

Patients and methods

Patients

Between October 1992 and May 2006, 53 patients underwent SHR for recurrence of HCV-related HCC. These patients had undergone first hepatic resection between April 1990 and March 2005. During that period, primary hepatic resection was performed for HCV-related HCC in 399 patients. Although the indications for SHR were almost the same as those for the first resection, we performed SHR only when the following two conditions were met [17]. First, the patient must have good liver function, comparable to that at the time of the initial resection. Second, the tumor must be localized so that it can be extirpated completely. For the exceptional patient with multiple recurrent tumors in the remnant liver who have not responded to other available treatments but whose liver function is adequate to tolerate SHR, we perform SHR as part of a multimodality treatment plan. In 2 of the 53 patients with multiple recurrent tumors, SHR was performed only for recurrent tumors for which transcatheter arterial embolization was not effective, as part of a multimodality treatment; the recurrent tumors could not be extirpated completely by SHR in the two patients. SHR was performed according to the standard indications in the remaining 51 patients who the subjects of this study (Table 1). The tumor-free survival rates were compared as a function of clinicopathologic variables. The median follow-up from operation until the detection of HCC recurrence or the study endpoint (31 July 2006) was 415 days (range = 62–4480 days). The median follow-up from operation until death or the study endpoint was 1264 days (range = 62–4480).

This study was conducted in accordance with the Helsinki Declaration and the guidelines of the Ethics Committee of our institution. Informed consent was obtained from each patient.

Interferon therapy

In 5 of 51 patients, primary HCC was detected after IFN therapy. In 11 patients, IFN therapy was performed after the first operation. IFN α was administered in 12 patients. IFN β , IFN α 2a, IFN α and IFN β , and IFN α and ribavirin were administered in one patient each.

The response to IFN therapy was classified on the basis of changes in the HCV RNA levels and serum alanine aminotransferase (ALT) activity during and immediately after IFN administration and for at least 1 year after IFN therapy. Six patients attained a sustained viral response (SVR) that was defined as return of ALT activity to within

Table 1 Subjects' demographics

Age (years)	66.2 \pm 6.3 (56–83)
Gender (male:female)	47:4
Alcohol abuse	12
Interferon therapy	16
Child-Pugh classification (A:B)	49:2
Total bilirubin (mg/dl)	0.9 \pm 0.3 (0.3–1.5)
Albumin (g/dl)	3.8 \pm 0.4 (2.8–4.6)
ICGR15 (%) ^a	16.7 \pm 6.9 (3.8–34.8)
Platelet count ($\times 10^4/\text{mm}^3$)	13.8 \pm 6.4 (5.8–43.4)
Aspartate aminotransferase (IU/L)	58 \pm 28 (18–164)
Alaine aminotransferase (IU/L)	65 \pm 36 (13–158)
α -Fetoprotein (>20 ng/ml)	17
Tumor size (cm)	2.6 \pm 1.4 (1.1–9.5)
Differentiation of main tumor (well, moderate: poor)	39:12
Tumor number (single:multiple)	36:15
Portal invasion	15
Grade (0:1:2:3)	2:14:34:1
Stage (0:1:2:3:4)	2:11:8:7:23
Operative methods (anatomic:nonanatomic)	4:47

Age, the results of laboratory tests, and tumor size are shown as mean \pm SD with range

ICGR15 = indocyanine green retention rate at 15 min

^a One patient who was intolerant of ICG was excluded

the reference range and no detectable serum HCV RNA for at least 1 year after IFN therapy. A biochemical response (BR), defined as normalized ALT activity for at least 1 year after IFN therapy with or without transient disappearance of serum HCV RNA, was attained in three patients. The other seven patients were defined as the nonresponse (NR) group because they had no decrease in their ALT activity and had persistent serum HCV RNA. The serum samples were tested for anti-HCV antibody using an enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Tokyo, Japan). Serum HCV RNA was assayed by a reverse transcriptase-nested polymerase chain reaction using primers derived from a conserved 5'-untranslated region of the viral genome [7] and by a branched DNA probe method (Quantiplex HCV-RNA, Chiron Corp., Emeryville, CA).

Detection of recurrence

Serum concentration of α -fetoprotein and protein induced by vitamin K absence and antagonist II were measured 1 month after surgery and every 3 months thereafter. Ultrasonography, computed tomography, chest radiography, or some combination of these tests was done 1 month after surgery and every 3 months thereafter. When a

recurrence of HCC was strongly suspected on the basis of tumor markers or imaging, selective hepatic angiography, ultrasound-guided biopsy, or both was conducted to establish a definitive diagnosis.

Histology

The system of the Liver Cancer Study Group of Japan [21] was used to categorize histologic findings. The histologic grade of differentiation (well, moderate, or poor) of HCC was determined according to a modification of Edmondson and Steiner [22]. The grade score (severity of active hepatitis) and stage score (degree of hepatic fibrosis) in noncancerous hepatic tissues were determined by the score of the histologic activity index [23], which was determined by four events, i.e., periportal necrosis with or without bridging necrosis, intralobular degeneration with focal necrosis, portal inflammation, and fibrosis.

Statistics

The survival rates were calculated by the Kaplan-Meier method and were compared with those from the log-rank test. The tumor-free survival time was measured from the date of resection until the detection of a recurrent tumor or the endpoint of this study (31 July 2006) in patients without recurrence. Cox's proportional hazard model with stepwise variable selection was used for multivariate analysis. A *p* value of less than 0.05 was considered significant. Variables were selected on the basis of previous studies or our own clinical experience. The variables chosen were age (≥ 65 or < 65), gender, history of alcohol abuse (intake of at least 86 g of ethanol daily for at least 10 years) [24], history and response to IFN therapy (SVR, BR vs. NR, and no treatment), total bilirubin (≥ 1 or < 1 mg/dl), albumin concentration (≥ 3.5 or < 3.5 g/dl), indocyanine green retention rate at 15 min (ICGR15, ≥ 20 or $< 20\%$), platelet count ($\geq 10 \times 10^4$ or $< 10 \times 10^4/\mu\text{l}$), aspartate aminotransferase (AST) activity (> 40 or ≤ 40 IU/L), ALT

activity (> 45 or ≤ 45 IU/L), serum α -fetoprotein concentration (AFP, > 20 or ≤ 20 ng/ml), the largest diameter of the main tumor (> 4 or ≤ 4 cm), the degree of differentiation of the main tumor (well-, moderate vs. poorly differentiated HCC), the number of tumors (single or multiple, including intrahepatic metastasis), microscopic portal invasion, the grading score (0, 1, or 2-4), the staging score (0-3 or 4), and operative method.

Results

There was no operative mortality. Intractable pleural effusion that called for use of diuretics and treatment by thoracentesis developed postoperatively in three patients, intra-abdominal infection in two patients, bile leakage in two patients, and gastric ulcer in two patients. The tumor-free and cumulative survival rates after SHR in the 51 patients are shown in Figure 1.

By univariate analysis, NR and lack of interferon therapy ($p = 0.0089$), high ICGR15 ($p = 0.0068$), high AST ($p = 0.0607$), high ALT ($p = 0.0930$), large tumor (> 4 cm, $p < 0.0001$), and multiple tumors ($p = 0.0105$) were risk factors for HCC recurrence after SHR (Table 2). Multivariate analysis showed that NR and lack of IFN therapy, high ICGR15, large tumor, and multiple tumors were independent risk factors for HCC recurrence after SHR (Table 3). The tumor-free survival rate was higher in SVR and BR patients than in other patients, including NR patients and patients who did not undergo IFN therapy ($p = 0.0089$, Fig. 2). The tumor-free survival rate was higher in patients with small tumor (≤ 4 cm) than in patients with large tumor (> 4 cm, $p < 0.0001$, Fig. 3). The clinicopathologic findings in patients who were sustained viral responders or biochemical responders and in other patients are shown in Table 4. The mean age and gender distribution were similar in the different groups. Although serum concentrations of total bilirubin, albumin, and ICGR15 were not significantly different between the groups, platelet count was significantly higher in patients who were sustained viral responders or biochemical

Fig. 1 Tumor-free and cumulative survival rates after second hepatic resection in all patients

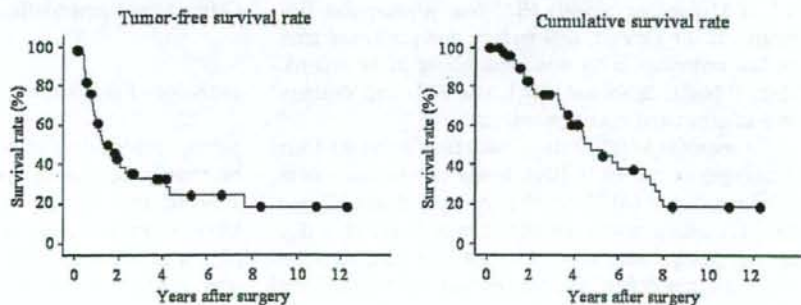


Table 2 Tumor-free survival rates after second hepatic resection for recurrent hepatocellular carcinoma by univariate analysis

Factors (numbers of patients)		Survival rates (%)			
		4 years	8 years	12 years	p value
Age (years)	≥65 (28)	36	27	27	0.914
	<65 (23)	31	22	–	
Gender	male (47)	36	26	26	0.514
	female (4)	–	–	–	
Alcohol abuse	+ (12)	45	33	33	0.678
	– (39)	31	23	23	
Interferon therapy	SVR + BR (9)	74	74	74	0.0089
	NR + no therapy	42	24	15	15
Total bilirubin (mg/dl)	≥1.0 (20)	–	–	–	0.286
	<1.0 (31)	38	28	28	
Albumin (g/dl)	≥3.5 (39)	36	27	27	0.156
	<3.5 (12)	19	–	–	
ICGR15 (%) ^a	≥20 (16)	15	–	–	0.0068
	<20 (34)	42	34	34	
Platelet count ($\times 10^4/\text{mm}^3$)	≥10 (35)	32	27	27	0.156
	<10 (16)	37	18	–	
AST (IU/L)	>40 (37)	23	16	16	0.0607
	≤40 (14)	56	44	44	
ALT (IU/L)	>45 (33)	23	16	–	0.0930
	≤45 (18)	52	41	41	
α -Fetoprotein (ng/ml)	>20 (17)	32	32	–	0.866
	≤20 (34)	34	21	21	
Tumor size (cm)	>4.0 (4)	0	0	0	<0.0001
	≤4.0 (47)	36	27	27	
Differentiation of main tumor	Well, moderate (39)	27	21	21	0.469
	Poor (12)	58	39	–	
Tumor number	Single (36)	41	28	28	0.0105
	Multiple (15)	15	–	–	
Portal invasion	+ (15)	47	36	36	0.690
	– (36)	26	18	18	
Grade	0, 1 (16)	42	42	42	0.264
	2–3 (35)	28	19	–	
Stage	0–3 (28)	36	36	36	0.334
	4 (23)	27	13	–	
Operative methods	anatomic (4)	25	–	–	0.649
	nonanatomic (47)	34	26	26	

ICGR15 = indocyanine green retention rate at 15 min; AST = aspartate aminotransferase; ALT = alanine aminotransferase; SVR = sustained viral response; BR = biochemical remission; NR = no response

^a One patient who was intolerant of ICG was excluded

responders than in other patients. The serum activities of AST and ALT were significantly lower in patients who were sustained viral responders or biochemical responders than in other patients. Thus, active hepatitis was controlled in SVR and BR patients. The proportion of patients with an elevated serum AFP concentration (>20 ng/ml), tumor size, differentiation of the main tumor, prevalences of portal invasion and multiple tumors, grading score, staging

score, and operative methods were similar in the different groups.

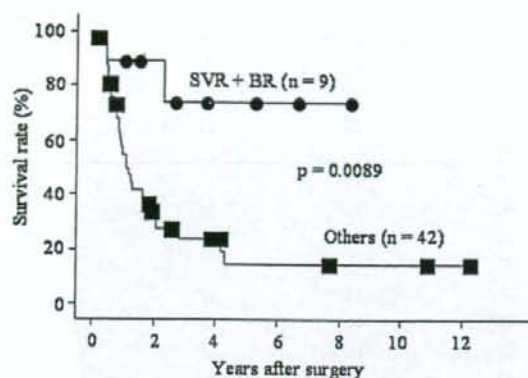
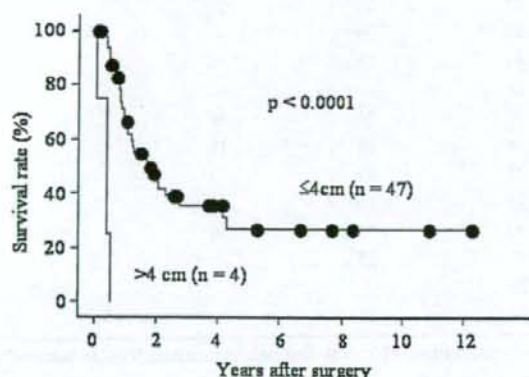
Discussion

Treatment for recurrent HCCs includes SHR, ablation therapy such as microwave coagulation therapy and

Table 3 Risk factors for recurrence after second hepatic resection for recurrent hepatocellular carcinoma by multivariate analysis

Factors	Risk ratio	95% confidence interval	p value
NR + lack of interferon therapy	17.660	2.252–138.496	0.0063
ICGR15 ($\geq 20\%$) ^a	0.396	0.179–0.878	0.0227
Tumor size (>4 cm)	0.140	0.030–0.643	0.0115
Multiple tumors	0.330	0.143–0.766	0.0098

ICGR15 = indocyanine green retention rate at 15 min

^a One patient who was intolerant of ICG was excluded**Fig. 2** Comparison of tumor-free survival rates in patients who were sustained viral responders or biochemical responders with other patients. Circle, 9 patients who were sustained viral responders or biochemical responders; square, other 42 patients**Fig. 3** Comparison of tumor-free survival rates in patients with large tumor and small tumor. Circle, 47 patients with small tumor (≤ 4.0 cm); another line, 4 patients with large tumor (>4.0 cm)

radiofrequency ablation therapy, and transarterial therapy such as transcatheter arterial embolization. Liver transplantation is another option in selected patients with impaired liver function. Although the value of SHR for recurrence has been demonstrated, indications for SHR are unclear. The tumor-free survival rate in patients with

chronic hepatitis is unsatisfactory because multicentric carcinogenesis persists as long as hepatitis remains active. Recurrence is not uncommon following SHR, even when the resection is considered curative.

Although prognostic factors after first resection for HCC have been evaluated by many investigators, there are a few studies about prognostic factors after SHR. In addition, viral status in patients has not been evaluated in the previous study. In this study univariate analysis showed that NR and lack of IFN therapy, high ICGR15, high AST, high ALT, large tumor, and multiple tumors are risk factors for recurrence. In addition, the tumor-free survival rate was higher in SVR and BR patients than in other patients, nonresponders, and patients who did not undergo IFN therapy. Multivariate analysis showed NR and lack of IFN therapy, high ICGR15, large tumor, and multiple tumors are independent risk factors for HCC recurrence after SHR. Previous studies have indicated that active hepatitis with a sustained increase in the serum aminotransferase activity is a risk factor for recurrence [1, 2, 6, 25]. Other recent studies found that HCC is less likely to develop in patients in whom IFN normalized serum ALT activity, even when HCV RNA remained detectable [11, 12]. IFN therapy also suppresses carcinogenesis after surgery [13, 14, 16, 26, 27]. In this study, active hepatitis had been suppressed in SVR and BR patients, and the tumor-free survival rate after SHR was significantly higher in these patients than in others. Thus, the induction of remission of active hepatitis by IFN therapy suppressed carcinogenesis after SHR. The study of IFN therapy after tumor ablation by Shiratori et al. [16] showed that the rate of second or third recurrence was lower in the IFN group than in the control group, although the incidence of first recurrence of a new foci of HCC was similar in the two groups. It is possible that what is called the first recurrent tumor had already developed by the time of the first operation, and the remission of active hepatitis by IFN therapy suppressed subsequent carcinogenesis. Thus, SHR is likely to be associated with a low incidence of recurrence when IFN therapy has controlled chronic hepatitis C. IFN α was the first therapy for chronic hepatitis C. The combination of IFN and ribavirin increases SVR rates compared with IFN alone. The combination of a

Table 4 Clinicopathologic findings in patients who were sustained viral responders or biochemical responders and other patients

	SVR + BR (n = 9)	Others (n = 42)	p value
Age (mean ± SD)	67.2 ± 6.7	65.9 ± 6.3	0.561
Gender (male:female)	8:1	39:3	>0.999
Total bilirubin (mg/dl)	0.8 (0.7, 1.0)	0.8 (0.5, 1.3)	0.822
Albumin (g/dl)	3.7 (3.6, 4.2)	3.8 (3.3, 4.1)	0.388
ICGR15 (%) ^a	16.4 (10.5, 21.5)	17.1 (6.3, 26.7)	0.695
Platelets (× 10 ⁴ /mm ³)	17.7 (15.2, 23.4)	11.1 (6.8, 20.5)	0.0016
AST (IU/L)	34 (25, 53)	56 (29, 104)	0.0091
ALT (IU/L)	25 (18, 64)	67 (23, 119)	0.0165
Tumor size (cm, mean ± SD)	2.4 ± 1.1	2.6 ± 1.5	0.615
Differentiation of main tumor (well:moderate:poor)	0:6:3	4:29:9	0.520
Multiple tumors	4	11	0.421
Portal invasion	3	12	>0.999
Grade (0:1:2:3)	1:3:5:0	1:11:29:1	0.574
Stage (0–I:4)	6:3	22:20	0.488
Operative methods (anatomic:nonanatomic)	0:9	4:38	>0.999

ICGR15 = indocyanine green retention test at 15 min; AST = aspartate aminotransferase; ALT = alanine aminotransferase

^a One patient who was intolerant of ICG was excluded

pegylated IFN and ribavirin significantly increases SVR rates and is now recognized as the standard therapy [28]. Such IFN therapy is recommended in patients with chronic hepatitis C, even after resection of primary HCC.

The independent risk factors for recurrence after SHR include large tumors and multiple tumors. Thus, a close follow-up after the first operation is important for detecting HCC recurrence at an early stage. When a small recurrent tumor is detected, liver resection or ablation therapy is indicated. We reported that anatomic resection is a favorable prognostic factor in patients in whom IFN was effective [29]. Thus, anatomic resection may be more useful than ablation therapy in such patients. On the other hand, SHR or transarterial therapy is usually used in patients with advanced recurrent tumors such as large tumors and multiple tumors because intrahepatic recurrence was strongly suspected to represent metastasis, in which case IFN therapy is unlikely to be effective. Such lesions require additional treatment such as anticancer chemotherapy.

In conclusion, patients in whom active hepatitis has been controlled by IFN therapy are the best candidates for SHR. IFN therapy should be recommended in patients undergoing resection of an HCV-related HCC because SHR prolongs life if there is a recurrence after liver resection.

References

- Kubo S, Nishiguchi S, Shuto T et al. (1999) Effects of continuous hepatitis with persistent hepatitis C viremia on outcome after resection of hepatocellular carcinoma. *Jpn J Cancer Res* 90:162–170
- Kubo S, Hirohashi K, Tanaka H et al. (2000) Risk factors for recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. *World J Surg* 24:1559–1565
- Kumada T, Nakano S, Takeda I et al. (1997) Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. *Hepatology* 25:87–92
- Kubo S, Kinoshita H, Hirohashi K et al. (1999) Patterns of and risk factors for recurrence after liver resection for well-differentiated hepatocellular carcinoma: a special reference to multicentric carcinogenesis after operation. *Hepatogastroenterology* 46:3212–3215
- Sakon M, Umeshita K, Nagano H et al. (2000) Clinical significance of hepatic resection in hepatocellular carcinoma: analysis by disease-free survival curves. *Arch Surg* 135:1456–1459
- Tarao K, Takemiya S, Tamai S et al. (1997) Relationship between the recurrence of hepatocellular carcinoma (HCC) and serum alanine aminotransferase levels in hepatectomized patients with hepatitis C virus-associated cirrhosis and HCC. *Cancer* 79:688–694
- Nishiguchi S, Kuroki T, Nakatani S et al. (1995) Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 346:1051–1055
- Kasahara A, Hayashi N, Mochizuki K et al. (1998) Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. *Hepatology* 27:1394–1402
- Imai Y, Kawata S, Tamura S et al. (1998) Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. *Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med* 129:94–99
- International Interferon-alpha Hepatocellular Carcinoma Study Group (1998) Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. *Lancet* 351:1535–1539
- Yoshida H, Shiratori Y, Moriyama M et al. (1999) Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. *IHIT Study Group. Inhibition of*

- Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 131:174–181
12. Ikeda K, Saitoh S, Arase Y et al. (1999) Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 29:1124–1130
 13. Ikeda K, Arase Y, Saitoh S et al. (2000) Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor—a prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 32:228–232
 14. Kubo S, Nishiguchi S, Hirohashi K et al. (2001) Effects of long-term postoperative interferon- α therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma: a randomized, controlled trial. *Ann Intern Med* 34:963–967
 15. Kubo S, Nishiguchi S, Hirohashi K et al. (2002) Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 89:418–422
 16. Shiratori Y, Shiina S, Teratani T et al. (2003) Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med* 138:299–306
 17. Shuto T, Kinoshita H, Hirohashi K et al. (1996) Indications for, and effectiveness of, a second hepatic resection for recurrent hepatocellular carcinoma. *HepatoGastroenterology* 43:932–937
 18. Shimada M, Takenaka K, Taguchi K et al. (1998) Prognostic factors after repeat hepatectomy for recurrent hepatocellular carcinoma. *Ann Surg* 227:80–85
 19. Nakajima Y, Ko S, Kanamura T et al. (2001) Repeat liver resection for hepatocellular carcinoma. *J Am Coll Surg* 192:339–344
 20. Minagawa M, Makuuchi M, Takayama T et al. (2003) Selection criteria for repeat hepatectomy in patients with recurrent hepatocellular carcinoma. *Ann Surg* 238:703–710
 21. Liver Cancer Study Group of Japan (2003) General rules for the clinical and pathological study of primary liver cancer, 2nd ed. Kanehara & Co, Tokyo
 22. Edmondson HA, Steiner PE (1954) Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer* 7:462–503
 23. Desmet VJ, Gerber M, Hoofnagle JH et al. (1994) Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 19:1513–1520
 24. Liver Cancer Study Group of Japan (1990) Primary liver cancer in Japan: Clinicopathological features and results of surgical treatment. *Ann Surg* 211:277–287
 25. Shirabe K, Takenaka K, Taketomi A et al. (1996) Postoperative hepatitis status as a significant risk factor for recurrence in cirrhotic patients with small hepatocellular carcinoma. *Cancer* 77:1050–1055
 26. Kubo S, Nishiguchi S, Hirohashi K et al. (2001) Influence of previous interferon therapy on recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. *Jpn J Cancer Res* 92:59–66
 27. Uenishi T, Kubo S, Hirohashi K et al. (2002) Relationship between response to previous interferon therapy and postoperative recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatol Res* 24:404–412
 28. Moreno-Otero R (2005) Therapeutic modalities in hepatitis C: challenges and development. *J Virol Hepat* 12:10–19
 29. Kubo S, Tanaka H, Takemura S et al. (2007) Surgical treatment for hepatocellular carcinoma detected after successful interferon therapy. *Surg Today* 37:285–290

Convenience of a Tape-Guiding Technique in Different Types of Hepatectomy

Hiromu Tanaka, Shigekazu Takemura, Kazuki Ohba, Seikan Hai, Tsuyoshi Ichikawa
Shintaro Kodai, Hiroji Shinkawa, Taichi Shuto¹, Kazuhiro Hirohashi¹, Shoji Kubo

Hepato-Biliary-Pancreatic Surgery, ¹General Practice and Medical Education

Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

Corresponding Author: Hiromu Tanaka, MD, Assistant Professor of Hepato-Biliary-Pancreatic Surgery

Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

Tel: +81 6 6645 3841, Fax: +81 6 6646 6507, E-mail: tanakahm@msic.med.osaka-cu.ac.jp

KEY WORDS:

Tape-guiding;
Hanging
maneuver;
Hepatectomy

ABBREVIATIONS:

Tape-Guiding
Technique (TGT);
Hepatocellular
Carcinoma (HCC);
Intrahepatic
Cholangio-
carcinoma (ICC);
Inferior Vena Cava
(IVC); Right
Hepatic Vein
(RHV); Middle
Hepatic Vein
(MHV); Left
Hepatic Vein
(LHV); Short
Hepatic Vein
(SHV)

ABSTRACT

Background/Aims: The liver hanging maneuver is widely used in right lobectomy to resect huge tumors and harvest living donors. The convenience of tape assistance in other types of hepatectomy is not well known.

Methodology: Tape-guiding technique (TGT) was applied in 30 hepatectomies of different type between April 2003 and April 2006. The indications were liver carcinoma in 22 and living-donor in 8. Hepatectomies included right lobectomy, 14; left lobectomy with caudate lobectomy, 8; left lobectomy without caudate lobectomy, 2; lateral segmentectomy, 3; central bisegmentectomy, posterior segmentectomy, and superior dorsal partial resection, 1 each. A tape was placed in front of the inferior vena

cava for right hepatectomy and left hepatectomy with caudate lobectomy. In other hepatectomies, the tape was positioned to be the target of parenchymal dissection.

Results: TGT was successfully performed in all 30 cases. Tape facilitated dissection by helping the surgeon maintain orientation, and traction on the tape flattened the parenchyma, making it easier to identify and manage vessels and ducts. With an assistant holding the tape, the surgeon's left hand was free, and ligation and suturing was easier and more secure.

Conclusions: The TGT is a convenient technique that is applicable to different types of liver resection.

INTRODUCTION

The liver hanging maneuver in right lobectomy was introduced by Belghiti (1) in 2001. Since then, it has become widely accepted as a safe and easy technique for managing huge tumors invading the diaphragm. Kokudo and co-workers (2) modified the maneuver into the sling suspension technique and have used it in donor right lobectomy, posterior segmentectomy with right hepatic vein (RHV), and left lobectomy with caudate lobectomy and middle hepatic vein (MHV). Although Belghiti's "hanging" maneuver means the technique using a tape positioned in front of the IVC, further modifications of the maneuver using a tape in different space, such as donor left lobectomy without caudate lobectomy with or without MHV (3,4) had been reported. We also felt that the tape-guiding technique has advantages and can be applied in various situations of hepatic parenchymal dissection. This report describes the use of the tape-guiding technique (TGT) in different types of liver resection.

METHODOLOGY

Between April 2003 and April 2006: 30 patients underwent hepatectomy using the TGT. The group consisted of 19 men and 11 women, with median age of 64 (20 to 80) years old. The surgical indications

were hepatocellular carcinoma (HCC) in 12, intrahepatic cholangiocarcinoma (ICC) in 9, metastatic tumor in 1, and living-donor for transplantation in 8. Among the 22 patients with liver tumor, the diameter of the main tumor ranged from 2.0 to 18.0 (median: 5.7) cm. In 7 patients, the tumor had invaded to the main portal veins, roots of the hepatic veins, main hepatic duct, or diaphragm. Types of hepatectomy included right lobectomy in 14, left lobectomy with caudate lobectomy in 8, left lobectomy without caudate lobectomy in 2, lateral segmentectomy in 3, central bi-segmentectomy, posterior segmentectomy, and partial resection of the superior dorsal region between the anterior segment and caudate lobe in 1. Hepatic anatomy and type of hepatectomy were classified based on Healey's segments (5).

This study was conducted in accordance with the Helsinki Declaration and the Guidelines of the Ethics Committee of our institution. Written informed consent for participation was obtained from each patient.

Surgical Approach

Tape-positioning: For right lobectomy, left lobectomy with caudate lobectomy, posterior segmentectomy with the right hepatic vein (RHV), and partial resection of superior dorsal region between the

TABLE 1 Operative Blood Loss, Transfusion, and Complications

Type of hepatectomy	No. of patients	blood loss (g)		Blood transfusion	Bile leakage	Ascites
		Range (median)				
Right lobectomy	14	50-5100 (1170)		4	2	0
Left lobectomy	10	700-3500 (1480)		2	1	0
Others	6	240-1500 (1110)		1	1	1
Total	30	50-5100 (1700)		7	4	1

anterior segment and caudate lobe, a tape was placed between the dorsal surface of the liver and the IVC (Figure 1A). In 17 of these 24 cases, mobilization of the resection side of the liver and division of the short hepatic veins (SHV) were performed so that the tape could be positioned under direct vision. In the other 7 cases, the tumor was too large, hard, or had invaded the diaphragm or the root of the hepatic vein, so that mobilization of the liver was difficult. In these cases, the tape was passed prior to hepatic mobilization. In such cases, we began the dissection by ligating and dividing several short hepatic veins from the caudate process and then proceeded to develop the anterior plane of the IVC with long forceps, according to Belghiti's (1) method. When the forceps were not long enough to pass through the space between the dorsal liver surface and the IVC because of a huge tumor, after additional dissection from both the caudal and cranial sides, a probe with a thick tip was passed caudal-to-cranial through the plane anterior to the IVC, the tip was tied with a ligature stitched to a tape, and the probe was withdrawn, pulling the tape into place (Figure 2).

For left lobectomy without caudate lobectomy or lateral segmentectomy, mobilization of the lateral segment, exposure of the anterior surface of the MHV and left hepatic vein (LHV), and division of the cranial side of the ligamentum venosum was performed, and the root of the LHV encircled. The left end of the tape was pulled inferiorly along the ligamentum venosum. When the middle and left hepatic veins joined deep in the hepatic parenchyma or when harvesting a living donor, the LHV was not encircled to avoid injury to the vein, and the cranial end of the tape was placed just to the left of the root of the LHV (Figure 1B).

Parenchymal dissection: Hepatic parenchyma was dissected with a Cavitron Ultrasonic Surgical Aspirator (CUSA Excel TM; Valley lab, Boulder, CO) and bipolar electrocautery (CMC TM III, Valley Forge Scientific Co. Oaks, PA), starting from the anterior surface of the liver and moving toward the tape. During dissection, the hepatoduodenal ligament usually was clamped for 15 minutes with 5-minute intervals of restored flow to minimize blood loss. Vascular obstruction was not performed when harvesting donor livers.

In right lobectomy without MHV and left lobectomy with caudate lobectomy and MHV, parenchymal

dissection was started from the anterior surface along the right side of the MHV, and was directed to the middle of the tape lying on the anterior surface of

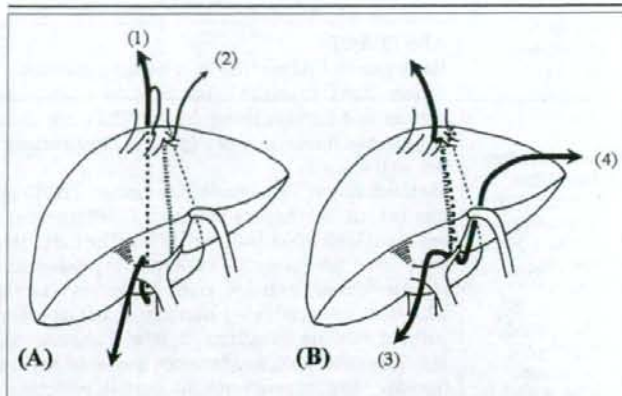


FIGURE 1 (A) Use of the tape-guiding technique in right lobectomy, left lobectomy with caudate lobectomy, posterior segmentectomy with the right hepatic vein, and partial resection of the dorsal liver. The tape is placed between the dorsal surface of the liver and anterior surface of the inferior vena cava (1). In right lobectomy with middle hepatic vein (MHV) and left lobectomy with caudate lobe without MHV, when possible, the root of the MHV is encircled prior to mobilization, and the cranial end of the tape is led to the left side of the MHV (2). (B) The course of the tape in left lobectomy without caudate lobectomy (3) and lateral segmentectomy (4).

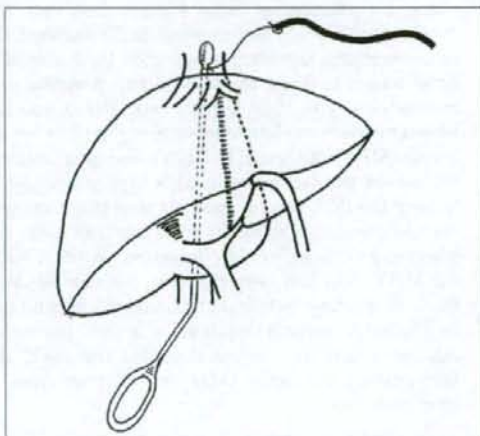


FIGURE 2 The tape-passing procedure between the dorsal surface of the liver and anterior surface of the inferior vena cava (IVC) in the patient with a large, hard tumor. A probe with a thick tip is passed caudal to cranial through the plane anterior to the IVC. The tip is tied with a ligature sutured to the tape, and the probe is withdrawn, leaving the tape in place.

the IVC. During deeper parenchymal dissection, the tape was gently elevated to help to control bleeding. In right lobectomy with MHV and left lobectomy with caudate lobectomy without MHV, the dissection was carried out along the left side of the MHV and directed towards the tape. When possible, the root of the MHV was encircled first, and the cranial end of the tape was pulled to the left side of the MHV (Figure 1A(2)).

In left lobectomy without caudate lobectomy and MHV, dissection began on the anterior surface between the two lobes along the left side of the MHV, and was slightly curved to the left as the plane developed. The tape placed along the ligamentum venosum was lifted gently to serve as a guide. In lateral segmentectomy, after ligation and division of hepatic

pedicles to the left lateral inferior and superior areas, parenchyma was dissected toward the root of the LHV while lifting the tape. Posterior segmentectomy including the RHV also was carried out from the anterior border of the anterior and posterior segments along the left side of the RHV and directed towards the tape placed on the IVC (Figure 3A).

For central bisegmentectomy, dissection between the left lateral and medial segments and division of the MHV and right anterior hepatic pedicle were the initial maneuvers. Then, a tape was placed at the bottom of the dissected plane, and remnant parenchyma between the anterior and posterior segments was dissected using the tape as a guide (Figure 3B).

In one patient with small HCC located between the right anterior superior area and the caudate lobe, the cranial half of the hepatic parenchyma between the right and left lobes was divided along the right side of the MHV using the tape described previously. Then, the vessels from the right hepatic pedicle to the area surrounding the tumor were divided, and the ischemic area was removed with the tumor (Figure 3C).

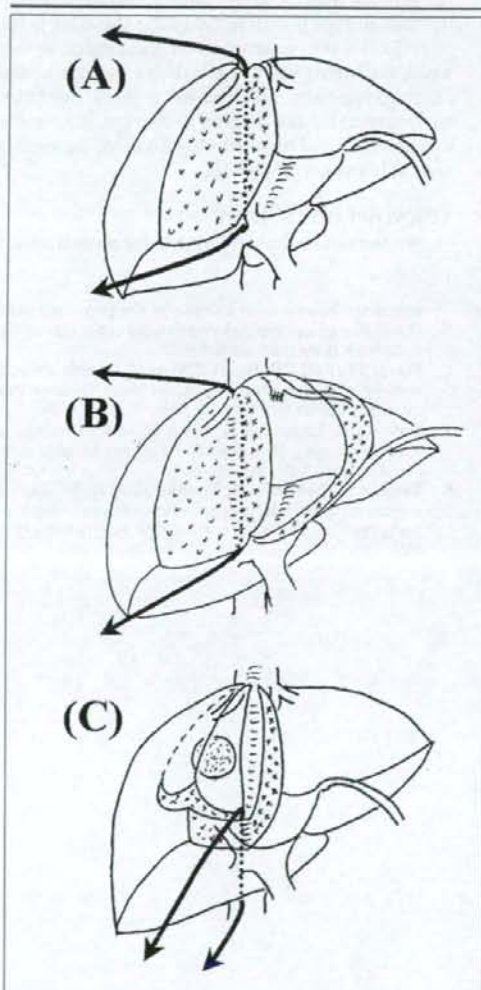


FIGURE 3 Parenchymal dissection in posterior segmentectomy with the right hepatic vein (A), central bisegmentectomy (B), and partial resection of the dorsal liver (C).

RESULTS

The TGT was safely and easily performed in all 30 cases. Blind dissection along the anterior surface of the IVC prior to hepatic mobilization was performed without complication whenever it was attempted (4 of 14 right lobectomies and 3 of 8 left lobectomies).

During the parenchymal dissection, the tape placed behind the liver clearly indicated the direction of the dissection. Traction flattened the section and protected any large vessels near the plane by making them easier to see and control. Free use of the left hand helped to perform maneuvers such as ligation and suturing vessels more securely. Estimated blood loss, transfusion, and complications are shown in Table 1. The patient with the greatest blood loss, 5100g, underwent right lobectomy without mobilization before parenchymal dissection for 13cm ICC. In this patient, clamping of the hepatoduodenal ligament was incomplete, since the patient had dense adhesion around the hepatic hilum due to previous total gastrectomy. When the destination of the dissection slid from the middle of the tape, bleeding from the injured short hepatic veins was troublesome.

Although all patients were discharged from hospital without major complications, 4 patients developed a biliary fistula, but all fistulas healed spontaneously. The sources of the leaks were the roots of the caudate branches of the caudate lobe in 2 patients. In the other 2 patients, the leaks were more peripheral branches. Intractable ascites developed in one patient who underwent partial resection of the dorsal area of the liver. This resolved in 2 months with conservative treatment.

DISCUSSION

This study demonstrated the many advantages of

the TGT in different types of hepatectomy. Compared with the usual left-hand-guiding technique (6), the target of the dissection was much less ambiguous. And since the surgeon's left hand was free, operative maneuvers such as ligation and suturing were performed easily and securely. The advantage is particularly clear when performing left-sided hepatectomy because it is laborious for the surgeon to keep his left hand behind the left lobe when standing on the right side of the patient.

Although bleeding from the deeper parenchyma near the IVC was controlled more easily by gentle lifting of the tape, every effort should be made to orient the dissection directly toward the middle of the tape to avoid injury of the short hepatic veins. Since the tape itself has no effect on hemostasis during superficial and middle-depth parenchymal dissection, use of standard techniques remains essential to minimize the bleeding.

A few comments regarding the blind dissection along the anterior plane of the IVC, the incidence of bleeding from an injured SHV has been reported to be 1.4 to 4% (1,2). Kokudo and co-workers (7) have proposed adjunctive intraoperative ultrasound to make the procedure safer and easier. Nevertheless, it is clear that tape-positioning is easier and safer after completion of routine hemi-liver mobilization and

division of SHVs. Ultimately, the surgeon must decide the best approach in each individual patient. According to our personal experience, division of all SHVs following only left-sided hepatic mobilization is more laborious than that following right-sided mobilization. Hence, we recommended tape passage as the initial maneuver when performing left hepatectomy with caudate lobectomy.

There were no deaths and only 4 biliary fistulas (13%). These results are consistent with our previous report of patients who had at least 2 liver segments resected (11/78, 14%) (8). Most biliary leaks were from caudate branches. These may have been difficult to identify because they were compressed by surrounding parenchyma when lifted by the tape. Therefore, it is essential to look for biliary branches from the caudate lobe by occasionally loosening tension on the tape during the dissection near the hilar plate.

TGT is a convenient tool for the hepatic surgeon, which facilitates exposure, reduces the risk of surgical misadventure, and creates a more comfortable environment for the surgeon to operate. It is useful in a wide variety of procedures, and other applications are likely to be forthcoming.

ACKNOWLEDGEMENT

We thank Dr. Steven Lazrove for critical reading.

REFERENCES

- 1 Belghiti J, Guevara O A, Noun R, et al: Liver hanging maneuver: a safe approach to right hepatectomy without liver mobilization. *J Am Coll Surg* 2001; 193:109-111.
- 2 Kokudo N, Sugawara Y, Imamura H, et al: Sling suspension of the liver in donor operation: a gradual tape-repositioning technique. *Transplantation* 2003; 76:803-807.
- 3 Suh KS, Lee HJ, Kim SH, et al: Hanging maneuver in left hepatectomy. *Hepatogastroenterology* 2004; 51:1464-1466.
- 4 Broering DC, Rogiers X, Malago M, Bassas A, Broelsch CE: Vessel loop-guided technique for parenchymal transection in living donor or in situ split-liver procurement. *Liver Transpl Surg* 1998; 14:241.
- 5 Healey JE, Jr., Schroy PC: Anatomy of the biliary ducts within the human liver; analysis of the prevailing pattern of branchings and the major variations of the biliary ducts. *AMA Arch Surg* 1953; 66:599-616.
- 6 Starzl TE, Bell RH, Beart RW, et al: Hepatic trisegmentectomy and other liver resections. *Surg Gynecol Obstet* 1975; 141:429-437.
- 7 Kokudo N, Imamura H, Sano K, et al: Ultrasonically assisted retrohepatic dissection for a liver hanging maneuver. *Ann Surg* 2005; 242:651-654.
- 8 Tanaka S, Hirohashi K, Tanaka H, et al: Incidence and management of bile leakage after hepatic resection for malignant hepatic tumors. *J Am Coll Surg* 2002; 195:484-489.

Response to Interferon Therapy Affects Risk Factors for Postoperative Recurrence of Hepatitis C Virus-Related Hepatocellular Carcinoma

TAKAHIRO UENISHI, MD,^{1*} SHUHEI NISHIGUCHI, MD,² SHOHO TANAKA, MD,¹
TAKATSUGU YAMAMOTO, MD,¹ SHIGEKAZU TAKEMURA, MD,¹ AND SHOJI KUBO, MD¹

¹Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, Osaka, Japan

²Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

Background: Interferon therapy might reduce recurrence after resection of hepatitis C virus-related hepatocellular carcinoma, especially among sustained virologic or biochemical responders.

Methods: Of 209 patients who underwent curative resection for early-stage hepatitis C virus related hepatocellular carcinoma, 70 patients underwent interferon therapy. A sustained virologic or biochemical response was achieved in 40 patients (SVR/BR group). Thirty no responders and 139 patients who had not received interferon therapy were classified as the NR/non-IFN group. Risk factors for postoperative recurrence in each group were analyzed.

Results: The tumor-free survival rates in the SVR/BR group were significantly higher than those in the NR/non-IFN group. By multivariate analysis, the presence of multiple tumors was independently associated with recurrence after resection in both groups, while histologic evidence of cirrhosis was another independent risk factor for postoperative recurrence in the NR/non-IFN group.

Conclusions: Newly multicentric carcinogenesis after resection could be suppressed when active hepatitis is controlled by interferon therapy. Patients with single hepatitis C virus related hepatocellular carcinoma detected after successful interferon therapy are good candidates for surgical resection. Adjuvant interferon therapy might be indicated for patients who undergo curative resection for single hepatocellular carcinoma associated with hepatitis C.

J. Surg. Oncol. 2008;98:358–362. © 2008 Wiley-Liss, Inc.

KEY WORDS: hepatocellular carcinoma; hepatitis C virus; interferon; postoperative outcome

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers in Asia and Africa, and its incidence is increasing in the Western world [1,2]. Since chronic infection with hepatitis C virus (HCV) is closely associated with development of HCC [3], surveillance of patients with HCV infection has made possible the detection of HCC at an early stage [4]. Patients with early-stage HCC should be considered for any of the available treatment options, including local ablation therapy, liver transplantation, and surgical resection. Recently, radiofrequency ablation (RFA) has been accepted as new technique for the treatment of HCC. Although several reports have shown that RFA for small HCC can provide effective local control of tumors and favorable survival rates, RFA cannot achieve complete necrosis of tumors in all cases [5,6]. Although the long-term outcomes after liver transplantation are satisfactory in patients with early-stage HCC [7,8], liver transplantation for HCC is limited because of the shortage of donors and the high associated costs [9]. Hepatic resection therefore has been the first-line therapeutic option for early-stage HCC [10]. However, the outcome after hepatic resection for HCC is still unsatisfactory because of the high incidence of tumor recurrence, especially in the liver remnant [11,12].

Persistent active hepatitis and extensive fibrosis play important roles in the development of HCV-related HCC [13–15]. In treating HCC, it is therefore necessary to control recurrences that originate from intrahepatic metastases and also to control newly multicentric carcinogenesis after surgery [16–24]. Previous studies have indicated that HCC is less likely to develop in those in whom interferon (IFN) therapy was effective at normalizing the serum alanine aminotransferase (ALT) activity, even when the HCV RNA did not disappear [25–32]. Similarly, the control of active hepatitis is important in

patients who have had a curative resection for HCV-related HCC, since continuous active hepatitis is strongly associated with recurrence after curative resection [33–36]. We have reported that patients in whom HCC is detected after IFN therapy are good candidates for hepatic resection because of the low incidence of postoperative recurrences [33], especially among virologic or biochemical responders [34,35]. Moreover, postoperative long-term IFN- α therapy appears to decrease the incidence of recurrences after a curative resection of HCV-related HCC [36]. We therefore hypothesized that IFN therapy might prevent recurrence due to new carcinogenesis after hepatic resection in the same way that IFN therapy decreases the incidence of HCV-related HCC.

Intrahepatic metastases and multicentric carcinogenesis after resection of HCC might be associated with different risk factors [16–21]. Tumor factors, such as venous invasion and the presence of multiple tumors, whose major mechanism is intrahepatic metastases, have been found to be risk factors for early recurrence [11,17,18]. On the other hand, several investigators have reported that newly multicentric carcinogenesis was closely linked to the status of the underlying liver [16–21]. In this retrospective study, we investigated whether different risk factors are associated with the postoperative recurrence of HCV-related HCC among each group based upon their response to IFN therapy.

*Correspondence to: Takahiro Uenishi, MD, Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan. Fax: +81-6-6646-6057. E-mail: m6877710@msic.med.osaka-cu.ac.jp

Received 26 April 2008; Accepted 27 May 2008

DOI 10.1002/jso.21111

Published online 21 July 2008 in Wiley InterScience (www.interscience.wiley.com).

PATIENTS AND METHODS

Patients

Between January 1993 and March 2007, 279 patients with anti-HCV antibody, but not hepatitis B surface antigen, underwent a curative hepatic resection for HCC in the Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Hospital. Curative surgery was defined as the complete removal of all macroscopic tumors. The absence of tumor cells along the parenchymal transection line was confirmed histologically. Four perioperative deaths occurred from postoperative liver failure. Of the 279 patients, exclusive of operative deaths, 209 patients had early-stage HCC who met the Milan criteria [7] (single tumor ≤ 5 cm, maximum of three total tumors with none > 3 cm, no major vessel invasion, and no extrahepatic involvement) were included in this study. Forty-three patients underwent a hepatic resection for HCC that had been detected after IFN therapy, and 27 patients received IFN therapy after curative resection. The type, dosage, and duration of IFN varied. The response to IFN therapy was determined virologically and biochemically. Of the 70 patients who received IFN therapy, 32 patients obtained a sustained viral response (SVR) that was defined as return of ALT activity to within the reference range and no detectable serum HCV RNA for at least 6 months after IFN therapy. A biochemical response (BR), which was defined as normalized ALT activity for at least 6 months after IFN therapy with or without the transient disappearance of serum HCV RNA, was obtained in eight patients. These 40 patients were defined as the responsive (SVR/BR) group, while the other 30 patients had no decrease in their ALT activity and had persistent serum HCV RNA. The NR/non-IFN group included these 30 nonresponders and 142 patients who had not received IFN therapy.

All patients were followed from resection until death or the end point of this study (December 31, 2007). The follow-up period ranged from 65 to 4,894 days (median, 1,491 days).

Detection of Recurrence

Serum alpha-fetoprotein was measured 1 month after surgery and every 3 months thereafter. Hepatic ultrasound scanning or CT was

performed 1 month after surgery and every 3 months thereafter. When a recurrence of HCC was strongly suspected on the basis of tumor markers or imaging, selective hepatic angiography, ultrasound-guided biopsy, or both were performed to establish a definitive diagnosis.

Surgical Procedure and Pathology

The type of operative procedure was described using the Brisbane 2000 system [37]. A hemihepatectomy, bi-segmentectomy, and segmentectomy were all assumed to be anatomic resections. The classification system of the Liver Cancer Study Group of Japan [38] was used to categorize the histological findings. The grade (grade of active hepatitis) and stage (degree of hepatic fibrosis) in the non-cancerous portions were determined by the score of the histologic activity index [39,40], which was determined by four events, that is, periportal necrosis with or without bridging necrosis, intralobular degeneration with focal necrosis, portal inflammation, and fibrosis. Two pathologists without any knowledge of the clinical and laboratory data examined all materials.

Statistics

Differences in clinicopathologic findings were analyzed by the Mann-Whitney *U*-test or Chi-squared test. The tumor-free survival rates were calculated by the Kaplan-Meier method, and were compared with the log-rank test. Covariates with *P* values < 0.1 in the log-rank test were entered into a Cox regression model with forward stepwise selection. The variables chosen were age (≥ 65 or < 65 years), gender, aspartate aminotransferase (AST) activity (≤ 40 or > 40 IU/L), ALT activity (≤ 45 or > 45 IU/L), total bilirubin (≤ 1.0 or > 1.0 mg/dl), albumin concentration (< 3.5 or ≥ 3.5 g/dl), platelet count (≥ 10 or $< 10 \times 10^9/\mu\text{l}$), serum alpha-fetoprotein concentration (≤ 20 or > 20 ng/ml), the largest diameter of the main tumor (< 3.0 or ≥ 3.0 cm), the degree of differentiation of the main tumor (well, moderate or poor), the number of tumors (single or multiple), the presence of portal invasion, the grading score (0-1 or 2-4), the staging score (0-3 or 4), and type of hepatic resection (anatomic resection or nonanatomic resection).

TABLE I. Clinicopathologic Findings in Patients With Hepatocellular Carcinoma

	SVR/BR (n = 40)	NR/non-IFN (n = 169)	<i>P</i> -value
Age (year)	65 (54, 71)	67 (56, 75)	0.010
Gender (M/F)	35:5	140:29	0.473
AST activity (IU/L)	43 (27, 91)	66 (36, 102)	< 0.001
ALT activity (IU/L)	43 (26, 139)	74 (32, 117)	0.022
Albumin (g/dl)	4.1 (3.6, 4.4)	3.6 (3.3, 4.1)	< 0.001
Total bilirubin (mg/dl)	0.8 (0.5, 1.3)	0.8 (0.5, 1.3)	0.436
Platelet count ($10^3/\text{mm}^3$)	14 (9, 21)	13 (8, 21)	0.100
Child-Pugh score (A/B)	39:1	156:13	0.238
High AFP (> 20 ng/ml)	15	88	0.096
Anatomic resection	13	57	0.882
Tumor size (cm)	2.0 (1.5, 3.8)	2.5 (1.5, 4.0)	0.082
Number of tumors			
Single/multiple	27:13	132:37	0.157
Portal invasion	8	33	0.946
TMN stage			
I/II/III	7:21:12	27:101:41	0.684
Tumor differentiation			
Well/moderate or poor	4:36	18:151	0.904
Histologic activity index score			
Grade 0-1/2-4	9:31	34:135	0.738
Stage 0-3/4 (cirrhosis)	28:12	82:87	0.014

Data are presented as the median with the 10th and 90th percentiles indicated in parentheses. AST, aspartate aminotransferase activity; ALT, alanine aminotransferase; AFP, alpha fetoprotein.

RESULTS

The clinical features, laboratory test results, and pathologic findings of the surgical specimens are summarized in Table I. The mean age in the SVR/BR group was significantly younger than those in the NR/non-IFN group. Although the serum concentration of albumin just before surgery was significantly higher in the SVR/BR group than in the NR/non-IFN group, there were no differences in the serum concentrations of total bilirubin, the platelet counts, and the proportion of Child-Pugh A cirrhosis between two groups. The serum activities of aspartate aminotransferase and ALT just before surgery were significantly lower in the SVR/BR group than in the NR/non-IFN group. Although there were no differences in tumor-related factors, the proportion of stage 4 fibrosis (cirrhosis) in the SVR/BR group was lower than that in the NR/non-IFN group.

The tumor-free survival rates for the patients in the SVR/BR group and the NR/non-IFN group were 71% and 46% at 3 years; and 54% and 23% at 5 years, respectively (Fig. 1). The recurrence rates in the NR/non-IFN group steadily increased over time. Conversely, only one patient in the SVR/BR group had a recurrence of HCC more than 4 years after resection. The tumor-free survival rate was higher in the SVR/BR group than in the NR/non-IFN group ($P < 0.001$).

Table II shows the univariate analysis of risk factors for postoperative recurrence in each group according to the response to IFN therapy. In the SVR/BR group, the low platelet count, the presence of multiple tumors and a nonanatomic resection were possible risk factors for recurrence after resection. In the NR/non-IFN group, low albumin concentration, the presence of multiple tumors, and histologic evidence of cirrhosis (stage 4 fibrosis) were associated with lower tumor-free survival rates. By multivariate analysis, the presence of multiple tumors was an independent risk factor associated with postoperative recurrence in both groups (Table III). In addition, histologic evidence of cirrhosis was another independent predictor of postoperative recurrence in the NR/non-IFN group.

DISCUSSION

Because of recent progress in surgical techniques and perioperative management, the perioperative outcome of hepatic resection for

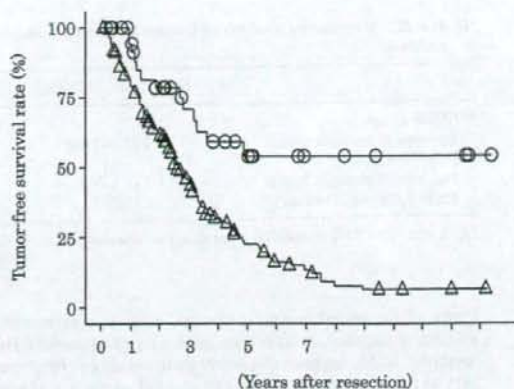


Fig. 1. Tumor-free survival rates after curative resection of hepatocellular carcinoma. Open circles: SVR/BR group (n=40); open triangles: NR/non-IFN group (n=169).

HCC has markedly improved [10]. However, the high incidence of recurrence after resection remains a serious problem in patients with HCV-related HCC [11,12]. Postoperative recurrence of HCV-related HCC is thought to result from the growth of intrahepatic metastases in the residual liver and metachronous, multicentric carcinogenesis due to the underlying liver disease [16-24]. Accordingly, different strategies may be needed for the prevention and management of these two patterns of HCC recurrence.

Cirrhosis is a major risk factor for the development of HCV-related HCC [25,26]. In patients with chronic active hepatitis, fibrosis of the liver is established through repetitive necroinflammation and regeneration. It has been reported that the risk for the development of HCC increases with the degree of liver fibrosis, from 0.5% among patients with stage 0 or 1 fibrosis to 7.9% among patients with stage 4 fibrosis (cirrhosis) [25]. Several investigators have reported that histologic

TABLE II. Univariate Analysis for Factors Associated With Postoperative Recurrence

Factors	Number of patients	Tumor-free survival rate (95% CI)			P-value
		3 years (%)	5 years (%)		
SVR/BR group					
Type of resection					
Anatomic	13	90 (71-100)	90 (71-100)		0.032
Limited	27	62 (42-82)	39 (16-61)		
Number of tumors					
Single	27	81 (64-98)	68 (45-90)		0.001
Multiple	13	49 (16-81)	24 (0-53)		
Platelet count ($10^3/\text{mm}^3$)					
≥ 10.0	35	81 (66-96)	61 (40-81)		0.010
< 10.0	5	20 (0-55)	20 (0-55)		
NR/non-IFN group					
Albumin (g/dl)					
≥ 3.5	113	52 (42-62)	26 (16-35)		0.021
< 3.5	56	33 (20-47)	17 (5-30)		
Number of tumors					
Single	132	49 (40-58)	26 (17-35)		0.007
Multiple	37	36 (20-52)	11 (0-25)		
Stage of fibrosis					
0-3	82	57 (45-68)	34 (22-46)		0.003
4 (cirrhosis)	87	35 (24-46)	13 (4-21)		

TABLE III. Multivariate Analysis of Factors Predicting Postoperative Recurrence

	Multivariate risk ratio*	P-value
SVR/BR group		
Presence of multiple tumors	3.9 (1.3-11.8)	0.018
NR/non-IFN group		
Presence of multiple tumors	1.7 (1.1-2.6)	0.014
Stage 4 fibrosis (Cirrhosis)	1.7 (1.2-2.4)	0.005

*Risk ratio with 95% confidence interval in parentheses.

status of the underlying liver disease, such as evidence of fibrosis and active hepatitis, is also associated with recurrence of HCC after resection, which suggests that newly multicentric carcinogenesis could play an important role in the development of recurrence after resection [16-23]. The incidence of newly multicentric carcinogenesis is considerably high, which was associated with approximately 50% of postoperative recurrences [24]. Several investigators have identified that IFN therapy suppresses the development of HCC by causing active hepatitis to go into remission and by improving hepatic fibrosis, which increases the survival rate in patients with HCV infection [25-32]. Moreover, SVR/BR to IFN therapy was identified as an independent factor for a lower incidence of postoperative recurrence [34,35]. In this study, the tumor-free survival rate was higher in the SVR/BR group than in the NR/non-IFN group. The majority of HCC recurrence was detected in the SVR/BR group within 4 years after surgery, while recurrence of HCC occurred throughout the follow-up period in the NR/non-IFN group. Histologic evidence of cirrhosis was a risk factor for postoperative recurrence in the NR/non-IFN group but not in the SVR/BR group. These findings support the hypothesis that controlling active hepatitis by IFN therapy can improve hepatic fibrosis and prevent the development of multicentric carcinogenesis after resection.

The growth of micrometastases via vascular invasion is considered another major form of HCC recurrence [24]. Because of the high likelihood of micrometastases of HCC spreading through the portal venous system, anatomic resection is theoretically effective for the eradication of micrometastases [41,42]. In this study, the tumor-free survival rate for patients who underwent an anatomic resection was much higher than that for patients who underwent nonanatomic resection in SVR/BR group. Although some studies have suggested that anatomic resection has a beneficial effect on recurrence-free survival after hepatic resection for single HCC [43,44], the superiority of an anatomic resection has been unclear. Previous studies identified that tumor-related factors such as tumor size, the presence of multiple tumors and portal invasion are significant factors associated with postoperative recurrence of HCC [11,16-18]. In this study, the presence of multiple tumors was an independent risk factor for postoperative recurrence, irrespective of the response to IFN therapy. Therefore, in patients with multiple tumors liver transplantation could be considered because the efficacy of adjuvant therapeutic approaches, such as postoperative hepatic arterial chemotherapy remains controversial. Conversely, curative resection provides the chance of cure for patients with single HCV-related HCC detected after successful IFN therapy. Patients who undergo curative resection for single tumor are good candidates for adjuvant IFN therapy.

CONCLUSIONS

Late recurrence of HCC after resection decreased and the histologic status of the underlying liver disease was not associated with postoperative recurrence in the SVR/BR group. These results suggest that newly multicentric carcinogenesis after resection could be suppressed when active hepatitis is controlled by IFN therapy.

However, there was a high incidence of postoperative recurrence in patients with multiple tumors irrespective of their response to IFN therapy. Patients with single HCV-related HCC detected after successful IFN therapy are good candidates for surgical resection. Adjuvant IFN therapy might be indicated for patients who undergo curative resection for single HCV-related HCC.

REFERENCES

- Parkin DM, Bray F, Ferlay J, et al.: Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001;94:153-156.
- El-Serag HB, Mason AC: Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999;340:745-750.
- Sherlock S: Viruses and hepatocellular carcinoma. *Gut* 1994;35:828-832.
- Trevisani F, De NS, Rapaccini G, et al.: Semiannual and annual surveillance of cirrhotic patients for hepatocellular carcinoma: Effects on cancer stage and patient survival (Italian experience). *Am J Gastroenterol* 2002;97:734-744.
- Lencioni R, Cioni D, Crocetti L, et al.: Early-stage hepatocellular carcinoma in patients with cirrhosis: Long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005;234:961-967.
- Komorzono Y, Oketani M, Sako K, et al.: Risk factors for local recurrence of small hepatocellular carcinoma tumors after a single session, single application of percutaneous radiofrequency ablation. *Cancer* 2003;97:1253-1262.
- Mazzaferro V, Regalia E, Doci R, et al.: Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693-699.
- Bruix J, Llovet JM: Prognostic prediction and treatment strategy in hepatocellular carcinoma. *Hepatology* 2002;35:519-524.
- Bismuth H, Chiche L, Adam R, et al.: Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. *Ann Surg* 1993;218:145-151.
- Fan ST, Lo CM, Liu CL, et al.: Hepatectomy for hepatocellular carcinoma: Toward zero hospital deaths. *Ann Surg* 1999;229:322-330.
- Nagasue N, Uchida M, Makino Y, et al.: Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. *Gastroenterology* 1993;105:488-494.
- Nagao T, Inoue S, Yoshimi F, et al.: Postoperative recurrence of hepatocellular carcinoma. *Ann Surg* 1990;211:28-33.
- Sherlock S: Viruses and hepatocellular carcinoma. *Gut* 1994;35:828-832.
- IcEDA K, Saitoh S, Koida I, et al.: A multivariate analysis of risk factors for hepatocellular carcinogenesis: A prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993;18:47-53.
- Takano S, Yokosuka O, Imazeki F, et al.: Incidence of hepatocellular carcinoma in chronic hepatitis B and C: A prospective study of 251 patients. *Hepatology* 1995;21:650-655.
- Kumada T, Nakano S, Takeda I, et al.: Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. *Hepatology* 1997;25:87-92.
- Poon RT, Fan ST, Ng IO, et al.: Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. *Cancer* 2000;89:500-507.
- Kubo S, Hirohashi K, Tanaka H, et al.: Risk factors for recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. *World J Surg* 2000;24:1559-1565.
- Kubo S, Nishiguchi S, Shuto T, et al.: Effects of continuous hepatitis with persistent hepatitis C viremia on outcome after resection of hepatocellular carcinoma. *Jpn J Cancer Res* 1999;90:162-170.
- Shirabe K, Takenaka K, Taketomi A, et al.: Postoperative hepatitis status as a significant risk factor for recurrence in cirrhotic patients with small hepatocellular carcinoma. *Cancer* 1996;77:1050-1055.
- Tarao K, Takemiya S, Tamai S, et al.: Relationship between the recurrence of hepatocellular carcinoma (HCC) and serum alanine

- aminotransferase levels in hepatectomized patients with hepatitis C virus-associated cirrhosis and HCC. *Cancer* 1997;79:688-694.
22. Ko S, Nakajima Y, Kanehiro H, et al.: Significant influence of accompanying chronic hepatitis status on recurrence of hepatocellular carcinoma after hepatectomy. Result of multivariate analysis. *Ann Surg* 1996;224:591-595.
 23. Ko S, Kanehiro H, Hisanaga M, et al.: Liver fibrosis increases the risk of intrahepatic recurrence after hepatectomy for hepatocellular carcinoma. *Br J Surg* 2002;89:57-62.
 24. Sakon M, Umeshita K, Nagano H, et al.: Clinical significance of hepatic resection in hepatocellular carcinoma: Analysis by disease-free survival curves. *Arch Surg* 2000;135:1456-1459.
 25. Imai Y, Kawata S, Tamura S, et al.: Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998;129:94-99.
 26. Yoshida H, Shiratori Y, Moriyama M, et al.: Interferon therapy reduces the risk for hepatocellular carcinoma: National surveillance program of chronic and nonchronic patients with chronic hepatitis C in Japan. *Ann Intern Med* 1999;131:174-181.
 27. Shiratori Y, Imazeki F, Moriyama M, et al.: Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000;132:517-524.
 28. Kasahara A, Hayashi N, Mochizuki K, et al.: Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. *Hepatology* 1998;27:1394-1402.
 29. Ikeda K, Saitoh S, Arase Y, et al.: Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: A long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999;29:1124-1130.
 30. International Interferon-alpha Hepatocellular Carcinoma Study Group: Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: A retrospective cohort study. *Lancet* 1998;351:1535-1539.
 31. Nishiguchi S, Kuroki T, Nakatani S, et al.: Randomised trial of effects interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995;346:1051-1055.
 32. Nishiguchi S, Shiomi S, Nakatani S, et al.: Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. *Lancet* 2001;357:196-197.
 33. Kubo S, Nishiguchi S, Hirohashi K, et al.: Influence of previous interferon therapy on recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. *Jpn J Cancer Res* 2001;92:59-66.
 34. Uenishi T, Kubo S, Hirohashi K, et al.: Relationship between response to previous interferon therapy and postoperative recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatol Res* 2002;24:404-412.
 35. Uenishi T, Nishiguchi S, Tamori A, et al.: Influence of interferon therapy on outcome after surgery for hepatitis C virus-related hepatocellular carcinoma. *Hepatol Res* 2006;36:195-200.
 36. Kubo S, Nishiguchi S, Hirohashi K, et al.: Effects of long-term postoperative interferon- α therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma: A randomized, controlled trial. *Ann Intern Med* 2001;134:963-967.
 37. Terminology Committee of the IHPBA. Terminology of liver anatomy and resections. *HPB* 2000;2:333-339.
 38. Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer (in Japanese). 4th ed. Tokyo: Kanehara; 2000. p. 19.
 39. Knodell RG, Ishak KG, Black WC, et al.: Formation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;1:431-35.
 40. Desmet VJ, Gerber M, Hoofnagle JH, et al.: Classification of chronic hepatitis: Diagnosis, grading and staging. *Hepatology* 1994;19:1513-1520.
 41. Makuuchi M, Hasegawa H, Yamazaki S: Ultrasonically guided subsegmentectomy. *Surg Gynecol Obstet* 1985;161:346-350.
 42. Castaing D, Garden OJ, Bismuth H: Segmental liver resection using ultrasound-guided selective portal venous occlusion. *Ann Surg* 1989;210:20-23.
 43. Regimbeau JM, Kianmanesh R, Farges O, et al.: Extent of liver resection influences the outcome in patients with cirrhosis and small hepatocellular carcinoma. *Surgery* 2002;131:311-317.
 44. Hasegawa K, Kokudo N, Imamura H, et al.: Prognostic impact of anatomic resection for hepatocellular carcinoma. *Ann Surg* 2005;242:252-259.

CASE REPORT

Hiroji Shinkawa · Takuya Nakai · Akihiro Tamori
Hiromu Tanaka · Shigekazu Takemura · Kazuki Ohba
Takahiro Uenishi · Masao Ogawa · Satoshi Yamamoto
Seikan Hai · Tsuyoshi Ichikawa · Shintaro Kodai
Kazuhiro Hirohashi · Kenichi Wakasa · Shoji Kubo

Hepatocellular carcinoma (HCC) recurring 10 years after clearance of hepatitis B surface antigen and 20 years after resection of hepatitis B virus-related HCC

Received: January 7, 2008 / Accepted: March 24, 2008

Abstract A 62-year-old man had been followed up for chronic hepatitis B (HB) since 1973. Hepatocellular carcinoma (HCC) was detected in 1985, at the age of 42 years. Serum HB surface antigen and anti-HB envelope antibody were positive at that time. A right hepatic lobectomy was performed. In 1995, serum HB surface antigen had cleared spontaneously and liver function had normalized. In March 2005, at the age of 62 years, a 1.5-cm diameter hepatic mass was detected in the left lateral segment. At that time, he was seropositive only for anti-HB core antibody. A diagnosis of recurrent HCC was made, and partial hepatectomy was performed. Covalently closed circular HBV DNA was detected in both cancerous and noncancerous tissues by nested polymerase chain reaction (PCR). Cassette-ligation-mediated PCR showed that HBV DNA was integrated into the telomerase reverse transcriptase gene located on chromosome 5p15.

Key words Hepatocellular carcinoma · Hepatitis B virus · Human telomerase reverse transcriptase (hTERT) · Liver resection

Introduction

It is well known that hepatitis B virus (HBV) can cause hepatocellular carcinoma (HCC). Persistent active hepatitis can result in progression to cirrhosis and the development of HCC. During the natural history of chronic hepatitis B (HB), seroconversion from HB surface antigen (HBsAg) to anti-HB surface antibody (anti-HBs) is associated with remission of active hepatitis and improvement of liver function and pathologic features.¹ Although it is thought that clearance of HBsAg from the serum indicates clinical cure and a decreased risk of carcinogenesis, HCC is sometimes detected after this seroconversion.^{2–6} It has also been reported that occult HBV infection is important in the development of HCC.^{4,7–10} In this report, we describe a case of HCC which recurred in 2005, 10 years after the clearance of HBsAg in 1995, and 20 years after resection of the first HCC while the patient was seropositive for HBsAg in 1985.

Case report

A 62-year-old man had been followed up for chronic HB since 1973. In 1985 (at age 42 years), a hepatic tumor was detected in the anterior superior segment (S8) by ultrasonography (US) and computed tomography (CT). Serum HBsAg was positive, serum anti-HBs was negative, HB envelope antigen (HBeAg) was negative, and anti-HB envelope antibody (anti-HBe) was positive (Table 1). Liver function tests indicated active hepatitis. Transcatheter arterial embolization and percutaneous transhepatic portal vein embolization were performed, followed by right lobectomy. He was transfused with 1500 ml whole blood and 880 ml fresh frozen plasma during the operation. The tumor measured 1.8 × 1.5 cm (Fig. 1A), and was classified as T1N0M0 according to the TNM system.¹¹ Pathologic examination revealed a moderately differentiated HCC and no microvascular invasion (Fig. 1B). Examination of the

H. Shinkawa (✉) · H. Tanaka · S. Takemura · K. Ohba · T. Uenishi · M. Ogawa · S. Yamamoto · S. Hai · T. Ichikawa · S. Kodai · S. Kubo
Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
Tel. +81-6-6645-3841; Fax +81-6-6646-6057
e-mail: m1297198@msic.med.osaka-cu.ac.jp

T. Nakai
Department of Surgery, Kinki University, Osakasayama, Japan

A. Tamori
Department of Hepatology, Osaka City University Graduate School of Medicine, Osaka, Japan

K. Hirohashi
Department of General Practice, Osaka City University Hospital, Osaka, Japan

K. Wakasa
Department of Pathology, Osaka City University Hospital, Osaka, Japan

Table 1. Results of laboratory tests at the times of the first and second operations

Test	First operation (Oct. 1985)	Second operation (March 2005)
Albumin (g/dl)	4.0	4.2
Total bilirubin (mg/dl)	0.6	0.6
Aspartate aminotransferase (IU/l)	50	24
Alanine aminotransferase (IU/l)	115	27
ICGR15 (%)	13.5	19.6
Prothrombin test (%)	105	113
HBeAg	-	-
Anti-HBe	+	+
HBsAg	+	-
Anti-HBs	-	-
Anti-HBc	+	+
HBV DNA	ND	+
Anti-HCV (titer)	ND	2.2
HCV RNA	ND	-
α -Fetoprotein (AFP; ng/ml)	57	4
AFP-L3 (%)	ND	0
PIVKA-II (AU/ml)	ND	21

ICGR15, indocyanine green retention rate at 15 min; HBeAg, hepatitis B envelope antigen; anti-HBe, anti-hepatitis B envelope antibody; HBsAg, hepatitis B surface antigen; anti-HBs, anti-hepatitis B surface antibody; anti-HBc, anti-hepatitis B core antibody; HBV DNA, hepatitis B virus DNA; anti-HCV, anti-hepatitis C virus antibody; HCV RNA, hepatitis C virus RNA; PIVKA II, protein induced by vitamin K absence or antagonist II; ND, not determined; INH, inhibition

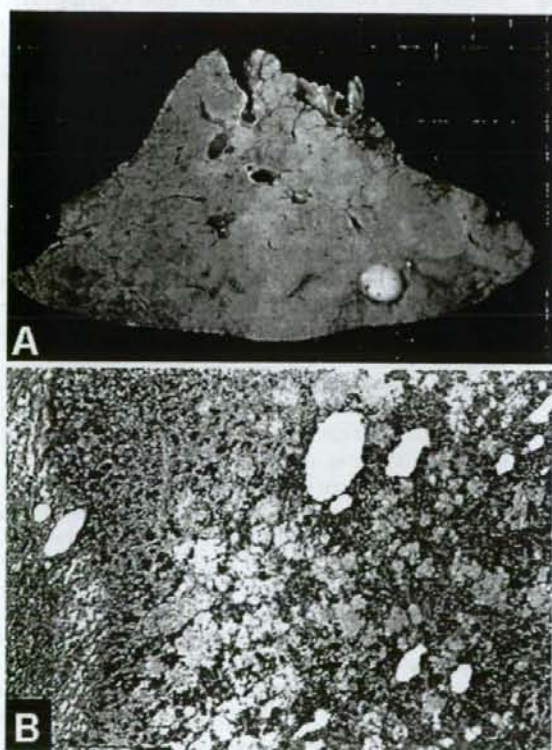


Fig. 1. **A** Resected specimen from the first operation. The tumor measured 1.8 cm \times 1.5 cm and was mostly necrotic. **B** Pathologic examination showing that the tumor is a moderately differentiated hepatocellular carcinoma. H&E, \times 20

nontumorous hepatic tissue showed minimal hepatitis activity (grade 1) and portal-portal septa without architectural distortion (stage 2) according to the histologic activity index score.¹² In 1995 (at age 52 years), serum HBsAg had cleared. In 1996, serum HBV DNA was negative by the polymerase chain reaction (PCR) method. Serum anti-hepatitis C virus antibody (anti-HCV; Ortho Diagnostic Systems, Tokyo, Japan) was positive, but serum hepatitis C virus RNA (Quantiplex HCV-RNA; Chiron, Emeryville, CA, USA) was negative. The anti-HCV titer was consistently low. The serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) had normalized in 1995. In March 2005 (at age 62 years), a hepatic mass measuring 1.5 cm in diameter was detected in the left lateral segment by CT and US. At that time, serum HBsAg, anti-HBs, and HBV DNA were all negative (Table 1). Anti-HB core antibody was positive. The anti-HCV titer was very low. Although the indocyanine green retention rate at 15 min was 19.6%, the results of other liver function tests were within the reference ranges. Renal function tests were abnormal because of renal failure following coronary artery bypass grafting and graft replacement of the ascending aorta at the age of 59 years. The levels of the tumor markers α -fetoprotein (AFP), AFP-L3 fraction, and protein induced by vitamin K absence or antagonist II were all within the reference ranges. In May 2005, partial hepatectomy was performed with a preoperative diagnosis of recurrent HCC. The tumor measured 2.5 \times 2.0 cm (Fig. 2A), and was classified as T2N0M0. Pathologic examination showed a moderately differentiated HCC and no microvascular invasion (Fig. 2B), with nonactive hepatitis (grade 0) and portal fibrous expansion (stage 1) in the noncancerous tissue. The HB surface (HBs) gene and covalently closed circular (ccc)

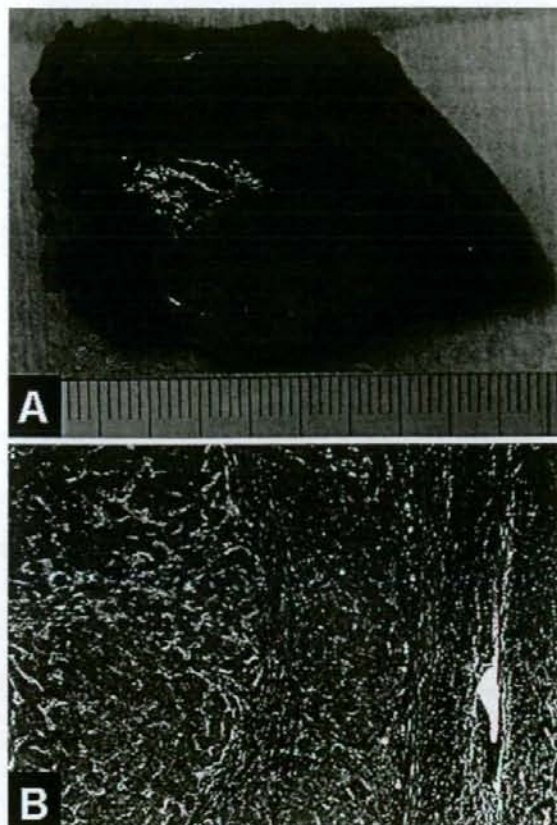


Fig. 2. A Resected specimen from the second operation. The tumor measured 2.5 cm \times 2.0 cm. B Pathologic examination showing that the tumor is a moderately differentiated hepatocellular carcinoma. H&E, \times 20

HBV DNA were detected in the tumor by PCR. The *HBs* gene, hepatitis B core (*HBc*) gene, and ccc HBV DNA were detected in the nontumorous tissue by PCR. The *HBx* gene was not detected in either tumor or nontumorous tissues by PCR (Fig. 3). Cassette-ligation-mediated PCR^{10,13} showed that HBV DNA was integrated into the telomerase reverse transcriptase gene (*hTERT* gene) located on chromosome 5p15 in the tumor (Fig. 4). The patient is in good health without recurrent tumor 18 months after the second operation.

Discussion

In this report, we describe a case of recurrent HCC 10 years after the clearance of HBsAg and 20 years after resection of the first HBV-related HCC. The duration between the first HCC and the recurrent HCC is the longest reported in the literature to date. The duration between the clearance of HBsAg and the detection of the recurrent tumor is the longest except for one case reported by Yoshino et al.⁶

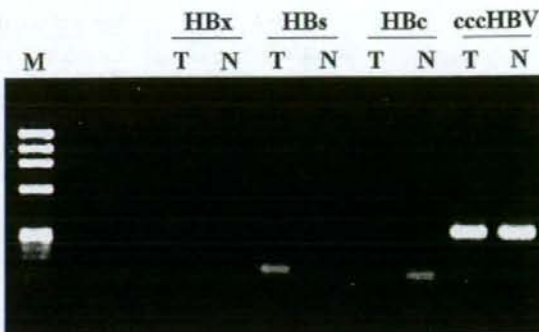


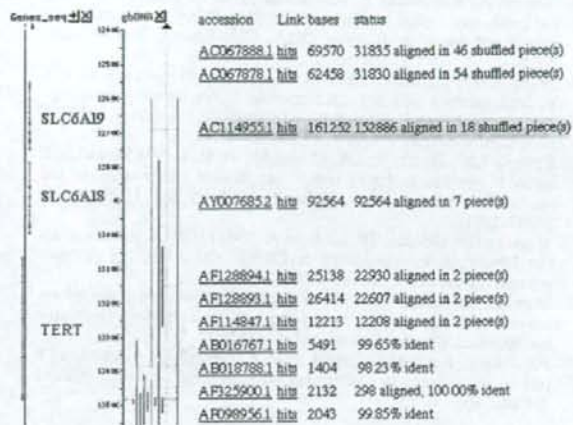
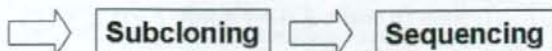
Fig. 3. Hepatitis B virus (HBV) DNA analysis of the resected sample. Detection of the *HBx* gene, *HBs* gene, *HBc* gene, and ccc HBV DNA was performed using specific primers in the tumor (T) and nontumorous (N) tissues. M, Marker (pBR322/AluI).

The mechanism of carcinogenesis in HCC associated with HBV includes mutagenesis and changes in proliferation and differentiation caused by the integration of HBV DNA.¹⁴⁻¹⁷ HBx protein binds with p53 protein and inhibits p53-mediated prevention of neoplastic transformation,^{18,19} and also activates cellular transcription factors.^{20,21} Chronic inflammation and oxidative stress due to HBV infection induce DNA damage in hepatocytes.²² Although we could not investigate the integration of HBV DNA at the time of the first operation in this patient, the first HCC is thought to have been caused by chronic HB, because he was seropositive for HBsAg and had active hepatitis at that time. At the time of the second operation, serum HBsAg and HBV DNA had cleared and liver histology had improved. Research on HBV DNA has shown that the *HBs* gene and ccc HBV DNA are detectable in HCC and nontumorous hepatic tissue, and that the *HBc* gene is detectable in non-cancerous hepatic tissue; the *HBx* gene was not detected in either tumor or nontumorous tissues in our patient. Integration of HBV DNA into the *hTERT* gene has also been detected. Previous reports showed that HBV DNA was integrated into the *hTERT* gene, and that it also could play an important role in hepatic carcinogenesis.^{23,24} These findings indicate that the patients have either occult HBV infection or integration of HBV DNA in their liver. Other possible mechanisms of hepatic carcinogenesis include inactivation of p53 by mutations and regional allelic deletions, although changes in the *p53* gene and p53 protein were not investigated in our patient.²⁵

Hepatitis B antigens have been detected in the hepatic tissues of some patients after the clearance of serum HBsAg associated with the remission of active hepatitis, especially in those with a high anti-hepatitis B core antibody (anti-HBc) titer.^{26,27} HBV DNA is often integrated into the host's chromosomes and plays an important role in hepatic carcinogenesis.²⁸ Thus, we speculate that, in our patient, the HCCs developed from multicentric origins, and that the integrated HBV DNA participated in the development of the recurrent HCC. In patients with chronic HB, screening for HCC is necessary even after clearance of serum HBsAg

Fig. 4. Cassette-ligation-mediated polymerase chain reaction (PCR). HBV DNA is integrated into the telomerase reverse transcriptase (*hTERT*) gene located on chromosome 5p15. Identical

Cassette-ligation-mediated PCR



and HBV DNA and remission of hepatitis, because the oncogenic potential due to occult HBV infection or the integration of HBV DNA is considered to continue.²⁹

Itsubo et al.³⁰ reported a case of recurrent HCC associated with HCV infection due to transfusion during the first liver resection. The first liver resection had been performed 18 years previously for HCC associated with HBV infection. In our patient, serum anti-HCV was positive and HCV RNA was negative in 1995. Although the HCV infection in the patient reported by Itsubo et al.³⁰ might have been caused by the transfusion during the first operation, it was transient because HCV RNA was negative and the levels of AST and ALT were within the reference ranges. Thus, the recurrent HCC was not directly caused by the HCV infection. However, an HCV superinfection might have played a role in the spontaneous HBsAg clearance in our patient.³¹

The rare case that we have reported indicates that long-term follow up is necessary for chronic HB, even after the clearance of serum HBsAg and HBV DNA and remission of hepatitis, because the oncogenic potential due to occult HBV infection or the integration of HBV DNA is considered to continue.

References

- Sherlock S, Dooley J (2002) Diseases of the liver and biliary system, 11th edn. Blackwell Science, Oxford, pp 285-303
- Adachi H, Kaneko S, Matsushita E, et al. (1992) Clearance of HbsAg in seven patients with chronic hepatitis B. *Hepatology* 16:1334-1337
- Okoshi S, Igarashi M, Suda T, et al. (2002) Remote development of hepatocellular carcinoma in patient with liver cirrhosis type B

serologically cured for HBs antigen with long-standing normalization of ALT values. *Dig Dis Sci* 47:2002-2006

- Ohba K, Kubo S, Tamori A, et al. (2004) Previous or occult infection with hepatitis B virus in hepatitis B surface antigen-negative and anti-hepatitis C negative patients with hepatocellular carcinoma. *Surg Today* 34:842-848
- Kubo S, Tanaka H, Shuto T, et al. (2005) Clinicopathologic features and outcome after liver resection for hepatocellular carcinoma in patients with concurrent versus previous chronic hepatitis B. *Surg Today* 35:216-222
- Yoshino S, Sohara N, Otsuka T, et al. (2004) Hepatocellular carcinoma developed 12 years after serologic clearance of HBs antigen (in Japanese). *Kanzo (Liver)* 45:206-210
- Perterlini P, Poussin K, Kew M, et al. (1995) Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 21:313-321
- Takeuchi M, Fujimoto J, Niwamoto H, et al. 1997 Frequent detection of hepatitis B virus X-gene DNA in hepatocellular carcinoma and adjacent liver tissue in hepatitis B surface antigen negative patients. *Dig Dis Sci* 42:2264-2269
- Matsuzaki Y, Sato M, Saito U, et al. (1999) The role of previous infection of hepatitis B virus in HBs antigen negative and anti-HCV negative Japanese patients with hepatocellular carcinoma: etiological and molecular biological study. *J Exp Clin Cancer Res* 18:379-389
- Tamori A, Nishiguchi S, Kubo S, et al. (2003) HBV DNA integration and HBV-transcript expression in non-B, non-C hepatocellular carcinoma in Japan. *J Med Virol* 71:492-498
- Spiessl B, Beahrs OH, Hermanek P, Hutter RVP, Scheibe O, Sobin LH, Wagner GB (eds) International Union Against Cancer (1992) TNM Atlas: illustrated guide to the TNM/pTNM classification of malignant tumours, 3rd edn, 2nd rev. Springer, Berlin Heidelberg New York Tokyo
- Desmet VJ, Gerber M, Hoofnagle JH, et al. (1994) Classification of chronic hepatitis diagnosis, grading and staging. *Hepatology* 19:1513-1520
- Tamori A, Nishiguchi S, Kubo S, et al. (1999) Possible contribution to hepatocarcinogenesis of X transcript of hepatitis B virus in Japanese patient with hepatitis C virus. *Hepatology* 29:1429-1434
- Kim CM, Koike K, Saito I, et al. (1991) HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 351:317-320

15. Meyer M, Wiedorn KH, Hofschneider PH, et al. (1992) A chromosome 17:7 translocation is associated with a hepatitis B virus DNA integration in human hepatocellular carcinoma DNA. *Hepatology* 15:665-671
16. Hino O, Shows TB, Rogler CE (1986) Hepatitis B virus integration site in hepatocellular carcinoma 17:18 translocation. *Proc Natl Acad Sci U S A* 83:8338-8342
17. Tamori A, Yamanishi Y, Kawashima S, et al. (2005) Alteration of gene expression in human hepatocellular carcinoma with integrated hepatitis B virus DNA. *Clin Cancer Res* 11:5821-5826
18. Wang XW, Forrester K, Yeh H, et al. (1994) Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Natl Acad Sci U S A* 91:2230-2234
19. Elmore LW, Hancock AR, Chang SF, et al. (1997) Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. *Proc Natl Acad Sci U S A* 94:14707-14712
20. Maguire HF, Hoeffler JP, Siddiqui A (1991) HBV X protein alters the DNA binding specificity of CREB and ATF-2 by protein-protein interactions. *Science* 252:842-844
21. Izumi H, Barrett JC (2003) Transcriptional regulation of the telomerase *hTERT* gene as a target for cellular and viral oncogenic mechanisms. *Carcinogenesis* 24:1167-1176
22. Pikarsky E, Porat MR, Stein I, et al. (2004) NF- κ B functions as a tumor promoter in inflammation-associated cancer. *Nature* 431:461-466
23. Ferber MJ, Montoya DP, Yu C, et al. (2003) Integrations of the hepatitis B virus (HBV) and human papillomavirus (HPV) into the human telomerase reverse transcriptase (*hTERT*) gene in liver and cervical cancers. *Oncogene* 22:3813-3820
24. Bonf KO, Young JK, Yount NP, et al. (2006) Quantitative assessment of *hTERT* mRNA expression in dysplastic nodules of HBV-related hepatocarcinogenesis. *Am J Gastroenterol* 101:831-838
25. Cougot D, Neuveut C, Buendia MA (2005) HBV induced carcinogenesis. *J Clin Virol* 34 (Suppl 1): S75-78
26. Kojima M, Udo K, Takahashi Y, et al. (1977) Correlation between titer of antibody to hepatitis B core antigen and presence of viral antigens in the liver. *Gastroenterology* 73:664-667
27. Omata K, Afroudakis A, Liew CT, et al. (1978) Comparison of serum hepatitis B surface antigen (HBsAg) and serum anticore with tissue HBsAg and hepatitis B core antigen (HBcAg). *Gastroenterology* 75:1003-1009
28. Joachim L, Eberhard H (2007) Hepatitis B virus-induced oncogenesis. *World J Gastroenterol* 13:74-81
29. Hu K-Q (2002) Occult hepatitis B virus infection and its clinical implications. *J Viral Hepat* 9:243-257
30. Itsubo M, Koike K, Osada M, et al. (2005) A case of recurrent hepatocellular carcinoma, based on different hepatic pathogenesis from initial condition, developed 18 years after hepatic resection (in Japanese). *Kanzo (Liver)* 46:277-283
31. Sheen IS, Liaw YF, Lin DY, et al. (1994) Role of hepatitis C and delta viruses in the termination of chronic HBsAg carrier state: multivariate analysis in a longitudinal follow-up study. *J Infect Dis* 170:358-361