Influence of Previous Interferon Therapy on Recurrence after Resection of Hepatitis C Virus-related Hepatocellular Carcinoma

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Interferon (IFN) therapy decreases the incidence of hepatocellular carcinoma (HCC) in patients with hepatitis C virus (HCV). One hundred and fifty-nine consecutive patients who underwent liver resection for HCV-related HCC were studied. In 17 (group 1) of the 159 patients, HCC was detected during or after IFN therapy. The incidences of recurrence after surgery in the group 1 patients and the other 142 patients (group 2) were compared. Eight patients had a complete response to IFN, 4 had a partial response, and 5 had no response. The proportion of patients without HCV viremia was significantly higher in the group 1 patients (*P*<0.0001). The tumor-free survival rate was significantly higher in the group 1 patients (*P*=0.0010). By multivariate analysis of various risk factors for recurrence, no previous IFN was a significant independent risk factor for recurrence (risk ratio=6.336; 95%CI, 1.512–26.50). The patients with HCC who underwent IFN therapy previously are good candidates for liver resection because recurrence after the operation was rarely observed.

Key words: Hepatocellular carcinoma — Hepatitis C virus — Interferon — Liver resection — Multicentric carcinogenesis

Hepatitis C virus (HCV) infection has been reported to be a cause of hepatocellular carcinoma (HCC). After the report by Hoofnagle *et al.*[1] on the use of interferon (IFN) in chronic hepatitis C, there have been numerous studies suggesting that IFN is effective in the treatment of chronic hepatitis C. Recent studies have shown that IFN causes HCV RNA to disappear not only from the serum, but also from hepatic tissue, and that IFN improves liver histology.[2–4] Since we reported that IFN decreases the incidence of HCC in patients with chronic hepatitis C and cirrhosis in a prospective randomized study,[5] several investigators have shown that IFN suppresses the incidence of HCC in patients with chronic hepatitis C.[6–13] However, HCCs are found even in some patients successfully treated with IFN.[6–14] We have reported that continuous active hepatitis with HCV viremia is a high risk factor for recurrence after the resection of HCV-related HCC and that the results after liver resection for HCC in patients with anti-HCV antibody (anti-HCV) and without HCV viremia were satisfactory.[15] However, the influence of previous IFN therapy on the patient's outcome after the resection of HCV-related HCC has not been evaluated. It is not practicable to carry out a randomized prospective study of preoperative IFN (after the detection of HCC) because the

therapy takes several months to 1 year to complete and therefore HCC may progress during treatment. In this retrospective study, we tried to determine whether previous IFN therapy improves the tumor-free survival rate after the resection of HCV-related HCC.

PATIENTS AND METHODS

Patients Between 1993 and December 1999, 178 consecutive patients with anti-HCV (lacking hepatitis B surface antigen) had a curative resection for HCC. Curative surgery was defined as the complete resection of all macroscopic tumors. The absence of tumor cells along the parenchymal transection line was confirmed histologically.

No tumors remained in the remnant liver by computed tomography (CT) at 3 to 4 weeks after surgery. Nineteen patients who died of operative complications, received IFN therapy after surgery, or had other malignancies were excluded from the study. Of the 159 remaining patients, 17 had visited the Second Department of Surgery, Osaka City University Medical School for treatment for HCC that had been detected during or after IFN therapy (group 1). Group 2 consisted of the 142 other patients who did not receive IFN therapy before the detection of HCC. The patients were examined preoperatively by ultrasonography, plain and enhanced CT (including dynamic CT), lipiodol CT (Ultra-Fluide, Laboratorie Guerbet, Villepointe,

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France), and angiography. CT during arteriography and arterioportography were performed if possible. Intraoperative sonography was performed in all patients.

The study was conducted in accordance with the Helsinki Declaration and the guidelines of the ethics committee of our institution. Written informed consent was obtained from each patient.

Viral markers Serum samples obtained before surgery from all patients were assayed for hepatitis B virus (HBV) and HCV. The serum was examined for HBV antigen (HBsAg) with an enzyme immunoassay (International Reagents Corp., Kobe). The samples were assayed for anti-HCV by a second- or third-generation ELISA (Ortho Diagnostic Systems, Tokyo). Serum HCV RNA was detected by a nested polymerase chain reaction using reverse transcription and primers derived from a conserved 5′-untranslated region of the viral genome,[5] as well as a branched DNA probe assay (Quantiplex HCV-RNA, Chiron Corp., Emeryville, CA). When HCV RNA was not detected in the sera by these two methods, the results were further confirmed by a single-enzyme, combined reverse transcription-polymerase chain reaction (Amplicor HCV test, Roche Diagnostic Systems/Nippon Roche Co., Branchburg, NJ). Sera in which HCV RNA could not be detected by these three methods were considered negative for HCV RNA.

IFN therapy The type and the dose of IFN used, and the period of IFN therapy in the group 1 patients are shown in Table I. Natural IFN-α (human lymphoblastoid interferon; Sumiferon, Sumitomo Pharmaceuticals, Osaka) was administered to 8 patients. Recombinant IFN-α2a (Canferon, Takeda Chemical Industries, Ltd., Osaka) was administered to 1 patient. Recombinant IFN-α2b (Intron A, Schering-Plough, Co., Kenilworth, NJ) was administered to 7 patients. Human IFN-β (Feron, Toray Industries Inc., Tokyo) was administered to 1 patient. The patients received IFN intramuscularly. In 1 patient (patient no. 2), IFN was stopped early because HCC was detected (total dose: 192 MU). The response to therapy was classified on the basis of changes in the HCV RNA levels and serum alanine aminotranferase (ALT) activity during and immediately after IFN administration, and for at least 6 months thereafter.[5] A complete response (CR) was defined as an ALT activity within the normal reference range and no detectable serum HCV RNA for at least 6 months after IFN therapy. A partial response (PR) was defined as a normalized ALT activity or the transient disappearance of serum HCV RNA. Non-response (NR) was defined as no decrease in the ALT activity and persistence of serum HCV RNA.

Detection of recurrence The serum concentrations of α-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist II were measured every 3 months. Ultrasonography, CT, magnetic resonance imaging, chest radiography, or some combination of these was performed every 3 months. When a tumor recurrence in the remnant liver was suspected on the basis of tumor markers or imaging, we performed angiography or a biopsy under

Table I. Previous IFN Therapy and Responses in Individual Patients

Patient no.	Age/sex	IFN therapy			Response[a]	Interval to tumor detection[b]
		Type	Dose (MU)	Period (weeks)		
1	67/M	α	520	12	NR	8 months
2	51/M	α	192	8	PR	During
3	60/M	α2b	240	8	CR	9 months
4	57/M	α	520	12	PR	1 year 5 months
5	65/M	α2a	378	12	CR	1 year
6	64/M	α2b	500	14	CR	2 years 10 months
7	54/M	α2b	500	14	PR	3 years 7 months
8	65/M	α	480	24	PR	2 years
9	61/M	α	783	24	NR	2 years 2 months
10	52/M	α2b	1024	40	CR	8 months
11	59/M	α2b	524	24	CR	2 years 2 months
12	62/M	α2b	752	28	NR	5 years 5 months
13	67/F	α	519	24	NR	3 years
14	59/M	α2b	800	24	CR	2 years 4 months
15	59/M	α	1272	68	CR	2 years 1 month
16	49/M	β	234	24	CR	2 years 7 months
17	57/M	α	504	24	NR	3 years 4 months

a) CR, complete response; PR, partial response; NR, no response.
b) Interval between the end of IFN therapy and the detection of HCC.

ultrasonographic guidance (or both) to establish a definitive diagnosis. Bone metastases were assessed by scintigraphy using ^{99m}Tc diphosphonate.

Pathologic examination The liver specimens before IFN therapy were obtained by biopsy. The resected specimens were cut into serial slices 5 mm thick, fixed in 10% formalin, and stained with hematoxylin and eosin. The number of sections examined was at least 10 for each patient, and the maximum number was 302. The histologic grade of tumor differentiation was assigned according to the modified classification[16] of Edmondson and Steiner[17] and Kondo et al.[18]

Noncancerous hepatic tissues were examined pathologically. A histologic activity index (HAI)[19] was used to evaluate the severity of active hepatitis and the degree of fibrosis. A HAI score (components 1 to 3) of 0 indicated no activity (grade 0), a score of 1 to 3 indicated minimal activity (grade 1), a score of 4 to 8 indicated mild activity (grade 2), a score of 9 to 12 indicated moderate activity (grade 3), and a score greater than 12 indicated severe activity (grade 4). The fibrosis score (the staging) was determined by component 4 in the HAI score. Stage 0 indicated no fibrosis, stage 1 indicated portal fibrous expansion, stage 2 indicated porto-portal septa without architectural distortion, stage 3 indicated portocentral septa with architectural distortion, and stage 4 indicated cirrhosis.

Statistics We used Student's t test to analyze the differences in age and the Mann-Whitney test to analyze the differences in the laboratory values and tumor size. χ^2 or Fisher's exact test was used to compare the categorical data between groups. The tumor-free survival rate was calculated by the Kaplan-Meier method, and the differences in the rates between the groups were compared by use of the log rank test. Cox's proportional hazard model with stepwise variable selection was used for multivariate analysis. The variables were selected for their potential relationship to recurrence on the basis of previous studies[20–31] or our own clinical experience. The variables chosen (except previous IFN therapy) were age (≥ 65 or <65 years), sex, history of intake of at least 86 g of ethanol daily for at least 10 years,[20] history of blood transfusion, Child-Pugh score[32] (A or B), HCV viremia, albumin concentration (<3.5 or ≥ 3.5 g/dl), aspartate aminotransferase (AST) activity (≤ 40 or >40 IU/liter), ALT activity (≤ 45 or >45 IU/liter), total bilirubin (≤ 1.0 or >1.0 mg/dl), platelet count (≥ 10 or $<10\times10^4/mm^3$), AFP (≤ 20 or >20 ng/ml), the largest diameter of the main tumor (<3 or ≥ 3 cm), the number of tumors (single or multiple), the degree of differentiation of the main tumor (well-differentiated or other), the presence of portal invasion, the grading score ($0-2$ or 3, 4), the staging score ($0-2$ or 3, 4), preoperative transcatheter arterial embolization, the operative method (major or minor hepatectomy), and the presence or

Table II. Laboratory and Histologic Findings in Patients with and without Preoperative IFN Therapy

	Group 1 (n=17) IFN therapy		Group 2 (n=142) No preoperative IFN
	Before[a]	After[a]	
Albumin (g/dl)	3.9 (3.5, 4.5)[b]	3.8 (3.6, 4.3)[f]	3.6 (3.2, 4.0)
AST (IU/liter)	89 (36, 135)[c]	40 (27, 90)[g, h]	66 (37, 120)
ALT (IU/liter)	121 (42, 172)[d]	42 (25, 136)[i]	74 (33, 130)
Total bilirubin (mg/dl)	0.9 (0.5, 2.0)	0.7 (0.5, 1.4)	0.8 (0.5, 1.3)
Platelet count ($\times10^4/mm^3$)	11.3 (6.3, 19.3)	13.9 (6.0, 19.7)	12.3 (7.4, 21.4)
ICGR$_{15}$ (%)	—	15.1 (8.0, 26.0)	16.6 (8.4, 26.2)
Grading 1	1[e]	6[j]	29
2	7	8	86
3	8	3	26
4	1	0	1
Staging 0–3	10	9	73
4	7	8	69

Results of laboratory tests are given as medians, with 10th and 90th percentiles. Results of the grading and the staging score are given as number of patients.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR$_{15}$, 15-min indocyanine green retention test.

a) The liver specimens before IFN therapy were obtained by biopsy. The liver specimens after IFN therapy were obtained at operation.

b) $P=0.0005$, c) $P=0.0397$, d) $P=0.0015$, e) $P=0.0082$, f) $P=0.0118$, g) $P=0.0074$, as compared with group 2.

h) $P=0.0093$, i) $P=0.0068$, j) $P=0.0748$, as compared with before IFN therapy.

Table III. Clinicopathologic Features of Patients with and without Preoperative IFN Therapy

	Group 1 IFN therapy (n=17)	Group 2 No preoperative IFN (n=142)	P
Age (year, mean±SD)	59±6	64±6	0.0022
Sex (M:F)	16:1	124:18	0.695
History of blood transfusion	3 (18%)	38 (27%)	0.563
Alcohol abuse	5 (29%)	44 (31%)	>0.999
Child-Pugh classification			
A	15 (88%)	100 (70%)	0.157
B	2 (12%)	42 (30%)	
Negative HCV RNA	8 (47%)	2 (1%)	<0.0001
HCV genotypes			
1b	12	98	>0.999
2a	4	22	
2b	0	5	
1b and 2a	0	1	
2a and 2b	0	4	
Unknown	1	12	
High α-fetoprotein (>20 ng/ml)	9 (53%)	81 (57%)	0.799
Tumor size (cm, mean±SD)	2.5 (1.5, 5.5)	3.0 (1.6, 7.0)	0.179
Number of tumors			
Single	11 (65%)	75 (53%)	0.444
Multiple	6 (35%)	67 (47%)	
Differentiation of primary tumor			
Well-differentiated	3 (18%)	14 (10%)	0.381
Moderately or poorly	14 (82%)	128 (90%)	
Portal invasion	4 (24%)	40 (28%)	>0.999
Preoperative TAE	3 (18%)	28 (20%)	>0.999
Type of resection			
Major hepatectomy	5 (29%)	45 (32%)	>0.999
Minor hepatectomy	12 (71%)	97 (68%)	
Positive margins	5 (36%)	68 (48%)	0.200

Tumor size is given as the median, with 10th and 90th percentiles.

IFN, interferon; AST, aspartate aminotransferase; ALT, alanine aminotransferase; $ICGR_{15}$, 15-min indocyanine green retention test; TAE, transcatheter arterial embolization.

absence of tumor-free margins. The clinical findings and the laboratory test results just before the operation were used for the analyses. Major hepatectomy included a tri-, bi-, or mono-segmentectomy, and minor hepatectomy included a subsegmentectomy or partial resection. When the tumor-free surgical margin by pathologic examination was less than 5 mm, it was defined as tumor-positive. A *P* value of less than 0.05 was considered significant.

RESULTS

Response to IFN therapy The response to IFN is summarized in Table I. A complete response was achieved in 8 patients, a partial response in 4 patients, and no response in 5 patients. In the 8 complete responders, serum HCV RNA was not detected at any follow-up appointment. The activities of AST and ALT were significantly lower just before liver resection (after IFN therapy) than before the therapy (*P*=0.0093, *P*=0.0068, respectively, Table II). The grading score decreased after IFN therapy in 9 patients. The grading score tended to be lower at the operation than before the therapy (*P*=0.0748). HCC was detected during IFN therapy in one patient, and 8 months to 5 years and 5 months after the end of IFN therapy in 16 other patients.

Clinicopathologic findings in patients with and without preoperative IFN therapy The clinical features, laboratory test results, and pathologic findings of the surgical specimens are shown in Tables II and III. The ages of the subjects ranged from 49 to 67 years in group 1 and 47 to 77 years in group 2 (Table III). The mean age was signifi-

cantly lower in the group 1 patients (P=0.0022). No significant differences in the proportion of patients with a history of blood transfusion or alcohol abuse were noted between the two groups. The proportion of patients without HCV viremia (negative HCV RNA in the sera) was significantly higher in the group 1 patients than in the group 2 patients (P<0.0001).

Although the serum AST activity was significantly higher before IFN therapy in the group 1 than group 2 patients (P=0.0397), the activity just before liver resection was significantly lower in the group 1 than group 2 patients (P=0.0074, Table II). Although the ALT activity was significantly higher before IFN therapy in the group 1 patients than in the group 2 patients (P=0.0015), the activity just before the operation was not different between the group 1 and group 2 patients. Although the grading score was significantly higher in the group 1 patients before IFN therapy than in group 2 patients (P=0.0082), the grading score in the resected specimens was not different between the group 1 and group 2 patients. The serum albumin concentration was significantly higher after IFN therapy in the group 1 patients than in the group 2 patients (P=0.0118). No significant differences were noted between the two groups in the serum total bilirubin, the platelet counts, the indocyanine green retention test, and the staging score. The proportion of patients with an elevated AFP concentration (>20 ng/ml) did not differ between the two groups (Table III). No significant differences were noted in tumor size, the number of tumors, the degree of differentiation of the main tumor, or the proportion of patients with portal invasion. The proportion of patients with preoperative transcatheter arterial embolization, the operative method, and the proportion of patients with a positive surgical margin were also not significantly different between the two groups.

Recurrence after surgery Recurrent tumor(s) was detected in 1 of the 8 complete responders, none of 4 partial responders, and 1 of 5 nonresponders. In the one complete responder with recurrence, the patient had not been followed up after the end of IFN therapy and then poorly differentiated HCC with portal invasion and intrahepatic metastases were detected at 3 years 9 months after completing IFN therapy. Among the group 2 patients, recurrences occurred in the liver in 80 patients and in other organ(s) in 5 other patients.

Tumor-free survival rates and factors related to recurrence The follow-up period in all patients was 639±536 days. The follow-up periods (from surgery to the detection of recurrence or the end point of this study) were 963±872 (median, 628 days; range, 59 to 2310 days) in group 1 and 600±470 days (median, 471 days; range, 61 to 2308 days) in group 2. The tumor-free survival rate was significantly higher in the group 1 patients (P=0.0010; Fig. 1). The presence of HCV viremia (P=0.0348), multiple tumors
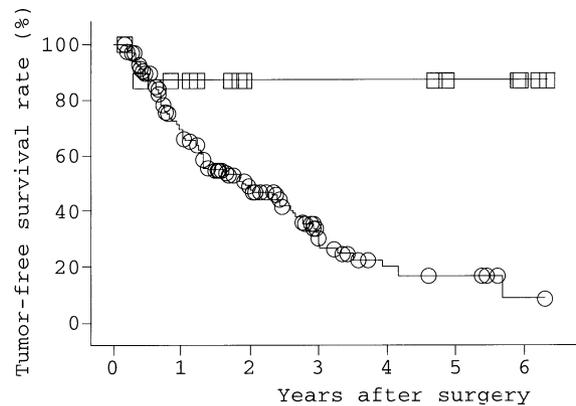

Fig. 1. Tumor-free survival rates in the patients with (group 1) and without (group 2) previous IFN therapy. □ group 1 (n=17), ○ group 2 (n=142). P=0.0010.

(P<0.0001), moderately or poorly differentiated HCC (P=0.0301), and high AST activity (P=0.0313) were found to be significant risk factors for recurrence (Table IV). A history of blood transfusion (P=0.0945), a low albumin concentration (P=0.0536), a Child-Pugh score of B (P=0.0706), and portal invasion (P=0.0562) tended to be significant risk factors for recurrence. All other factors examined were not significant risk factors. By multivariate analysis, no preoperative IFN (risk ratio=6.336; 95%CI, 1.512–26.50; P=0.0115) and multiple tumors (risk ratio=2.959; 95%CI, 1.894–4.630; P<0.0001) were significant independent risk factors for recurrence.

DISCUSSION

In patients with chronic hepatitis C, chronic inflammation, liver cell necrosis and regeneration, and extensive fibrosis are important in the development of HCC.[33] Moriya et al.[34] have shown in an experimental model that the HCV core protein has a significant role in the development of HCC. Recent studies have shown that IFN is effective in eliminating HCV and in suppressing active hepatitis and may decrease the incidence of HCC in patients infected with HCV[5–13] although this is still controversial.[35] Some of these studies have indicated that HCC was less likely to develop in patients in whom IFN was effective at normalizing the ALT, even if the HCV was not eradicated.[7, 9, 10–13] These results suggest that the anticarcinogenic activity of IFN is closely related to its ability to suppress active hepatitis.

Recurrences after the resection of a primary HCC are thought to result from intrahepatic spread through the portal vein and from multicentric (multifocal) carcinogenesis after surgery. Shirabe et al.[30] and Ko et al.[31] have reported

Table IV. Tumor-free Survival Rates after Operation

Variable	(n)	Survival rates			P
		1-year	3-year	5-year	
Age (years)					
≥65	(81)	66	33	28	0.313
<65	(78)	75	37	24	
Sex					
Male	(140)	71	30	22	0.856
Female	(19)	67	49	37	
History of blood transfusion					
−	(118)	73	40	27	0.0945
+	(41)	64	23	18	
Alcohol abuse					
−	(110)	76	39	27	0.180
+	(49)	57	26	18	
HCV viremia					
−	(10)	80	71	71	0.0348
+	(149)	69	34	20	
Previous IFN therapy					
−	(142)	69	30	17	0.0010
+	(17)	86	86	86	
Child-Pugh score					
A	(115)	71	38	33	0.0706
B	(44)	72	24	10	
Albumin (g/dl)					
≥3.5	(120)	71	41	32	0.0536
<3.5	(39)	68	16	5	
Aspartate aminotransferase (IU/liter)					
≤40	(34)	82	42	42	0.0313
>40	(125)	67	32	20	
Alanine aminotransferase (IU/liter)					
≤45	(43)	72	31	31	0.996
>45	(116)	70	33	21	
Total bilirubin (mg/dl)					
≤1.0	(110)	70	37	28	0.486
>1.0	(49)	71	28	18	
Platelet count (×10^4/mm^3)					
≥10	(115)	67	35	23	0.779
<10	(44)	79	33	23	
α-Fetoprotein (ng/ml)					
≤20	(90)	71	36	25	0.883
>20	(69)	71	31	24	
Tumor size (cm)					
<3	(76)	72	39	30	0.506
≥3	(83)	67	28	20	
Number of tumors					
Single	(86)	88	52	39	<0.0001
Multiple	(73)	97	15	8	
Differentiation of main tumor					
Well-differentiated	(17)	86	66	56	0.0301
Other	(142)	67	29	20	
Portal invasion					
−	(115)	77	36	27	0.0562
+	(44)	51	30	16	
Grading score					
0–2	(129)	67	38	25	0.167
3, 4	(30)	84	39	27	
Staging score					
0–2	(51)	65	45	40	0.200
3, 4	(108)	79	26	16	
Preoperative TAE					
−	(126)	70	37	22	0.413
+	(33)	70	42	30	
Operative method					
Major	(50)	59	31	25	0.598
Minor	(109)	76	36	24	
Surgical margin					
Negative	(86)	77	39	27	0.199
Positive	(73)	62	28	21	

TAE: transcatheter arterial embolization.

that active hepatitis is a risk factor for recurrence, including multicentric carcinogenesis, although their studies included patients infected with HBV. It has also been reported that a sustained increase in ALT activity is a risk factor for recurrence,[15, 30, 36] and we have reported that continuous active hepatitis with HCV viremia is a risk factor for recurrence.[15] In this study, we again confirmed by univariate analysis that the presence of HCV viremia and high AST activity are risk factors for recurrence. Thus, persistent HCV infection and continuous active hepatitis strongly affect the likelihood of recurrence, especially in patients with multicentric carcinogenesis, after resection. In this study, the tumor-free survival rate was significantly higher in the group 1 patients than in the group 2 patients and absence of previous IFN therapy was an independent risk factor for recurrence. In group 1, 8 patients were complete responders and 4 patients were partial responders. The activities of AST and ALT and the histologic severity of the active hepatitis were improved by IFN therapy. As a result, after IFN therapy, the AST activity was significantly lower in the group 1 patients. In addition, the proportion of patients with HCV viremia was significantly lower in group 1 than in group 2. These findings indicate that previous IFN therapy may halt or improve chronic active hepatitis and suppress postoperative carcinogenesis in the same way that IFN therapy decreases the incidence of HCC in patients infected with HCV, resulting in a higher tumor-free survival rate. Suppression of metastasis by improvement of active hepatitis is a possible factor in the low recurrence rate in group 1 because active hepatitis enhances upregulation of adhesion molecules on the sinusoidal lining cells of the liver.[37] Another possible mechanism for the anticarcinogenic activity of IFN is the immunologic and biologic effects of IFN, including the induction of IFN regulatory factor,[38] its growth inhibitory effect,[39] and its activation of natural killer cells[40] and T-cell activity.[41] These effects may suppress both intrahepatic metastasis and multicentric carcinogenesis. Lai *et al.*[42] reported that IFN treatment of patients with advanced HCC improved their survival rates.

As described in the introduction, it is not feasible to carry out a randomized prospective study of preoperative IFN. Although this study was not a randomized prospective study, the proportions of complete responders and nonresponders were similar to those of a national surveillance program in Japan.[13] In 7 complete responders (except 1 patient with advanced HCC) and 4 partial responders, no recurrence has been detected up to the end of this study. The results indicate that a satisfactory result can be expected after a curative resection for HCC that develops after IFN therapy. The patients with HCC who underwent IFN therapy previously are good candidates for liver resection because recurrence after the operation was rarely observed.

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