology is regarded as IPH clinically. When diagnosing IPH, one must rule out liver cirrhosis, obstruction of the extrahepatic portal vein and the inferior vena cava, blood diseases, parasitic diseases, granulomatous hepatic disease, congenital liver fibrosis, chronic viral hepatitis, primary biliary cirrhosis, and other known diseases [12]. IPH has been found in 2.1–2.6% of livers at autopsy [13, 14]. IPH is associated with a

Table 1 Summary of case reports of severe IPH requiring liver transplantation (LT)

Author	Year	Number of patients	Gender	Age at LT	Diagnosis before LT	Period between manifestation and LT	Explanted liver weight	Pathological diagnosis	Prognosis
McDonald et al. [2]	1990	1	Male	47	Cirrhosis	10 years	973 g	NRH	Died (4 months)
Le Bail et al. [3]	1990	1	Male	48	Recurrent HCC	N/A	N/A	HCC	Well
					Alagille syndrome			NRH	
de Sousa et al. [4]	1991	1	Female	24	Multiple liver tumors	1 year	2,970 g	NRH	Well
								Budd–Chiari syndrome	
Elariny et al. [5]	1994	1	Female	44	Cirrhosis	3 years	1,850 g	NRH	Well
Nadir et al. [6]	1994	2	Male 1	60	Cirrhosis 2	4 years	N/A	NRH 2	Both well
			Female 1	55–64					
Bernard et al. [7]	1995	1	Male	34	Cirrhosis	14 years	1,070 g	ISC	Well
Loinaz et al. [8]	1998	4	Male 4	35	NRH 1	0.5 years	1,100 g	NRH 3	Died 2 (1 month, 3 months)
				25–41	Cirrhosis 1	2 months–1 year	110–2,400 g	Partial nodular transformation 1	Recurrence 1 (7 years)
					Nodular liver 1				
					Chronic liver disease 1				
Radomski et al. [9]	2000	4	Male 3	46	Cirrhosis 4	7.7 years	N/A	NRH 4	All well
			Female 1	39–55		4–13 years			
Dumortier et al. [10]	2001	8	Male 8	45	IPH 8	4.5 years	1,045 g	ISC 5	All well
				20–63		3–10 years	(630–1,520 g)	NRH 3	
Krasinskas et al. [11]	2005	16	Male 11	47	Cirrhosis 13	4.3 years	1,100 g	NRH 15	Died 1 (5 months)
			Female 5	31–64	Azathioprine-associated liver injury 2	1–11 years	600–1,550 g	ISC 9	Recurrence 2 (3.5 months, 7 months)
					NRH 1				
Our patient		1	Female	65	IPH	5 years	620 g	IPH	Died (5 months)
Total		40	Male 30	45.5 (mean)		4.6 years (mean)	1,150 g (mean)		
			Female 10						

HCC: hepatocellular carcinoma, *IPH*: idiopathic portal hypertension, *ISC*: incomplete septal cirrhosis, *NRH*: nodular regenerative hyperplasia, *N/A*: not available

spectrum of histological lesions, including incomplete septal cirrhosis, nodular regenerative hyperplasia, and partial nodular transformation [14–16]. The lesions show some degree of uneven fibrosis but may coexist in the same liver. In our patient, typical findings of incomplete septal cirrhosis, nodular regenerative hyperplasia, or partial nodular transformation were not seen. The pathogenesis of IPH is unclear, but IPH can occur in association with a variety of developmental, vascular, collagen vascular, or biliary tract diseases [6, 8, 11]. Renal failure with or without transplantation and the toxic effects of some drugs may be related to IPH [5, 8, 10, 11]. Common symptoms of IPH include splenomegaly, pancytopenia, gastrointestinal bleeding, ascites, and encephalopathy.

Patients with IPH can be treated by the usual approaches: (a) long-term medical therapy, along with balloon tamponade and vasoactive drugs for bleeding esophageal varices; (b) endoscopic variceal ligation and sclerotherapy for both long-term and short-term treatment, and (c) portosystemic shunt procedures (portocaval, mesocaval, or radiological) or evascularization surgery [8]. In general, IPH does not progress to liver cirrhosis or hepatocellular carcinoma. The prognosis for patients with IPH is mostly good, if the gastrointestinal bleeding can be controlled. However, some cases of IPH progress to end-stage liver failure.

In our case, the patient developed symptoms of the common cold and rapidly progressive abdominal distension. Collagen diseases and autoimmune disorders are known to be associated with IPH [10, 11]. The immune system plays a role in IPH, so infection may be one of the progressive factors for IPH. Though a decrease in hepatic blood flow is considered to relate to IPH progression, it is unclear whether a decrease in hepatic blood flow affected acute deterioration in this case [11]. Portal vein thrombus is one of the causes for decreased hepatic blood flow, but it was not found during the operation or in the explanted liver specimen.

Some patients with IPH which is resistant to all other therapies may be successfully treated by orthotopic liver transplantation (OLT). Several case reports dealing with OLT for severe IPH are summarized in Table 1 [2–11]. If these previous reports and ours are taken into account, there were 40 patients (30 men, 10 women) with a mean age at transplantation of 45.5 years (range 20–65 years). Gastrointestinal varices, splenomegaly, and ascites were commonly seen in patients with severe IPH who required OLT, but these symptoms are common in IPH (Table 2). No specific symptoms characterize severe IPH. Eleven patients were treated for varices, seven by sclerotherapy and four by endoscopic variceal ligation, and nine patients underwent pretransplantation portosystemic shunting procedures (six transjugular intrahepatic portosystemic shunts and three mesocaval shunts) (Table 3). The time between the clinical manifestations of portal hypertension and liver transplantation ranged from 2 months to 14 years, with a mean of 4.6 years. Patients underwent OLT because of rapidly progressing, life-threatening, complications of portal hypertension and liver disease and the inefficacy of surgical or radiological portosystemic shunting. Combined liver and kidney transplantation is performed in patients with associated severe renal failure [5, 8, 10]. Most patients with severe IPH described in the literature were well following OLT. Four patients died in the early posttransplantation period, due to herpes zoster encephalitis, suicide, sudden rupture of an unsuspected splenic aneurysm, and pneumonia [2, 8, 11]. Three patients showed evidence suggestive of recurrence of IPH in the graft liver. One patient developed symptoms 1 year after OLT [11]; two patients were asymptomatic, though their liver biopsy findings after OLT suggested recurrence [8, 11].

Making a pretransplantation diagnosis of IPH is difficult; 23 of the 40 patients with IPH were believed clinically to have cirrhosis, based on the clinical presentation, the

Table 2 Symptoms before liver transplantation in literatures

Symptom	Number of patients (%)
Gastrointestinal varices	33(83)
Splenomegaly	26(65)
Ascites	24(60)
Gastrointestinal bleeding	15(38)
Encephalopathy	11(28)
Cytopenia	6(15)
Hepatopulmonary syndrome	3(8)
Liver atrophy	1(3)
Retroperitoneal collateral rupture	1(3)
Pleural effusion	1(3)
Hepatomegaly	1(3)
Recurrent HCC	1(3)
Bacterial peritonitis	1(3)

HCC: hepatocellular carcinoma

Table 3 Treatments before liver transplantation in literatures

Treatment	Number of patients (%)
Sclerotherapy	7(18)
Transjugular intrahepatic portosystemic shunts	6(15)
Endoscopic variceal ligation	4(10)
Mesocaval shunt	3(8)
Splenectomy	3(8)
Tumorectomy for HCC	1(3)

HCC: hepatocellular carcinoma

pretransplantation biopsy findings, or the radiological images. The diagnosis was made only after posttransplantation examination of the explanted liver. Some patients who had died from what appeared to be liver cirrhosis clinically, had hidden IPH. In patients with symptoms of severe portal hypertension without liver dysfunction, IPH should be considered.

In conclusion, appropriate treatment can provide IPH patients with a good prognosis, but some patients with IPH develop end-stage liver failure. Some patients who are clinically diagnosed as having cryptogenic cirrhosis might have IPH. Patients with severe IPH which is resistant to other therapies may be good candidates for OLT, after which a favorable outcome can be expected.

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SURGERY TODAY

Review Article

What Is the Real Contribution of Extrahepatic Cells to Liver Regeneration?

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Abstract
Extrahepatic cells, especially bone marrow (BM) cells, might contribute to liver repair, but recent published evidence suggests that they do not play a role in the normally regenerating liver. The mechanism by which extrahepatic cells express a liver-specific function in the liver, whether by transdifferentiation or by cell fusion, remains unclear. In this review, we investigate the status of findings on this controversial subject and summarize the recent research.

Key words Extrahepatic stem cells · Liver regeneration · Transdifferentiation · Cell fusion

Introduction

It has been reported that extrahepatic cells, especially bone marrow (BM)-derived cells, are mobilized and involved in the repair of liver tissue, including after injury.[1–17] However, there are few details about the involvement of extrahepatic cells and how much they contribute to normal liver regeneration. Even if such involvement exists, it remains unclear whether liver-specific function is achieved through transdifferentiation or cell fusion. Thus, we reviewed the findings of recent published research on this subject.

Contribution of Extrahepatic Cells to Liver Regeneration from Injury

After partial hepatectomy, the liver mass is usually restored within 1–2 weeks in rats and 1–3 months in humans, in order to catch up with liver-specific function. DNA synthesis and cell division occurs first in hepatocytes, and then in nonparenchymal cells.[17,18] It was originally believed that only cells in the liver participate in this restoration; however, recent findings in liver transplant recipients who received BM transplantation have prompted investigations into the contribution of BM cells to liver repopulation, especially liver regeneration after liver damage or partial hepatectomy, but with controversial results (Tables 1 and 2).

An in vivo experiment in 2000 revealed that hepatocytes could be derived from BM cells after irradiation in the absence of severe acute injury.[1] Then, in 2001, Baccarani et al.[2] reported that replacement of female liver venous endothelium with male BM in humans showed the possible involvement of BM cells in liver rearrangement. This was followed by the finding by Körbling et al. of the differentiation of circulating stem cells into mature hepatocytes.[3] Since 2002, research has advanced in this area with the advent of green fluorescent protein (GFP) transgenic mice, which express green fluorescent protein throughout their bodies. The GFP-positive cell-transplant model allows researchers to detect transplanted or mobilized cells without complicated molecular biological methods. Using this model, after GFP-positive BM transplantation, Fujii et al. found that although BM cells participated in liver regeneration after hepatectomy, the majority was committed to sinusoidal endothelial cells (Fig. 1), probably through endothelial progenitor cell mobilization.[4,5] Using their GFP/carbon-tetrachloride (CCl_4) mouse model, in 2003 Terai et al. reported that autologous BM cells were an effective treatment for liver failure caused by persistent liver damage. They found the same results for liver cirrhosis.[6,7,16] In 2005, am Esch also reported that CD133, used as a hematopoietic stem cell marker, plus BM stem cells infused into the portal vein, accelerated hepatic regeneration.[8] Even more recently, Conzelmann et al., using their reduced-size liver transplantation model, reported that recipient-derived

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Table 1. Relationship between extrahepatic cells and liver regeneration/impairment (1)

First author[Ref.]	Year	Patient or model	Findings
Baccarani[2]	2001	Human	Replacement liver venous endothelium in livers of BM transplant recipients
Fujii[4]	2002	GFP transgenic mouse	BM cells participated in LR. The majority was committed to sinusoidal endothelial cells
Wu[25]	2003	Human	Only rare, isolated, and tentatively identified recipient hepatocytes
Cantz[20]	2004	GFP transgenic mouse	No evidence of BM cells in LR
Terai[6]	2005	GFP transgenic mouse	Autologous BM cells were effective for treatment of liver failure
Di Campli[12]	2005	Human	No evidence of hematopoietic stem cells in LR

BM, bone marrow; GFP, green fluorescent protein; LR, liver regeneration

Table 2. Relationship between extrahepatic cells and liver regeneration (2)

First author[Ref.]	Year	Patient or model	Findings
am Esch[8]	2005	Human	CD133(+) BM stem cells infused into portal vein accelerating LR
Moritoki[13]	2006	GFP transgenic mouse	BM cells transfer did not contribute to the differentiation of cholangiocytes in chronic cholestasis model. Scattered GFP(+) cells in hepatic parenchyma
Tomiyama[14]	2007	Rat	Limited contribution of cells of intact extrahepatic tissue origin to LR in transplanted liver. Liver injury did not increase the percentage of GFP(+) using LT model
Conzelmann[9]	2007	GFP transgenic mouse	Using reduced-size LT, recipient-derived progenitor cells were present and might contribute to LR
Beaudry[21]	2007	GFP transgenic mouse	Contribution of circulating endothelial progenitor cells with exogenous vascular endothelial growth factor

BM, bone marrow; GFP, green fluorescent protein; LR, liver regeneration; LT, liver transplantation

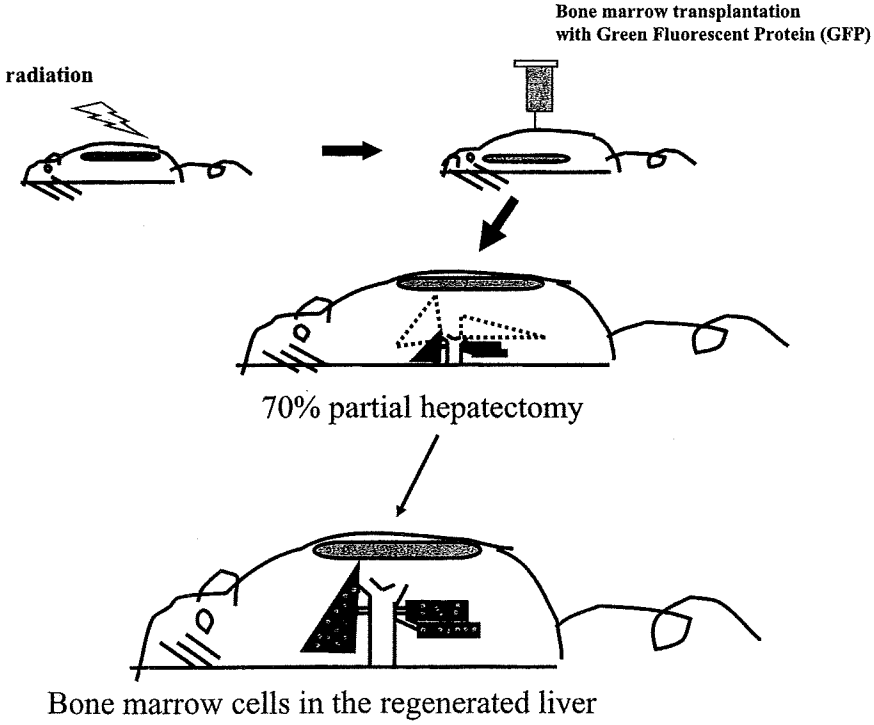

Fig. 1. Involvement of extrahepatic cells in liver regeneration. Transplanted bone marrow cells were involved in liver regeneration after partial hepatectomy in rats. The majority was committed to sinusoidal endothelial cells. Adapted from Fujii et al.[4]

progenitor cells were present and might contribute to liver regeneration in mice.[9] All these reports constitute encouraging data to support the notion that extrahepatic cells, and especially BM cells, are potent therapeutic resources for impaired liver regeneration. Furthermore, studies on partial hepatectomy using rats with liver regeneration impaired by retrorsine have revealed some positive results.[10,11] However, there is

Table 3. Controversies on involvement of extrahepatic cells in liver regeneration and repair

First author[Ref.]	Year	Species	Cells differentiated from extrahepatic cells
Yes			
Baccarani[2]	2001	Human	Hepatic endothelial cells
Fujii[4]	2002	Rat	Hepatic endothelial cells
Conzelmann[9]	2007	Mouse	9% of liver comprised within 28 days
Beaudry[21]	2007	Mouse	Hepatic endothelial cells
No			
Wu[25]	2003	Human	No endothelial cells from BM cells
Cantz[20]	2004	Mouse	Limited or no contribution
Di Campli[12]	2005	Human	No evidence of BM mobilization
Moritoki[13]	2006	Mouse	No cholangiocytes from BM cells
Tomiyama[14]	2007	Rat	Limited contribution

BM, bone marrow

still controversy regarding the degree and mechanism of involvement of the cells. Thus, we investigated the studies focusing on this issue (Table 3). In 2005, Di Campli et al. reported no evidence of hematopoietic stem cell mobilization in patients who underwent hepatectomy or in patients with acute liver failure. They observed no CD34-positive cells in the blood after hepatectomy for acute decompensation of a cirrhotic liver.[12] Similarly in 2006, a study by Moritoki et al. using GFP transgenic mice demonstrated that BM cell transfer seemed not to contribute to the differentiation of cholangiocytes in a chronic cholestasis model. These authors also found scattered GFP-positive cells in the hepatic parenchyma.[13] In 2007, Tomiyama et al. reported the limited contribution of cells originating from intact extrahepatic tissue in hepatocyte regeneration in transplanted rat livers. They reported that even in the non-injured liver, GFP-positive hepatocytes increased by 0.0048% per week: in other words, 5×10^3 were generated per day. However, liver injury did not trigger an increase in the percentage of GFP-positive hepatocytes in their liver transplantation model,[14] as Popp et al. reported similar findings in 2007.[15]

Based on these findings, it seems that limited involvement is possible in normal liver regeneration after partial hepatectomy. However, evidence from an in vivo liver injury model suggests that BM cells may be involved in the regeneration of an impaired liver. Conversely, an investigation on liver regeneration using a specific model in which liver cells cannot divide, using retrorsine, showed no contribution of multipotent mesenchymal stromal cells in liver regeneration. Whether extrahepatic cells migrate to the regenerating liver to function as liver cells and if so, how long they can survive, are still subjects of debate. Nevertheless, preliminary clinical studies with autologous BM cells or $CD34^+$ cells have been conducted to treat liver insufficiency, with moderate effects observed.[19]

Transdifferentiation or Cell Fusion

It has been intensely debated whether transdifferentiation or fusion accounts for the mechanism by which BM cells become hepatocytes. In 2004, Lee et al. described the differentiation of human mesenchymal stem cells into hepatocytes in vitro.[23] According to the transdifferentiation theory, the phenotype of BM cells changes to that of hepatocytes through coordinated changes in the transcriptional activities of many genes. The mesenchymal stem cell component in BM cells or other specific stem cells are candidates for this ability to transdifferentiate. Although transdifferentiation of the peripheral blood monocyte-derived subset into hepatic transdifferentiated cells has been described, it has not been established which cells are involved. Using their mouse model, Brulport et al. presented evidence not for transdifferentiation, but for a complex situation including partial differentiation and possible horizontal gene transfer.[24] In 2005, Wu et al. also reported minimal evidence of transdifferentiation from recipient BM to parenchymal cells, regenerating with long-term survival in human allografts.[25] On the other hand, "cell fusion" between BM stem cells and hepatocytes was reported and is thought to be a major mechanism based on an experiment repeated by many researchers, in which new hepatocytes appear after the infusion of BM cells.[26–30] We are still unsure if hepatocytes cannot be produced more effectively through "transdifferentiation" or "fusion." Further research on liver regeneration is needed to resolve these issues.[31]

In conclusion, based on our review of the recent literature, extrahepatic cells, especially BM cells, might contribute to repair of the injured liver but not to repair of the normally regenerating liver. The mechanism by which extrahepatic cells express a liver-specific function, whether transdifferentiation or cell fusion, has not been established.

Search Strategy

Recent data for this review were collected by PubMed searches.

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Acta Med. Nagasaki 53: 97－104

Underlying histological activity of hepatitis Plays an Important Role for Tumor Recurrence After Curative Resection of Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) commonly develops in patients with chronic hepatitis. This situation is one of the reasons why intrahepatic recurrence frequently occurs even after curative resection. There are two different components of such recurrences, which occurs within 12 months (the early recurrence group) and at more than 12 months after resection (the late recurrence group). The present study was conducted to clarify the factors contributing to these different types of HCC recurrence.

Methods: Ninety seven patients who underwent curative resection for HCCs were followed for initial recurrence, and predictive factors of recurrence were examined.

Results: Early and late intrahepatic recurrences developed in 30 and 42 patients, respectively. In the former group, univariate analyses showed the serum AFP level (>100ng/ml, P=0.045), higher inflammatory activity (Grading) (p=0.048) and status of fibrosis (Staging) (p=0.027) in non-cancerous liver tissues to be significant risk factors, while the serum AFP level (>100ng/ml) was the only independent risk factor based on a multivariate analysis (RR: 2.78). In the latter group, only the presence of hyperplastic foci (HPF) was found to be a significant risk factor (p=0.005). Higher Grading tended to be linked to shorter disease-free survival time, although not significant. In the non-cancerous liver tissues with HPF, the level of Grading, Staging, and PCNA labeling index was significantly higher (p=0.033, 0.003, 0.040, respectively). *Conclusion:* Not only the tumor factors but also the underlying hepatic status including HPF, Grading, and Staging were significant risk factors for intrahepatic recurrence after curative resection for HCC.

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Keywords: Hepatocellular carcinoma; Early and late recurrence; HPF; Grading; Staging

Introduction

Hepatocellular carcinoma (HCC) is one of the common causes of cancer death in Asia. Hepatic resection has been established as a curative treatment for hepatocellular carcinoma. Nevertheless, the prognosis remains poor because postoperative recurrences frequently occur (50 - 60%).[1-3] Such recurrences could originate from the intrahepatic metastases of the primary HCC[4,5] and the multicentric occurrence of new tumors in the postoperative liver remnant.[6-8] These unique features might be due to underlying liver diseases such as chronic active hepatitis with hepatitis B and C viral infection. There were several reports that HCC development was significantly linked to underlying liver diseases.[6,9,10] Shuto et al.[11] suggested that hyperplastic foci (HPF), which are defined as a focal hepatic parenchymal lesion where the hepatocytes have dense and small nuclei as well as eosinophilic cytoplasm, was an important predictor of recurrence of HCC after hepatic resection. Therefore, not only HCC tumor factors but also the underlying liver status should be carefully examined in order to select the optimal treatments and also better predict tumor recurrence after curative resection.

The present study was conducted to clarify the risk factors associated with intrahepatic recurrences in HCC patients who underwent curative hepatic resection by investigating tumor factors, op-

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erative factors, and patient characterization giving special attention to the underlying status of non-cancerous liver tissues.

Patients and methods

Patients and follow up

Ninety-seven patients underwent curative resection for HCC was closely followed up for more than 12 months after resection at Department of Transplant and Digestive Surgery, Nagasaki University Graduate School of Biomedical Sciences. They included 81 males and 16 females, with a mean age of 61 years old (range: 20-80). Twenty-four patients (24.7%) were positive for hepatitis B virus surface antigen (HBV) only, 48 (49.5%) for hepatitis C virus anti body (HCV) only, 4 for both (4.1%), and 21 were negative for both. The serum chemistry and the serum levels of alpha-fetoprotein (AFP) were measured monthly, and ultrasonography as well as com puted tomography scan were performed at a 3-month interval. Magnetic resonance imaging, angiography and liver biopsies were also performed to make a definitive diagnosis of recurrence, if needed.

Pathological examination

Resected liver specimens were fixed in 10% formaldehyde solution. After a macroscopic examination, a slice containing the maximum tumor diameter and other slices of lesions suspicious for metastases or venous invasion were embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H & E). According to the classification of Primary Liver Cancer by the Liver Cancer Study Group of Japan[12], the histological findings of each tumor were examined regarding the histological stage, the degrees of portal venous invasion and hepatic venous invasion as well as the presence of intrahepatic metastases.

Inflammatory activity (Grading), fibrotic status (Staging) and hyperplastic foci (HPF) in non-cancerous liver tissues

To classify the degree of hepatic inflammation (hepatitic activity), we used the Histological Activity Index (HAI) score as described by Knodell et al.[13] Based on their criteria, the H & E stained specimens of the non-cancerous liver tissues were examined and classified into four categories according to Desmet's method[14]; G0 (normal, or minimal chronic hepatitis), G1 (mild chronic hepatitis), G2 (moderate chronic hepatitis), and G3 (severe chronic hepatitis). To classify fibrosis, Scheuer's method[15] were used. The stage of fibrosis was categorized as F0 (non-fibrosis), F1 (enlarged, fibrotic portal tracts), F2 (fibrosis in periportal or portal-portal septa, but an intact architecture), F3 (fibrosis with architectural distortion, but not obvious cirrhosis) and F4 (probable or definite cirrhosis). Figure 1 shows a liver with inflammatory and fibrotic change (G2 and F3).

Hyperplastic foci (HPF) were defined as a focal hepatic parenchymal lesion where the hepatocytes have dense and small nuclei as well as eosinophilic cytoplasm[11,16] (Figure 2). The presence of HPF was examined in H&E-stained specimens of non-cancerous liver tissues. The liver tissue with at least one lesion of hyperplastic cells was defined as positive for HPF. The pathological findings were independently judged by two pathologists (K.K. and S.O.).

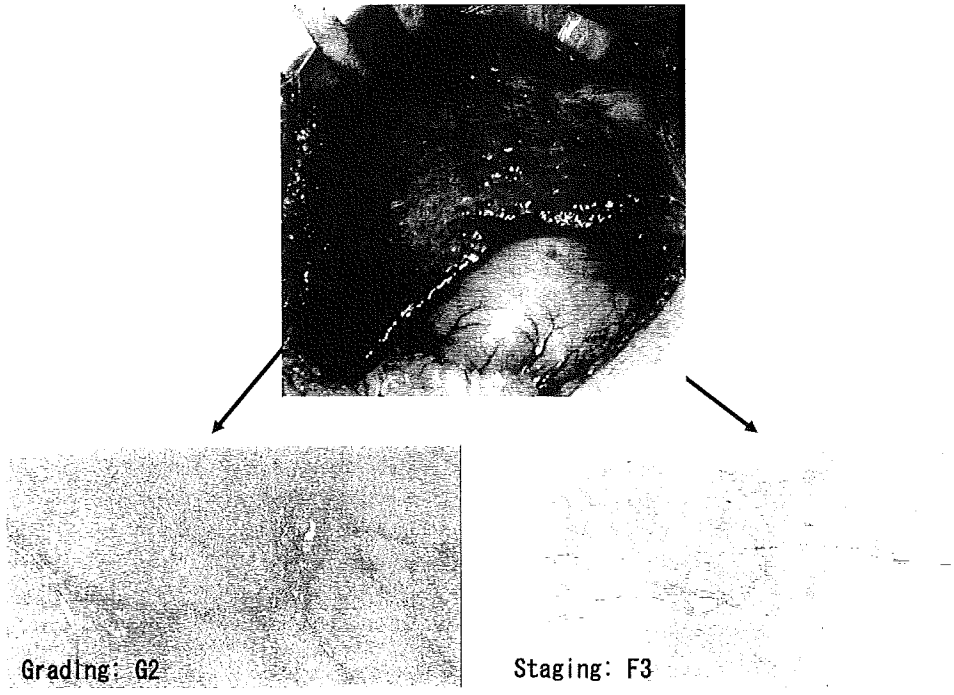

Figure 1. The liver with inflammatory and fibrotic change (G2 and F3).

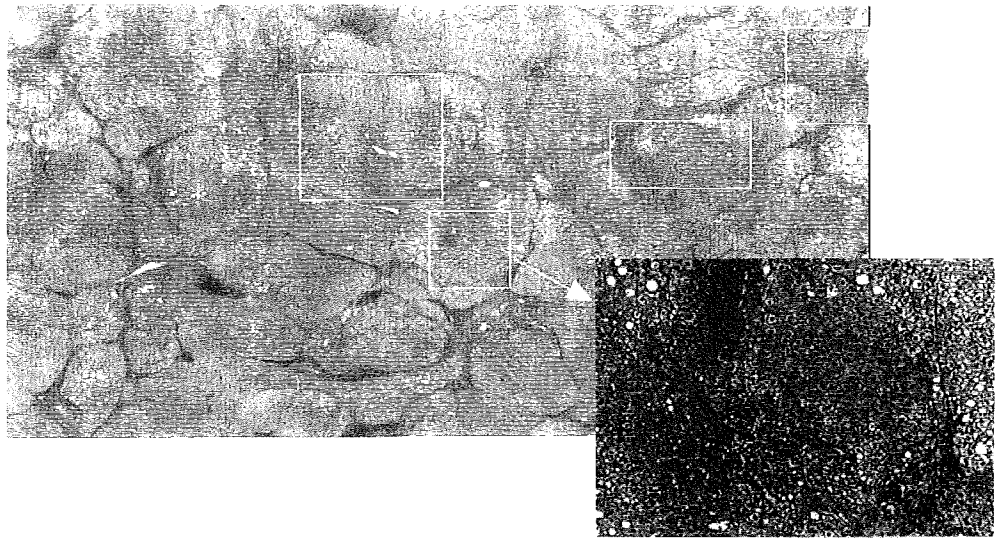

Figure 2. Hyperplastic foci is defined as a focal hepatic parenchymal lesion where the hepatocytes have dense and small nuclei as well as eosinophilic cytoplasm (in boxes) (x200, x400)

Proliferating cell nuclear antigen labeling index (PCNA L.I.)

The labeling index of PCNA was examined according to a method described in a previous report[17], which was determined by the ratio of PCNA-positive hepatocytes per 1000 hepatocytes.

Statistical analyses

Possible risk factors for early recurrence were compared using the chi-square test with Yates' correction (or Fisher's exact test where appropriate) for nominal variables and/or unpaired Student *t* test for continuous variables. Risk factors in either the chi-square or Student *t* test for early recurrence were consecutively analyzed based on multivariate logistic regression models. Possible risk factors for late recurrence were entered into Cox's multivariate proportional hazard model. The cumulative recurrence-free survival curves were analyzed using the Kaplan-Meier method and then were compared with the log rank test. A p value of less than 0.05 was considered to be statistically significant.

Results

Figure 3 shows the cumulative recurrence-free survival curve among 97 patients in the present study. Seventy-two of them (74.2 %) developed intrahepatic recurrence while the remaining 25 had no recurrence during the follow-up period. Two different components were observed in the recurrence-free survival curve. The first component was rapidly decreased within 12 months after curative hepatectomy, and these patients were classified as the early recurrence group. The second component was slowly decreased thereafter, and this group was classified as the late recurrence group.

Five patients (5.2 %) developed extra-hepatic recurrence, 7 (7.2 %) underwent a re-resection, and 27 (27.8 %) received chemolipiodolization. The 1-, 3-, and 5-year recurrence-free survival rates were 69 %, 38 % and 23 %, respectively. The median recurrence-free survival time was 30.7 months (range: 4.5 - 106.5 months). Preoperative patient characterization, tumor factors, and operative factors associated with HCC recurrences are shown in Table 1. The early and late recurrences developed in 30 and 42 patients, respectively.

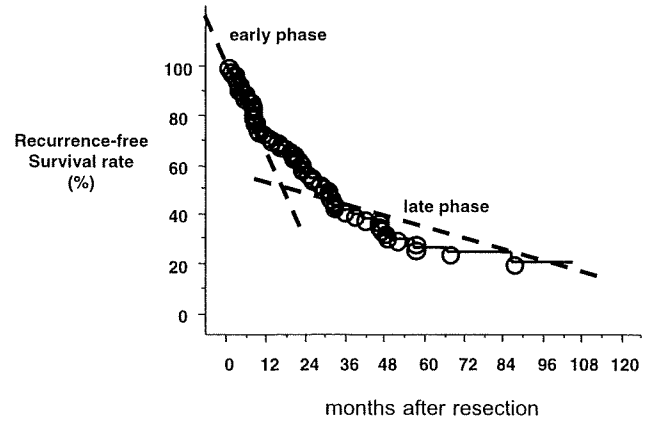

Figure 3. The cumulative recurrence-free survival curve after curative resection of HCC. There were two different components as follows; 1) rapid decrease within 12 months and 2) slow decrease more than 12 months.

Risk factors for early intrahepatic recurrence (Tables 1-3)

Thirty of 72 patients with recurrent HCCs (41.7 %) developed early intrahepatic recurrence within 12 months after a curative resection. The median recurrence-free survival time in the early phase was 5.9 months. Higher Grading (G2+G3; p=0.048), Staging

(F3+F4; p=0.027), and serum AFP levels (>100ng/ml; p=0.045) were significant risk factors based on a univariate analysis, whereas no operative factors were found to be significant. Eleven of 30 patients suffered from the early recurrence had HPF in the non-cancerous liver tissues. The presence of HPF was not significantly related to the early recurrence. A multivariate analysis demonstrated only the serum AFP levels to be independently significant (RR: 2.78).

Table 1. Differences between early, late, or no recurrence

Charasteristics	Categorization	Early recurrence (n=30)	Late recurrence (n=42)	No recurrence (n=25)
(Host factors)				
Gender	M	26	34	21
	F	4	8	4
Age (yrs)	≦62	19	19	12
	>62	11	23	13
Virus	B	8	9	7
	B+C	1	2	1
	C	15	22	11
	None	6	9	6
ICGR15 (%)	≦10.0	10	11	10
	>10.0	20	30	15
PT (%)	≦91.0	18	21	11
	>91.0	12	20	13
Alb (g/dl)	≦3.5	23	34	22
	>3.5	7	7	3
T.Bil (mg/dl)	≦1.0	24	35	21
	>1.0	6	7	4
ALT (IU/L)	≦80.0	23	34	22
	>80.0	7	8	3
Plt ($x10^4$/ml)	≦10.0	9	10	6
	>10.0	21	32	19
staging	F0+1+2	10	23	17
	F3+4	20	19	8
grading	G0+1	10	20	18
	G2+3	20	22	7
HPF	(-)	19	21	20
	(+)	11	21	5
(Tumor factors)				
Tumor differentiation	well	4	3	3
	moderate	20	32	20
	poorly	2	4	1
	necrosis	4	3	1
Tumor diameter (cm)	≦3.0	11	18	9
	>3.0	19	24	16
venous invasion or satellite lesion	(-)	13	23	19
	(+)	17	19	6
AFP (ng/dl)	≦100	18	33	21
	>100	12	9	4
(Operative factors)				
Type of resection	subsegmentectomy or less	16	13	11
	segmentectomy	4	14	9
	lobectomy or more	10	15	5
surgical margin (cm)	≦1.0	14	25	6
	>1.0	16	17	19
intraoperative blood loss (g)	≦1300	12	20	18
	>1300	18	22	7

Table 2. Univariate analysis for early recurrence

Characteristics	Categorization	Chi-square	p-value
(Host factors)			
Gender	M / F	0.315	0.7691
Age (yrs)	≦62 / >62	2.416	0.1311
Virus	HCV / Others	0.005	0.9999
ICGR15 (%)	≦10.0 / >10.0	0.022	0.9999
PT (%)	≦91.0 />91.0	0.955	0.3809
Alb (g/dl)	≦3.5 / >3.5	0.429	0.5569
T.Bil (mg/dl)	≦1.0 / >1.0	0.184	0.7738
ALT (IU/L)	≦80.0/ >80.0	0.656	0.4130
Plt ($x10^4$)	≦10.0 / >10.0	0.406	0.6169
Staging*	**F0+1+2 / F3+4**	**5.768**	**0.0271**
Grading*	**G0+1 / G2+3**	**4.532**	**0.0476**
HPF	Negative / Positive	0.040	0.9999
(Tumor factors)			
Tumor differentiation	well+moderate / poorly	0.0020	0.9999
Tumor diameter (cm)	≦3.0 / >3.0	0.115	0.8237
venous invasion	Negative / Positive	3.161	0.0822
AFP* (ng/ml)	**≦100 / >100**	**4.595**	**0.0445**
(Operative factors)			
Type of resection	less than segmentectomy / lobectomy or more	0.118	0.8133
surgical margin (cm)	≦1.0 / >1.0	0.001	0.9999
intraoperative blood loss (g)	≦1300 / >1300	2.318	0.1869

* significant factor

Table 3. Multivariate analysis for early recurrence

variables	Risk ratio	95% confidence interval	p-value
AFP* (ng/ml)	**2.779**	**1.016-7.600**	**0.0464**
Grading	0.472	0.166-1.338	0.1579
Staging	0.51	0.183-1.426	0.1995

* significant factor

Risk factors for late intrahepatic recurrence

Forty-two of 72 patients with recurrent HCCs (58.3 %) developed late intrahepatic recurrence and the median recurrence-free survival time was 46.1 months. Regarding the cumulative recurrence-free survival, patients with higher grading (G2+G3) tended to have a shorter recurrence-free survival time, although the difference was not statistically significant (Figure 4). On the other hand, Staging in non-cancerous liver tissues did not affect the late recurrence (Figure 4). Twenty-one of 42 patients suffered from the late recurrence had HPF in the non-cancerous liver tissues. The presence of HPF was a significant risk factor for the late recurrence (Table 4, Figure 4), while neither operative factors nor tumor factors were not significant risk factors for the late recurrence (Table 4). In addition, HPF-positive livers demonstrated not only higher Grading and Staging but also higher score of PCNA L.I. than those in HPF-negative livers (Figure 5).

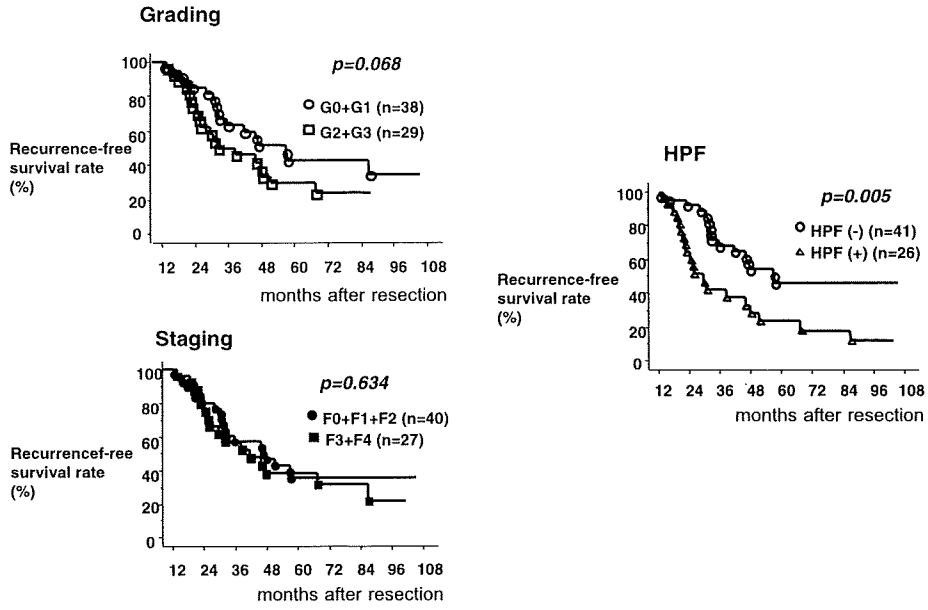

Figure 4. Relationship of recurrence-free survival time with Grading, Staging, and HPF.
The presence of HPF was significantly related to shorter recurrence-free survival time. Higher Grading (G2+G3) tended to be associated with shorter recurrence-free survival time, whereas Staging was not associated.

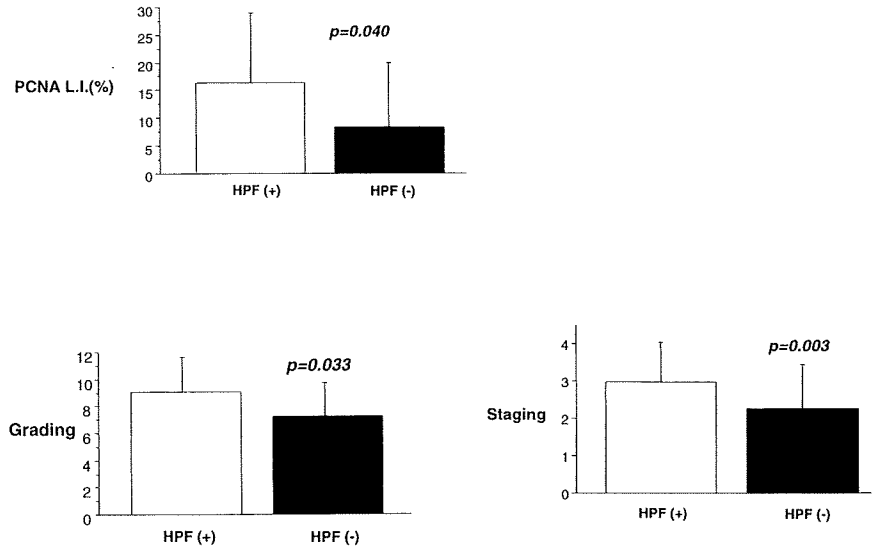

Figure 5. Relationship between HPF and Grading, Staging, PCNA L.I.
HPF is significantly associated with higher Grading, Staging and PCNA L.I.

Table 4. Analysis for late recurrence by Cox's proportional hazard model

Characteristics	categorization	Hazrd ratio	95% confidence interval	p-value
(Host factors)				
Gender	M / F	1.000	0.438-2.285	0.9970
Age (yrs)	≦62 / >62	1.090	0.566-2.100	0.7973
Virus	C / others	1.656	0.699-3.923	0.2520
Staging	F0+1+2 / F3+4	0.852	0.441-1.467	0.6346
Grading	G0+1 / G2+3	0.611	0.316-1.180	0.1425
HPF*	(-) / (+)	**2.611**	**1.346-5.067**	**0.0045**
ICGR15 (%)	≦10.0 / >10.0	1.507	0.721-3.147	0.2753
PT (%)	≦91.0 />91.0	0.633	0.320-1.251	0.1885
Alb (g/dl)	≦3.5/ >3.5	0.629	0.240-1.647	0.3449
T.Bil (mg/dl)	≦1.0 / >1.0	1.288	0.534-3.109	0.5733
ALT (IU/l)	≦80.0 / >80.0	1.365	0.595-3.130	0.4620
Plt ($x10^4/\mu l$)	≦10.0 / >10.0	0.694	0.322-1.494	0.3501
(Tumor factors)				
Tumor differentiation	well+moderately / poorly	0.4430	0.097-2.021	0.2929
Tumor diameter (cm)	≦3.0 / >3.0	1.162	0.603-2.238	0.6531
fc	(-) / (+)	0.683	0.282-1.655	0.3981
fc-inf	(-) / (+)	1.019	0.466-2.226	0.9629
venous invasion	(-) / (+)	1.650	0.852-3.195	0.1373
AFP (ng/ml)	≦100 / >100	0.955	0.417-2.189	0.9141
(Operative factors)				
Type of operation[a)]	A+B/C	1.657	0.737-3.727	0.2219
surgical margin (cm)	(-) / (+)	1.845	0.949-3.587	0.0708
Intraoperative blood loss (g)	≦1300 / >1300	1.831	0.944-3.552	0.0735

a) A: subsegmentectomy or less; B: segmentectomy; C: lobectomy or more. *significant factor

Discussion

In the present study, the recurrence-free survival curve after curative resection for HCC had different components including the early recurrence within 12 months after resection and late recurrence after more than 12 months. This finding was consistent with the report by Poon et al.[4] In the early recurrence group, a multivariate analysis revealed that the significant risk factor was higher serum AFP level (>100ng/ml). This finding suggests that tumor factors such as malignant potential of HCC cells are one of the important risk factors for the early recurrence.[18-20] In addition, univariate analyses in the risk factors of early recurrence also showed higher Grading and Staging of non-cancerous liver tissues to be significant risk factors, suggesting that higher hepatitic activity also enhanced early intrahepatic recurrence.[6] This finding also suggests that one of the mechanisms for early recurrence is due to metachronous occurrence of new tumors (multicentric origin, referred to as MO). However, the rate of intrahepatic recurrence due to MO is not so high (14 - 25%). [17,21] Therefore, high incidence of early recurrence in the active hepatitis group (G2+G3, F3+F4) compared to the other group (G0+G1, F1+F2) could not be explained by the MO-mechanism alone. It was previously reported that the expression of vascular adhesion molecules including endothelial leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 and CD-44 in the hepatocytes and/or hepatic sinusoidal lining cells