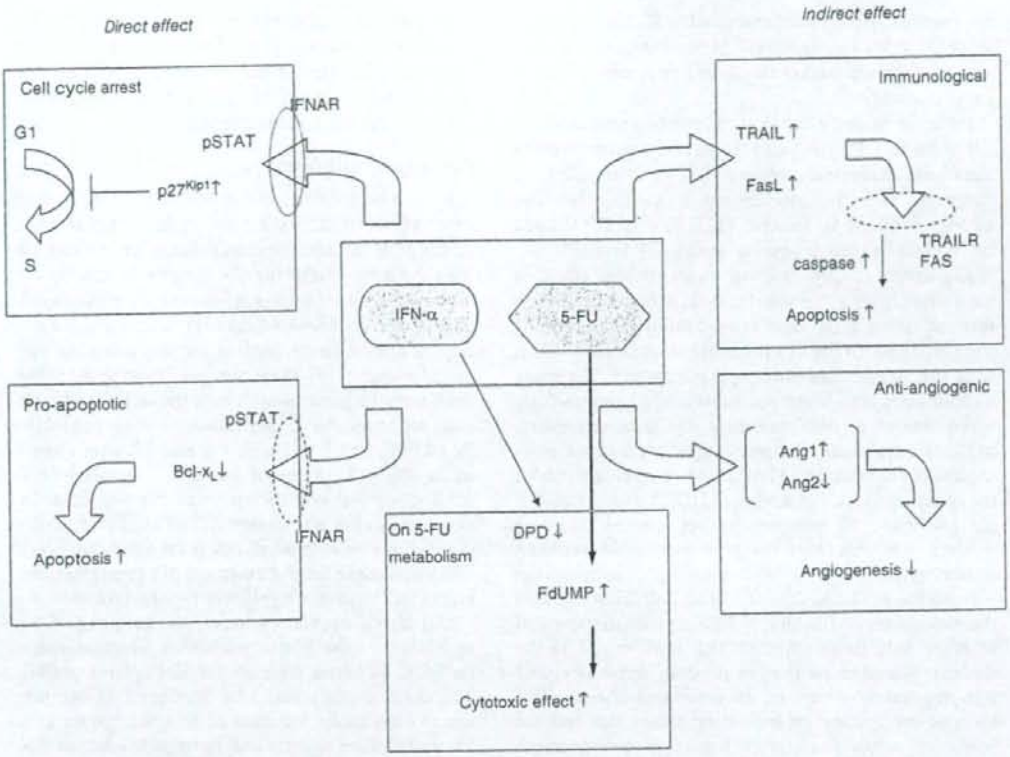

Figure 2 FAIT mechanism (version 1).

treated.[21,41,42,43] They lose crucial survival time by precluding their chance to undertake other treatment options. Moreover, FAIT induces side-effects (see Adverse effects). Therefore, it is vital that the patients be appropriately selected for FAIT and that sensitivity to this chemotherapy is predicted accurately.

Several studies have attempted to distinguish between responders and non-responders to FAIT. To investigate the role of clinical and pathological parameters in clinical effects of therapy, we compared some factors of responders ($n = 8$) with those of non-responders ($n = 5$).[41] The results showed that patient age, gender, serum AFP, protein induced by vitamin K absence or antagonist II (PIVKA-II), Child–Pugh, Okuda scores and Cancer of the Liver Italian Program (CLIP) scores did not correlate with the response to combination therapy. On the other hand, IFNAR2 expression in tumors correlated significantly with the response to the therapy ($P = 0.007$). Moreover, survival analysis showed the significant role of IFNAR2 expression on prognosis; IFNAR2-positive cases had better prognosis than negative cases.[41,76] Thus, the expression level of IFNAR2 was the sole factor that influenced the response to the combination therapy and that might be a potentially useful predictor of the response to FAIT.

The investigators from Kyoundo Hospital noted that the CR rate was higher among patients with HCV infection (22%) than among others (5%).[43] The final response to treatment was predictable, with the early response of tumor biomarker levels at the second week among patients whose markers were positive before treatment (AFP, L3 fraction of AFP, and des-γ-carboxy prothrombin; prediction with 90% sensitivity and 80% specificity). Otherwise, Yamamoto *et al.* reported that

among HCC patients who received FAIT, the expression of TRAIL mRNA in peripheral blood mononuclear cells was significantly higher in clinical responders than in non-responders.[75]

From the study by Patt *et al.*, all patients who achieved CR or PR to 5-FU (i.v.) and IFN-α had a corresponding significant decrease in serum AFP level (<50 ng/mL) as compared with the baseline value (median decrease, 82.5%), while most patients (11/14) with DP showed an increase in their follow-up serum AFP levels.[39]

The advent of DNA microarray technology could be used directly as a therapeutic tool. A novel prediction method using gene expression profiling has recently been reported for the treatment of breast cancer patients with the taxanes, docetaxel and paclitaxel.[80] The study by Kurokawa *et al.* identified subsets of 63 genes which, when analyzed simultaneously by gene expression profile analysis using adaptor-tagged competitive polymerase chain reaction (ATAC-PCR) technology, predict the response to FAIT in advanced HCC.[81] Further studies for prediction of the therapy are needed adopting modern methods, such as gene expression profiling technology.

From the available data, it will be helpful to examine the expression of IFNAR2 in HCC before therapy and whether only those with IFNAR2 must enroll in the therapy. We advocate that, in practice, responders and non-responders should be discriminated after the first cycle of the therapy by evaluating tumor size and the levels of tumor markers. For early non-responders, therapy should not proceed to the next cycle and instead a different therapeutic option should be explored.

Questions regarding non-responders to FAIT

Despite the prominent improvement in survival among complete responders, we must admit that the CR rate was not satisfactory, and that we need to enhance the response among partial responders by modifying the protocol. The studies show that approximately half of the patients with HCC will remain unsusceptible to the combination therapy.

We reported previously that FAIT had no effect in IFNAR2-negative cases.[35] Upregulation of IFNAR2 may be considered in order to induce a better response to the therapy in such cases. In our recent study involving *in vitro* experiments, we showed that IFNAR2 gene transfer is effective for augmenting the biological activity of IFN-α/5-FU combination therapy in HCC cells.[71] Thus, IFNAR2 gene transfection might enhance the response to FAIT in IFNAR2-negative patients. On the other hand, not all IFNAR2-positive cases benefited from FAIT. In such patients, increasing the doses or modifying t combination therapy (e.g. addition of other antitum agents) might increase the RR. Other parameters, ap from the expression of IFNAR2, might be important a necessary for the response to the therapy.

Further challenges

What can be done to make combination chemothera more effective? Increasing the doses or modifying t protocol of the combination therapy, monitoring ca fully the toxic effects, may be considered initially.

Higher concentrations of cancer chemotherapeu agents can be delivered directly to the HCC via t hepatic arterial route, considering that this is the maj vascular supply of these tumors. Yuen *et al.* show that, from 18 patients with inoperable HCC who we recruited to receive i.a. high doses of IFN-α (10 MU/n 30 MU/m², or 50 MU/m²), CR and PR were observ in 28.6% and 33.3% of patients, respectively.[21] T median survival was 15.9 months. No significant liv decompensation was observed. This pilot study show that transcatheter arterial IFN-α embolization was effective method for the treatment of patients with ino erable HCC without significant hepatic toxicity.

It is also a problem whether the coupling of 5-F and IFN-α is the best combination as chemothera for HCC. A better regimen for IFN-α/5-FU combin tion chemotherapy could be developed. In our pre ous *in vitro* study, we showed that the spectra of t antiproliferative activity and synergistic effect of IFN when combined with anticancer drugs (5-FU, DC and CDDP) were more potent than those of IFN-α.[61] Thus, combinations of IFN-β with other anticanc drugs may provide a better treatment of HCC wh FAIT is ineffective.

SUMMARY

CURATIVE THERAPIES SUCH as hepatic resectio liver transplantation, transcatheter arterial emb lization or percutaneous ablation have led to improv ment in survival of patients with HCC. Howev standard therapy for advanced, inoperable HCC was n established.

Both clinical and preclinical studies suggest effica of combination of 5-FU with IFN-α for such high advanced HCC. The efficacy of FAIT ranged 21.5–63 (overall RR, 46.4%), which was better than the previo reports with other combination chemotherapies f patients of a similar stage. Generally, the prognosis such patients is extremely poor and survival is general

limited to a few months after diagnosis. The FAIT markedly decreased tumor size and levels of tumor markers with an encouraging RR and prolonged survival time in the responders. Furthermore, the clinical response completely reflected the survival benefits.

It will be helpful to examine the expression of IFNAR2 in HCC before therapy and if only those with IFNAR2 should enroll. At present, we recommend starting the combination therapy with close monitoring of response, preferably that of tumor biomarkers, and treatment should be continued if there is a response after the first cycle of chemotherapy. For early non-responders, therapy should not proceed to the next cycle and instead a different therapeutic option should be explored.

Although the limitations in comparing the clinical response between some studies cannot be neglected, the marked effect and acceptable toxicity of the therapy for HCC patients with extremely poor prognosis suggests that FAIT is a potential, promising treatment regimen. To obtain conclusive evidence of the effect of this treatment, a large phase II trial and further investigation are essential.

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CONFLICT OF INTEREST

NO CONFLICT OF interest statement has been received from the authors.

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Interferon-α and 5-Fluorouracil Combination Therapy After Palliative Hepatic Resection in Patients With Advanced Hepatocellular Carcinoma, Portal Venous Tumor Thrombus in the Major Trunk, and Multiple Nodules

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BACKGROUND. The authors reported previously the beneficial effects of interferon (IFN)-α/5-fluorouracil (5-FU) combination therapy for patients with advanced hepatocellular carcinoma (HCC) who have tumor thrombi in the major portal branches. In this report, the authors describe the results from IFN/5-FU chemotherapy for patients who underwent palliative hepatic resection for advanced HCC with tumor thrombus in the main trunk of the portal vein and multiple nodules in the whole liver. In addition, they evaluated the correlation between the response to such therapy and expression of IFN-α type 2 receptor (IFNAR2).

METHODS. From October 1999 to December 2004, 30 patients with advanced HCC, tumor thrombi in the main trunk of the portal vein, and multiple nodules in the whole liver (Vp4 and grade 3 intrahepatic metastases) were recruited for this study. They underwent palliative hepatic resection followed by at least 2 courses of IFN/5-FU. IFNAR2 expression levels were determined by immunohistochemistry.

RESULTS. No major treatment-related complications were noted. An objective response was noted in 10 patients (33.3%) and included a complete response in 6 patients (20%), a partial response in 4 patients (13.3%), no response in 1 patient (3.3%), and progressive disease in 19 patients (63.4%). IFNAR2 expression was detected in 20 of 30 patients (66.7%). There was a significant difference in overall survival between patients with positive and negative IFNAR2 expression cases ($P < .0025$), and a significant correlation was observed between IFNAR2 expression and response to IFN/5-FU combination therapy ($P = .0199$).

CONCLUSIONS. Adjunct IFN/5-FU therapy is a promising modality for patients with advanced HCC, tumor thrombi in the major trunk, and multiple nodules after palliative hepatic resection. The results from this study indicated that the response to such therapy seemed to be correlated with IFNAR2 expression. *Cancer* **2007;110:2493–501.** © *2007 American Cancer Society.*

KEYWORDS: hepatocellular carcinoma, hepatic resection, interferon type 2 receptor, portal vein thrombosis, arterial infusion chemotherapy.

The prognosis for patients with advanced hepatocellular carcinoma (HCC) remains poor, particularly in patients who have tumor thrombi in the major trunk of the portal vein (Vp4).[1–3] The mortality rate is very high in patients with unresectable tumors, and their quality of life is poor because of intractable ascites or esophageal bleeding. Even in patients who have resectable HCC, the prognosis is extremely

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poor despite aggressive surgery.[4,5] In such a situation, conventional therapies generally have no clinical effect on HCC associated with portal vein tumor thrombi (PVTT) because of poor efficacy and possible complications.[6,7] Arterial infusion chemotherapy also has been attempted, but its effectiveness still is unsatisfactory.[9,10] Therefore, a new strategy is required for patients who have intractable HCC and tumor thrombi in the major branch of the portal vein.

Several recent studies have indicated the beneficial effects of interferon (IFN)-α-based combination chemotherapies for HCC[10–15] despite the lack of satisfactory results from IFN-α monotherapy.[16] We also reported on the clinical efficiency of IFN-α and 5-fluorouracil (5-FU) combination therapy for advanced HCC with PVTT and intrahepatic metastasis.[17–20] IFN-α suppresses the proliferation of all type I IFN receptor type 2 (IFNAR2)-positive cancer cell lines in vitro, an effect that is mediated through its high affinity to IFNAR2. Studies from our laboratories demonstrated that IFNAR2 expression in HCC tissues may be a useful predictor of response to IFN/5-FU combination therapy.[19]

The current study was an extension to our previous work,[18–20] in which we examined the clinical effects of the combination therapy of subcutaneous IFN-α and arterial infusion of 5-FU after palliative hepatic resection in 30 patients who had HCC associated with Vp4 and multiple intrahepatic metastases (IM3). We also investigated the correlation between response to this therapy and expression of IFNAR2.

MATERIALS AND METHODS

Patients and Selection Criteria

The current investigation was a single-arm, open-label study that was based on our previous reports.[18–20] Between October 1999 and December 2004, 30 patients with advanced HCC were enrolled. All patients had radiologically confirmed tumor thrombi in the main trunk of the portal vein (Vp4) and IM3. The diagnosis was based on liver function tests, serum α-fetoprotein (AFP), serum protein induced by vitamin K absence or antagonist-II (PIVKA-II), and imaging techniques, which included computed tomography (CT) scans, magnetic resonance imaging (MRI), hepatic angiography, and arterial portography. Consequently, these 30 patients underwent palliative reduction surgery with tumor thrombectomy in the main trunk of the portal vein to reduce tumor volume and to reopen the portal blood flow. IFN-α and 5-FU combination therapy for remnant multiple hepatomas was carried out after surgery. We used the following eligibility criteria for the selection of patients. 1) absence of extrahepatic metastases, 2) granulocyte count > 2500/μL or < 12,000/μL, 3) erythrocyte count > 8.0 g/dL, 4) platelet count > 8×10^4/μL, 5) glutamic oxaloacetic and pyruvic transaminase levels < 100 IU/L, 6) total bilirubin < 1.4 g/dL, 7) blood urea nitrogen < 30 mg/dL, 8) serum creatinine < 1.5 mg/dL, 9) successful implantation of intra-arterial catheter and drug delivery system, and 10) an Eastern Cooperative Oncology Group (ECOG) performance status from 0 to 2.[21] These eligibility criteria were based on our previous studies.[18,19] All patients signed informed consent documents that were approved by the Institutional Review Board attesting that they were aware of the investigational nature of the study and were willing to try the combination therapy.

Treatment Protocol of IFN/5-FU Combination Therapy

In each of 30 patients, an intra-arterial catheter was inserted through the gastroduodenal artery during surgery or through the subclavian or femoral artery with a subcutaneously implanted drug-delivery system.[22] Each patient received subcutaneous IFN-α (OIF; Otsuka Pharmaceutical Company, Tokushima, Japan) and an intra-arterial infusion of 5-FU (Kyowa Hakko Company, Tokyo, Japan). One cycle of treatment consisted of 4 weeks. IFN-α (5×10^6 U [5 MU]) was administered subcutaneously on Days 1, 3, and 5 of each week, resulting in a total dose of 60 MU per cycle. Continuous infusion chemotherapy (5-FU, 300 mg/m^2 per day) through the proper hepatic artery was administered in the first and second weeks through a catheter connected to a subcutaneously implanted drug-delivery system. A 2- or 3-week rest period (cessation of drug therapy) separated the treatment cycles. All anticancer therapies were discontinued when adverse effects reached level 2 of the ECOG classification[21] (with the exception of platelet and leukocyte counts < 40,000/mm^3 and < 2000/mm^3, respectively, because these parameters often were low before treatment because of associated cirrhosis).[18]

Evaluation of Response to IFN/5-FU Combination Therapy

A pretreatment evaluation was conducted at the commencement of the IFN-α/5-FU protocol, and a posttreatment evaluation was conducted after the completion of 2 cycles of treatment, almost 3 months later. The evaluation included CT or MRI studies and an assessment of changes in serum tumor markers, such as AFP and PIVKA-II. All patients had their results compared at these 2 time points for the evaluation of antitumor effects. The objective response was classified according to ECOG criteria.[21] A complete response (CR) was defined as normalization of tumor marker

levels and disappearance of all tumors and portal vein thrombosis on CT and/or MRI studies. A partial response (PR) was defined as a decrease in tumor marker levels and a decrease between 50% and 99% in 2-dimensional tumor measurement. No change (NC) represented a decrease < 50% or an increase < 25% in tumor measurements, and progressive disease (PD) represented an increase > 25%. In addition, we evaluated progression-free and overall survival rates. Follow-up was from 15 to 75 months.

Reagents and Immunohistochemistry

Rabbit polyclonal antihuman IFNAR2 antibody (OCT4813; Otsuka Pharmaceutical Company) and its blocking peptide were prepared according to the report by Novick et al.[23] The expression of IFNAR2 was examined in all 30 resected tumor samples by immunohistochemistry, which was carried out according to the method described previously by investigators in our laboratories.[19,24-26] All slides were interpreted by 1 of 2 investigators (H.W. or H.O.) in a blinded manner without knowledge of the clinical or pathologic parameters.

Statistical Analysis

The Breslow-Gehan-Wilcoxon univariate test was used to examine the possible correlations between the effect of therapy (CR/PR vs NC/PD), Child-Pugh score, serum AFP, serum PIVKA-II, Okuda score, Cancer of the Liver Italian Program (CLIP) score,[3] and the expression of IFNAR2. Survival curves were constructed using the Kaplan-Meier method. Differences in distribution between groups were compared using the chi-square test, and differences in mean values were calculated with the Student t test. All data were expressed as the mean ± standard error of the mean (SEM). A P value < .05 denoted a statistically significant difference.

RESULTS

Patient Characteristics

The preoperative clinical characteristics of the participating patients are summarized in Table 1. The median age of patients was 56 years (range, 29–71 years). AFP and/or PIVKA-II expression levels were abnormal in all 30 patients. Preoperative liver function tests (mean ± SEM values) were as follows; serum albumin, 3.6 ± 0.4 g/dL; serum total bilirubin, 1.0 ± 0.1 mg/dL; prothrombin time, 71.7% ± 12.3%; hepaplastin test, 75.4% ± 16.2%; and indocyanine green retention rate at 15 minutes, 18.5% ± 11.0%.

Clinical Response to Combination Therapy

Table 2 summarizes the operative procedure, postoperative pathologic diagnosis, and clinical response to IFN/5-FU combination therapy. All patients completed at least ≥2 cycles of IFN/5-FU combination therapy after palliative surgery. For patients who had a clinical response, we continued this combination therapy; whereas, for patients who had no response, we stopped treatment after the completion of the second cycle because of extensive progression of HCC.

At the start of IFN/5-FU treatment, all 30 patients had multiple intrahepatic lesions in the residual liver after palliative resection. The average and median tumor size of the largest nodule were 16 mm and 15 mm, respectively (n = 30 patients; range, 10–32 mm), as detected on CT or MRI studies. With regard to the clinical effect, 10 patients (33.3%) had an objective response, 6 patients (20%) had a CR, 4 patients (13.3%) had a PR, 1 patient (3.3%) had NC, and 19 patients (63.3%) had PD. With respect to the time to disease progression, the median progression-free survival was 2 months, and the 1-, 2-, and 3-year progression-free survival rates were 20%, 16%, and 0%, respectively. Furthermore, the median overall survival was 9.5 months, and the 1-, 2-, and 3-year survival rates were 40%, 28.5% and 21.4%, respectively. The median progression-free survival for patients who had a response (CR/PR; n = 10 patients) was 17.5 months, and the median progression-free survival of patients who had NC/PD (n = 20 patients) was 2 months. The 1-, 2-, and 3-year progression-free survival rates of patients who had CR/PR were 60%, 48%, and 0%, respectively; and all 3 rates were 0% for the patients who had NC/PD. The tumor burden generally was very small because of the excision of the main tumor by reduction surgery. However, there was no correlation between antitumor effect and tumor size in the remnant liver.

The median survival was 29 months for patients who achieved a CR/PR (n = 10 patients) and 6 months for patients who had NC/PD (n = 20 patients). The median follow-up of the patients who survived was 32 months. The 1-, 2-, and 3-year survival rates for patients who achieved a CR/PR were 100%, 77.8%, and 58.3%, respectively; and the rates for patients who had NC/PD were 10%, 0%, and 0%, respectively. The time to progression and overall survival curves are shown in Figures 1 and 2, respectively. There were significant differences in the time to progression and overall survival between responders (CR/PR) and nonresponders (NC/PD; $P < .0001$).

Adverse Effects

None of our patients developed side effects related to catheter insertion or subcutaneous implantation of the drug-delivery system. Grade 1 leukopenia, thrombocytopenia, or myelosuppression was ob-

TABLE 1
The Demographics of the Patients in the Current Study (n = 30)*

Patient	Age	Sex	T	M	N	Vp	IM	Stage	Alb, g/dL	T.Bil, mg/dL	PT/HPT, %	ICGR-15, %	AFP, ng/mL	PIVKA-II mAU/mL	Virus
1	51	Woman	4	0	0	4	3	IVA	2.9	1.1	69/130	9	138	49,300	—
2	49	Woman	4	0	0	4	3	IVA	4.3	1.2	92/98	16	6741	< 40	B
3	47	Man	4	0	0	4	3	IVA	3.5	0.9	73/100	11	61820	256	B
4	56	Man	4	0	0	4	3	IVA	4.3	1.2	82/92	ND	< 5	4583	B/C
5	69	Man	4	0	0	4	3	IVA	3.5	1.1	68/70	17	59	209,220	B/C
6	66	Man	4	0	0	4	3	IVA	3.7	1.1	55/63	11	555	13,257	B/C
7	65	Man	4	0	0	4	3	IVA	3.4	0.8	75/69	ND	3612	71	C
8	53	Man	4	0	0	4	3	IVA	3.9	1.2	75/70	17	97,000	1110	B
9	52	Man	4	0	0	4	3	IVA	3.6	1.1	62/72	8	15,800	6496	B
10	66	Man	4	0	0	4	3	IVA	3.6	1.1	61/67	ND	1659	181,770	C
11	39	Man	4	0	0	4	3	IVA	3.7	1.3	51/48	14	15,100	37,274	B
12	56	Man	4	0	0	4	3	IVA	4.1	0.9	67/67	1	276	921	B/C
13	53	Man	4	0	0	4	3	IVA	3.7	1.1	80/70	14	450	8365	B
14	67	Man	4	0	0	4	3	IVA	3.9	0.9	84/89	27	3366	3602	C
15	47	Man	4	0	0	4	3	IVA	4.1	0.9	71/79	3	423,300	49,392	B
16	70	Man	4	0	0	4	3	IVA	3.5	1.1	88/82	25	20	5820	C
17	70	Man	4	0	0	4	3	IVA	3.5	1.2	103/83	17	108,990	239,409	B/C
18	70	Man	4	0	0	4	3	IVA	4.1	1.1	66/68	43	198	498	B/C
19	29	Man	4	0	0	4	3	IVA	3.7	0.8	59/62	11	471,000	357,528	B
20	53	Man	4	0	0	4	3	IVA	3.3	0.9	71/70	29	113,660	205,074	B
21	42	Man	4	0	0	4	3	IVA	3.5	0.9	67/60	30	268	3023	B
22	53	Man	4	0	0	4	3	IVA	3.8	1.2	75/78	13	1710	298,176	B
23	32	Man	4	0	0	4	3	IVA	4.1	1.2	75/62	23	1,280,000	48,636	B
24	53	Man	4	0	1	4	3	IVA	3.5	1.1	97/89	20	17920	3872	C
25	54	Man	4	0	0	4	3	IVA	4.2	0.9	65/66	40	20	99	B
26	51	Woman	4	0	1	4	3	IVA	3	0.9	54/76	32	209	497	—
27	65	Man	4	0	0	4	3	IVA	3.6	1.2	65/58	7	332	7346	B
28	67	Man	4	0	0	4	3	IVA	3.8	1.1	61/54	11	330	20,256	—
29	68	Man	4	0	0	4	3	IVA	3.7	1.1	67/74	14	786	85,974	B
30	71	Man	4	0	0	4	3	IVA	2.8	0.9	74/78	37	137,300	10,200	C

T indicates tumor classification; N, lymph node status; M, metastasis; Vp, grade of portal vein thrombus; IM, intrahepatic metastases; Alb, serum albumin; T.Bil, serum total bilirubin; PT, prothrombin time; HPT, hepaplastin test; ICGR-15, indocyanine green retention rate at 15 minutes; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence; ND, not determined.
* TNM stage and the grade of portal vein thrombus were classified according to the 4th edition of the *General Rules for the Clinical and Pathological Study of Primary Liver Cancer* (Liver Cancer Study Group of Japan, 2000[3]).

served in 8 patients, but none of those episodes forced the termination of therapy or required treatment with granulocyte-colony stimulating factor. Other adverse effects, including stomatitis or diarrhea, were mostly grade 1 and clinically manageable in general. Fever was observed commonly but was controlled easily by nonsteroidal anti-inflammatory drugs before IFN injection. No depression because of IFN administration was observed in any of the 30 patients.

Correlation Between IFNAR2 Immunostaining Pattern and Prognosis

For each tissue section, the intensity of IFNAR2 immunostaining was scored on a scale from 0 to 2, in which 0 represented no or faint immunostaining, 1 represented moderate staining, and 2 indicated strong staining, based on our previous reports.[19,24,25] Table 2 shows the IFNAR2 expression level in each of the 30 patients. IFNAR2 expression was noted in 10 of 30 patients (33.3%). The median progression-free survival rate was 8 months for IFNAR2-positive patients and 2 months for IFNAR2-negative patients. The time to progression survival rates at 1, 2, and 3 years for IFNAR2-positive patients (n = 20 patients) were 30%, 24%, and 0%, respectively, and were significantly higher than the respective rates for IFNAR2-negative patients (n = 10 patients; 0% for all 3 rates; $P = .0038$) (Fig. 3A). The median overall survival rate was 16 months for IFNAR2-positive patients and 5.5 months for IFNAR2-negative patients. The overall survival rates at 1 year, 2 years, and 3 years among IFNAR2-positive patients (n = 20 patients; 60%, 42.8%, and 32.1%, respectively) were

TABLE 2
Operation, Postoperative Histology, Response to Interferon-α/5-Fluorouracil and Expression of Type I Interferon Receptor 2 in Patients With Hepatocellular Carcinoma

Patient	Operation	Histology		Response to IFN/5-FU	IFNAR2
		Cancer	Noncancer		
1	Extended left lobectomy	Ed III (poor)	Chr glisonitis	PR	1
2	Right lobectomy	Ed IV (undiff)	Normal	PD	1
3	Extended posterior segmentectomy	Ed III (poor)	B′−	PD	1
4	Extended posterior segmentectomy	Ed II (mod)	B′−	PR	1
5	Extended left lobectomy	Ed III (poor)	CIH	CR	1
6	Extended posterior segmentectomy	Ed III (poor)	B′+	CR	1
7	Left lobectomy	Ed III (poor)	B′+	PD	0
8	Right lobectomy	Ed III (poor)	B−	PD	1
9	Extended right lobectomy	Ed III (poor)	CIH	CR	1
10	Anterior segmentectomy	Ed III (poor)	B+	PD	1
11	Extended left lobectomy	Ed III (poor)	B′+	PD	1
12	Left lobectomy	Ed III (poor)	B−	PR	2
13	Left lobectomy	Ed IV (undiff)	B′−	PD	1
14	Right lobectomy	Ed III/IV (poor)	B+	PD	0
15	Extended left lobectomy	Ed III/IV (poor)	CIH	PD	0
16	Right lobectomy	Ed III/IV (poor)	CIH	CR	2
17	Right lobectomy	Ed III (poor)	CIH	CR	1
18	Right lobectomy	Ed III (poor)	CIH	PD	0
19	Extended right lobectomy	Ed III (poor)	CIH	PD	1
20	Right lobectomy	Ed III (poor)	B′−	PD	0
21	Right lobectomy	Ed III (poor)	CIH	PD	0
22	Left lobectomy	Ed III (poor)	CIH	CR	1
23	Right lobectomy	Ed III (poor)	CIH	PD	0
24	Right lobectomy	Ed III (poor)	B′−	PR	1
25	Extended left lobectomy	Ed III (poor)	B+	NC	1
26	Right lobectomy and pancreatoduodenectomy	Ed IV (undiff)	Normal	PD	0
27	Right lobectomy	Ed III (poor)	CIH	PD	0
28	Right lobectomy	Ed III/IV (poor)	B′−	PD	1
29	Right lobectomy	Ed III (poor)	CAH+	PD	1
30	Right lobectomy	Ed III (poor)	CAH+	PD	0

IFN/5-FU indicates interferon-α/5-fluorouracil; IFNAR2, type I interferon receptor 2; Ed, Edmondson grade; poor, poorly differentiated; Chr, chronic; PR, partial response; undiff, undifferentiated; PD, progressive disease; mod, moderately differentiated; CIH, chronic inactive hepatitis; CR, complete response; NC, no change; CAH, chronic active hepatitis.

significantly higher than the respective rates among IFNAR2-negative patients (n = 10 patients; 0% for all 3 rates; $P < .0025$) (Fig. 3B).

Clinical and Pathologic Correlations

Finally, we compared the responders (CR/PR; n = 10 patients) with the nonresponders (NC/PD; n = 20 patients) in terms of serum AFP (within normal range; < 5 ng/mL), serum PIVKA-II (normal range; < 45 mAU/mL), Child-Pugh scores, Okuda scores, CLIP scores, and IFNAR2 expression in univariate analysis (Table 3). Serum AFP, PIVKA-II, Child-Pugh scores, Okuda scores, and CLIP scores did not correlate with the response to combination therapy. Conversely, IFNAR2 expression correlated significantly with the response to IFN/5-FU combination therapy ($P = .0199$). Thus, the expression level of IFNAR2 was the sole factor that influenced the response to the combination therapy.

DISCUSSION

With regard to the patient selection criteria followed in the current study[27] and in our previous investigations,[18–20] we considered the presence of 3 types of advanced HCC with PVTT in the main trunk for the analysis of tumor progression (Fig. 3). The 3 types were defined as follows: type I, PVTT with multiple nodules in the bilateral lobes; type II, PVTT with a huge mass in 1 lobe and no intrahepatic metastatic nodules in the other lobe; and type III, PVTT with a huge mass in 1 lobe and multiple intrahepatic metastatic nodules in the other lobe. Patients with type I PVTT received IFN/5-FU combination treatment: An antitumor effect was noted in 43.7% of patients, and

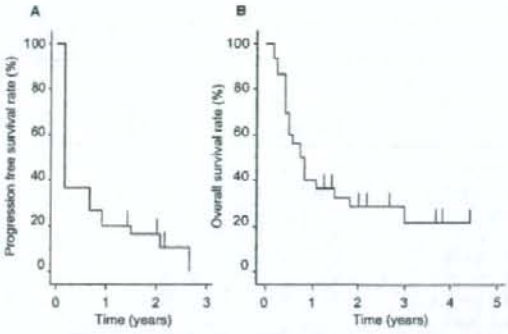

FIGURE 1. Kaplan-Meyer analysis of the efficiency of interferon-α/5-fluorouracil combination therapy. (A) The median progression-free survival was 2.0 months, and the 1-, 2-, and 3-year progression-free survival rates were 20%, 16%, and 0%, respectively. (B) The median overall survival was 9.5 months, and the 1-, 2-, and 3-year survival rates were 40%, 28.5%, and 21.4%, respectively.

a significant survival benefit was noted in 55 patients from our previous study.[19] Patients with type II PVTT underwent hepatic surgery to remove the huge mass followed by IFN/5-FU combination treatment as a postoperative adjunct. In this series, 100% survival rate at 1 year was achieved in 15 patients.[20] The patients in the current study had type III PVTT, which is considered the most advanced stage of HCC. In such patients, the main trunk of the portal vein already is packed with PVTT, and they have rapid worsening to liver failure because of the decrease in portal blood flow. These patients are prone to rupture of esophageal varices because of increased portal venous pressure. In general, most patients with advanced HCC can be treated only with best supportive care. However, for selected patients with type III PVTT who have liver function good enough to endure hepatic lobectomy, a multimodal treatment that includes surgery may be possible. Consequently, the 30 patients in the current

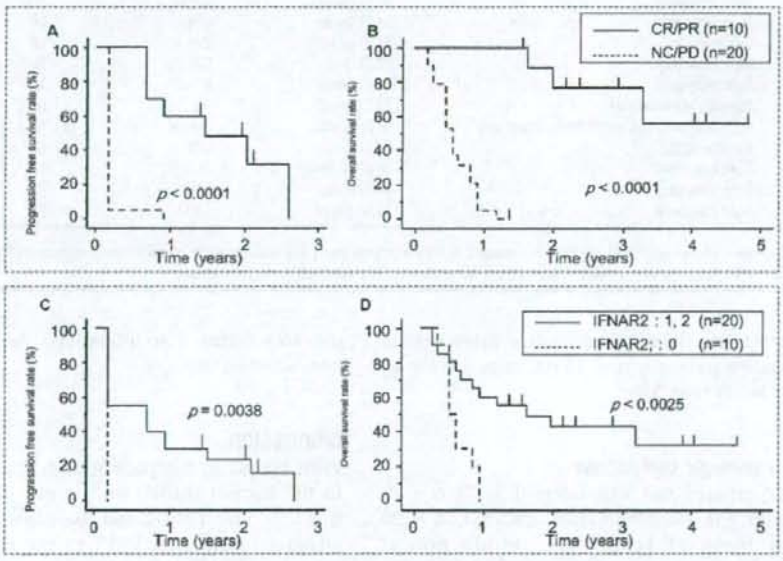

FIGURE 2. (A,B) Kaplan-Meyer analysis of the efficiency of interferon-α(IFN-α)/5-fluorouracil combination therapy. (A) The 1-, 2-, and 3-year progression-free survival rates of patients who attained a complete response/partial response (CR/PR) were 60%, 48%, and 0%, respectively; and all 3 rates were 0% for patients who had no change/progressive disease (NC/PD). (B) The 1-, 2-, and 3-year survival rates for patients who attained a CR/PR were 100%, 77.8%, and 58.3%, respectively; and the rates for patients who had NC/PD were 10%, 0%, and 0%, respectively. (C,D) Kaplan-Meyer analysis of the expression of IFN-α/type 2 interferon receptor (IFNAR2). (C) The 1-, 2-, and 3-year progression-free survival rates were 30%, 24%, and 0%, respectively, for IFNAR2-positive patients; and all 3 rates were 0% for IFNAR2-negative patients. There was a significant correlation between positive patients and negative patients ($P = .0038$). (D) The overall 1-, 2-, and 3-year survival rates for IFNAR2-positive patients (n = 20; 60%, 42.8%, and 32.1%, respectively) were significantly higher than the rates (0% for all 3 periods) for IFNAR2-negative patients (n = 10). There was a significant difference between positive patients and negative patients ($P = .0025$).

FIGURE 3. Schematic diagram of the 3 types of advanced hepatocellular carcinoma (HCC) with portal venous tumor thrombus (PVTT) in the main trunk of the portal vein according to the progression. Type I indicates PVTT with multiple nodules in both lobes; type II, PVTT with a huge mass in 1 lobe and no intrahepatic metastatic nodules in the other lobe; type III, PVTT with a huge mass in 1 lobe and multiple intrahepatic metastatic nodules in the other lobe.

study underwent palliative reduction surgery, which consisted of bisegmentectomy or trisegmentectomy with extirpation of PVTT to reduce tumor volume and to reopen the portal blood flow. IFN/5-FU combination therapy for remnant multiple hepatomas in the residual liver was carried out after surgery. With regard to these 30 patients, *none* developed any major complications, and they started the IFN/5-FU combination therapy from 3 to 5 weeks after surgery. We also demonstrated the beneficial effects of IFN/5-FU combination therapy in our patients. The efficacy of such treatment was 33.3% in our patients with highly advanced HCC. Thus, the combination treatment with IFN-α and 5-FU after hepatic palliative surgery had a marked antitumor effect with an encouraging response rate. Furthermore, the clinical response translated into survival benefits, as shown in Figures 1 and 2.

It should be noted, however, that the remaining 20 of 30 patients (66.7%) in our study did not respond to the combination therapy. Among the 20 nonresponders, there was only 1 patient with NC despite the mostly chemoresistant disease. We believe that this finding may be attributed as follows: The HCC in this series was far advanced, and HCC progression was extremely rapid and aggressive despite palliative reduction surgery. Under such conditions, almost all nonresponders died within 12 months; 12 of 20 patients (60.0%) died within 6 months. For nonresponders to this treatment, however, the survival was too short to allow the receipt of another treatment modality. Therefore, accurate prediction of chemosensitivity is desirable not only to prevent the loss of a limited chance for another possible treatment but also to avoid potentially serious side effects. However, currently, there are no suitable markers with which to distinguish between patients who are likely and patients who are unlikely to respond to this combination chemotherapy.

Several mechanisms for the anticancer effects of IFN-α, with or without 5-FU, have been proposed.[28–37]

TABLE 3
Univariate Analysis for Efficacy of Interferon-α/5-Fluorouracil Combination Therapy Based on α-Fetoprotein, Protein Induced by Vitamin K Absence, Child-Pugh Score, Cancer of the Liver Italian Program Score, and Type I Interferon Receptor 2 Expression

Characteristic	No. of patients: CR/PR (n = 10)	NC/PD (n = 20)	P
Age, y			
< 60	6	8	.9999
≥60	4	12	
Sex			
Men	10	18	.7958
Women	0	2	
Child-Pugh score			
A	7	12	.8934
B, C	3	8	
Cirrhosis			
Present	4	10	.8971
Absent	6	10	
AFP, ng/mL			
< 400	5	6	.503
≥400	5	14	
PIVKA-II, mAU/mL			
< 65	0	1	.9999
≥65	10	19	
Okuda score			
1	4	7	.7137
2-3	6	13	
CLIP score			
0-3	5	4	.2049
4-6	5	16	
IFNAR2			
Negative	0	10	.0199
Positive	10	10	

CR indicates complete response; PR, partial response; NC, no change; PD, progressive disease; AFP; α-fetoprotein, PIVKA-II; protein induced by vitamin K absence; CLIP, Cancer of the Liver Italian Program; IFNAR2, type I interferon receptor 2.

We demonstrated previously that IFN-α and 5-FU synergistically inhibited tumor cell proliferation with cell cycle arrest[38] and induced apoptosis by regulating apoptosis-related molecules.[39] We also reported that tumor necrosis factor-related apoptosis inducing ligand, its receptor pathway,[40] and Fas and the Fas-ligand pathway[41] partially contributed to the antitumor effects of IFN-α and 5-FU combination therapy. Moreover, IFN-α suppressed proliferation in all type I IFNAR2-positive HCC cell lines in vitro through mechanisms related to apoptosis or cell cycle inhibition.[42] The importance of IFNAR2 expression for the anticancer effect of IFN/5-FU was highlighted in a similar situation in our previous report.[38,39,43] These findings suggest that the antineoplastic effects of IFN-α are likely to be mediated through its high-affinity

membrane type I receptor, IFNAR2.[44] In this regard, we postulated that IFNAR2 expression in HCC tissues may be a useful predictor with which to distinguish between potential responders and nonresponders to IFN/5-FU combination therapy. On the basis of these results, we investigated the correlation between IFNAR2 expression and the effect of IFN/5-FU combination therapy using immunohistochemical analysis, and the results showed a good correlation.

Several markers for the prediction of tumor recurrence and prognosis have been identified for patients with HCC. Levy and Sherman[45] reported that the CLIP classification for HCC is easier to implement and more accurate than the Okuda classification. In addition, Koike et al.[46] suggested that the serum PIVKA-II level is the most useful clinical parameter for predicting the development of portal vein invasion. To investigate the applicability of these clinical parameters, AFP, PIVKA-II, Okuda scores, and CLIP scores were used in the current study to assess the clinical effects of IFN/5-FU combination therapy. The results indicated that expression of IFNAR2 was the only significant predictor of clinical outcome of IFN/5-FU combination therapy; and our survival analysis indicated a significant role of IFNAR2 expression on prognosis. These results suggest that the expression of IFNAR2 may be a potentially useful predictor of response to IFN/5-FU combination therapy. In our recent report using microarray analysis, several genes involved in IFN signaling transduction were identified as useful for molecular prediction of response to IFN/5-FU combination therapy.[47]

In conclusion, the current study has demonstrated the efficacy of IFN/5-FU combination therapy after surgery for patients with advanced HCC who have tumor thrombi in major branches of the portal vein. The results also indicated that the clinical response to such therapy is correlated significantly with the expression of IFNAR2 in patients with HCC.

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Case report

Complete remission of hepatocellular carcinoma with portal vein tumor thrombus and lymph node metastases by arterial infusion of 5-fluorouracil and interferon-α combination therapy following hepatic resection

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We report two cases of hepatocellular carcinoma (HCC) with portal vein tumor thrombus (PVTT) and lymph node (LN) metastases successfully treated by hepatic arterial infusion of 5-fluorouracil (5-FU) combined with systemic injection of interferon (IFN)-α following hepatic resection for the liver tumor. Complete remission was obtained. Case 1 was a 51-year-old man who had HCC in the right lobe of the liver with PVTT and multiple intrahepatic metastases. He also had abdominal and mediastinal LN metastases. Case 2 was a 53-year-old man who had diffuse-type HCC in the right lobe of the liver with PVTT and intrahepatic metastases. A chest computed tomography scan revealed lymph nodes enlarged to 1.0 cm from the mediastinum to the left supraclavicular space. Both patients underwent the hepatectomy to reduce the tumor volumes and remove the PVTT to relieve portal vein obstruction. Following the surgery, the patients underwent IFN-α/5-FU combination therapy. Three months after this combined therapy, tumor markers (both α-fetoprotein and protein induced by vitamin K absence or antagonist II) returned to the normal range and residual tumors in the liver disappeared. The patients are alive without any recurrence more than 1 year after initial treatment. IFN-α/5-FU combined therapy following hepatic resection is a promising modality for the treatment of advanced HCC with LN metastasis.

Key words: hepatocellular carcinoma, extrahepatic metastasis, lymph node metastasis, chemotherapy, interferon

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and the fifth leading cause of cancer-related death.[1] In recent years, the development of a diagnostic modality has brought about earlier diagnosis of small HCC, and the introduction of new therapeutic modalities, such as microwave coagulation therapy and radio frequency ablation therapy, has produced various options for the treatment of small HCC.[2,3] However, the overall prognosis of HCC is still poor, since half of HCC patients have a portal vein tumor thrombus (PVTT) or intrahepatic metastasis at the time of diagnosis. Tumor progression into the major branch of the portal vein is commonly considered as the most advanced and noncurable stage,[4] and HCC with lymph node metastasis is usually considered to be a rare and far advanced stage.[5] In fact, the prognosis of HCC patients with tumor thrombus in the main portal branch (PVTT) is very poor and a standard treatment regimen for HCC with PVTT has not yet been established.[6,7] We previously reported excellent efficiency of arterial infusion of 5-fluorouracil (5-FU) and interferon (IFN)-α combination therapy for HCC with PVTT.[8,9] The eligibility criteria for this combination therapy are histologically or radiologically confirmed HCC, the presence of a tumor thrombus that has invaded at least one of the main branches of the portal vein, and the absence of hematological extrahepatic metastasis.

In this paper, we report two cases of HCC patients with PVTT and distant lymph node metastases successfully treated by 5-FU/IFN-α combined therapy following hepatic resection. This is the first report showing effective chemotherapy for patients with HCC with PVTT and extrahepatic lymph node metastasis.

Case reports

Case 1

A 51-year-old man complaining of acute upper right abdominal pain visited his neighborhood hospital, and his illness was diagnosed as chronic hepatitis due to infection with hepatitis C virus and HCC with PVTT. He was referred to our hospital for treatment of the disease. From abdominal computed tomography (CT), an early enhanced lesion (6.0 cm in size) with a nonenhanced area inside was recognized in the right lobe of the liver with multiple intrahepatic metastases around the main tumor, and PVTT was developing from the anterior and posterior branch into the main portal trunk (Fig. 1A). Furthermore, the paraaortic lymph node was enlarged to 2.5 cm (Fig. 1B). A chest CT scan showed the subclavicular lymph node enlarged to 2.0 cm (Fig. 1C). We diagnosed the disease as huge HCC with PVTT in the right lobe of the liver and extrahepatic lymph node metastases.

Laboratory data were as follows: total bilirubin, 0.7 mg/dl; aspartate aminotransferase (AST), 22 U/l; alanine aminotransferase (ALT), 21 U/l; prothrombin time, 64%; indocyanine green 15 min retention test (ICG-R15), 16.0% (normal value, <10%), α-fetoprotein (AFP), 28 ng/ml; protein induced by vitamin K absence or antagonist II (PIVKA-II), 1847 mAU/l; and albumin, 3.1 g/dl.

To improve the long-term outcome, we performed volume reduction surgery for the hepatic tumor and paraaortic lymph node metastasis and removed the PVTT to relieve portal vein occlusion. Histological examinations showed that the swelling lymph node and main tumor in the liver were HCC (Fig. 2). Furthermore, we investigated the immunohistochemical expression of type I interferon receptor (IFNAR2) in the primary liver tumor, PVTT, and metastatic lymph node. The immunohistochemical procedure was performed using an EnVision+ peroxidase kit (Dako, Glostrup, Denmark) as previously described.[10,11] Briefly, formalin-fixed paraffin-embedded sections were used. After deparaffinization and rehydration, the sections were treated for antigen retrieval and blocked endogenous peroxidase. Primary antibody, rabbit anti-IFNAR2 (Otsuka Pharmaceutical, Tokushima, Japan, diluted 1:60) was applied to slides and incubated overnight at 4°C. Immunostaining was performed according to the instructions supplied by the manufacturer. For evaluation of immunostaining, the bile duct epithelium expresses moderate levels of IFNAR2, and these levels of staining were used as an endogenous positive control within the

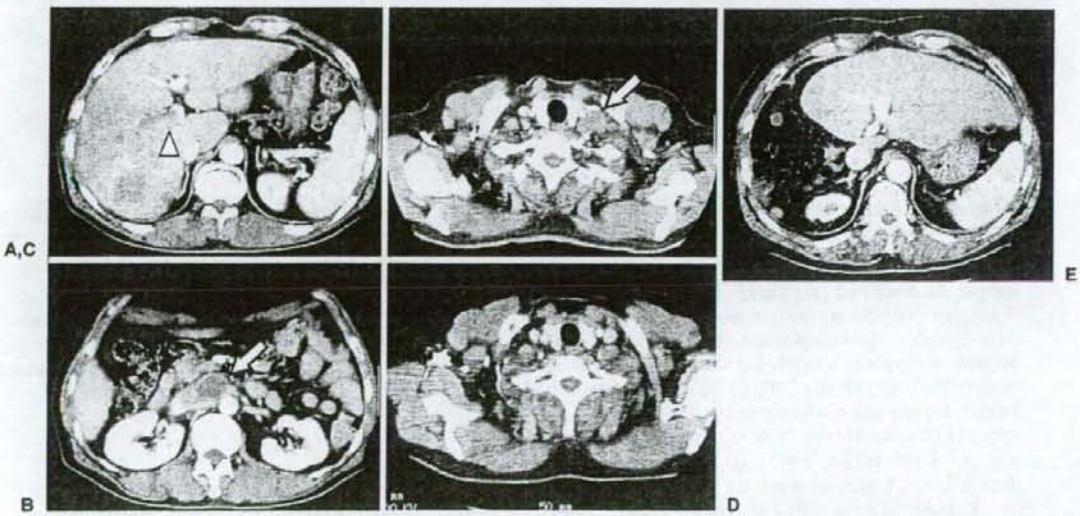

Fig. 1A–E. Computed tomography (CT) of case 1. **A** The main tumor, 6.0 cm in size, is located in the right lobe of the liver, and the tumor thrombus has invaded the right main branch of the portal vein (*arrowhead*). **B** Paraaortic lymph node enlarged, 2.5 cm in size (*white arrow*). **C** Chest CT scan shows an enlarged subclavicular lymph node to 2.0 cm (*white arrow*). **D** After three cycles of interferon-α/5-fluorouracil combination therapy, the subclavicular lymph node had vanished. **E** There was no residual tumor or recurrence in the remnant liver 56 months after the first treatment

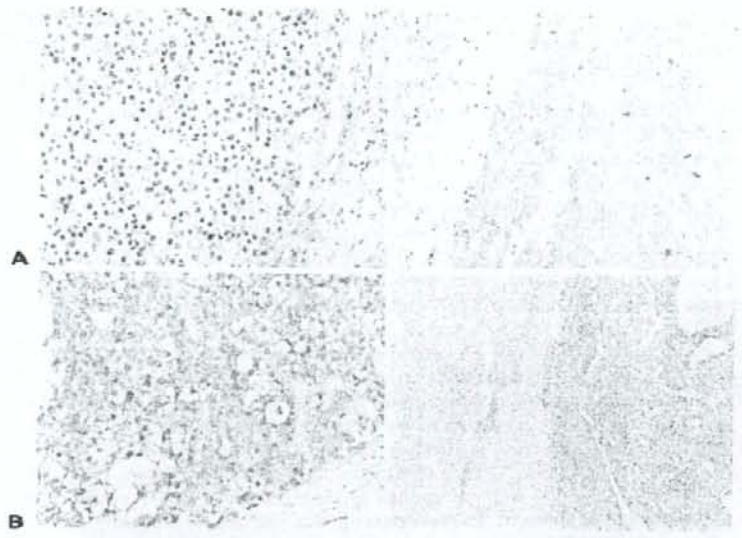

Fig. 2. A Microscopic finding of a metastatic lymph node of case 1 showed moderately differentiated hepatocellular carcinoma (hematoxylin and eosin, ×200). **B, C** Immunohistochemical staining for type I interferon receptor (IFNAR2) in the metastatic lymph node of case 1 (**B**) and in the main tumor of case 2 (**C**) showed strong expression of IFNAR2 (×200). **D** Microscopic finding of the main tumor in the liver showed poorly differentiated hepatocellular carcinoma (hematoxylin and eosin, ×100)

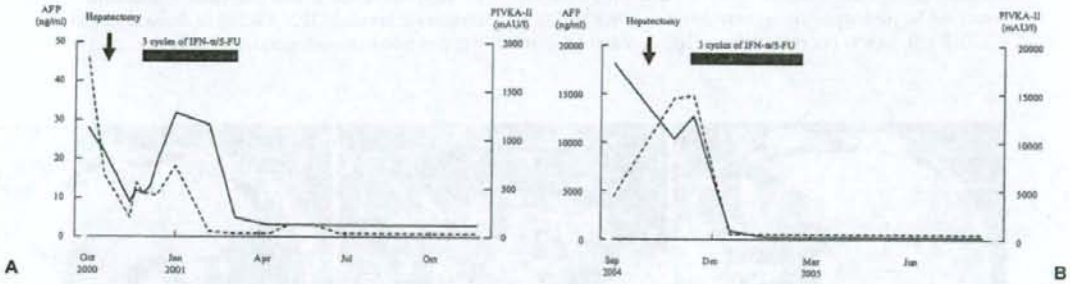

Fig. 3A,B. Clinical courses of cases 1 (**A**) and 2 (**B**). Serum α fetoprotein (*AFP*) and protein induced by vitamin K absence or antagonist II (*PIVKA-II*) are indicated by the *solid line* and *dashed line*, respectively. IFN-α/5-FU, interferon-α/5-fluorouracil combination therapy

sample, as described previously. The expression of IFNAR2 in the primary liver tumor, PVTT, and metastatic lymph node were positive (Fig. 2). Following the surgery, the patient was treated with subcutaneous administration of IFN-α (OIF; Otsuka Pharmaceutical, Tokyo, Japan) and continuous hepatic arterial infusion of 5-FU (Kyowa Hakko, Tokyo, Japan). The regimen was as follows: IFN-α (5×10^6 U) was administered on days 1, 3, and 5 of every week for 4 weeks and continuous hepatic arterial infusion chemotherapy (5-FU, 300 mg/m^2) was performed every 2 weeks for 4 weeks via a catheter connected to a subcutaneously implanted drug delivery system. Three months after the initial chemotherapy, tumor markers (both AFP and PIVKA-II) returned to normal range (Fig. 3A) and a CT scan showed that the subclavicular lymph node had disappeared (Fig. 1D). The patient still lives without any residual tumor or recurrence in either the liver or the extrahepatic sites, 56 months after the first treatment (Fig. 1E).

Case 2

A 53-year-old man had been regularly followed up for chronic hepatitis C at a local hospital. Regular follow-up abdominal CT revealed a single mass, 1.0 cm in diameter, in the right posterior superior segment (segment 7) of the liver in July 2003. He was diagnosed as having solitary HCC, and percutaneous ethanol injection therapy (PEIT) and transcatheter arterial embolization

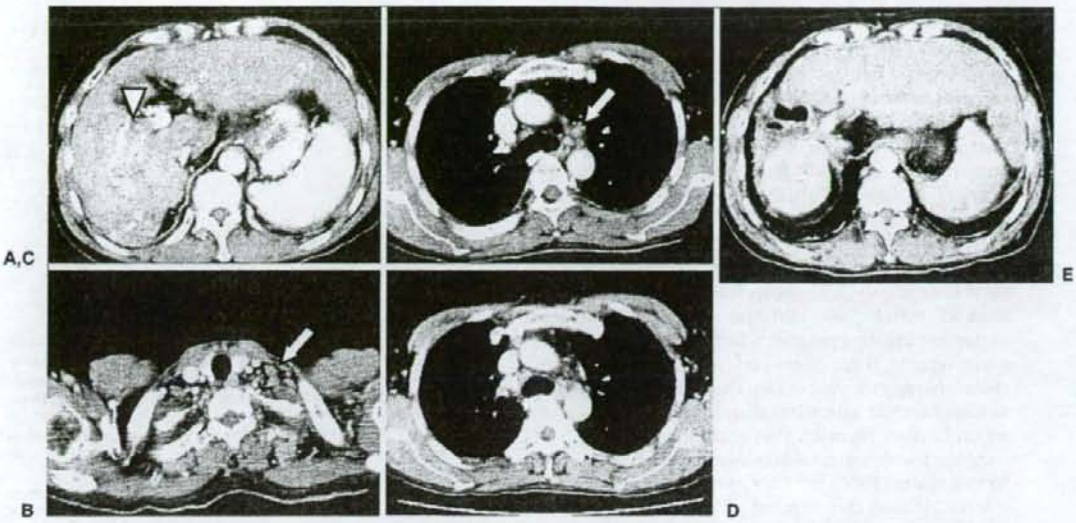

Fig. 4A–E. CT of case 2. **A** The main tumor is an early enhanced area occupying the whole right lobe of the liver, and the tumor thrombus has invaded the right main branch of the portal vein (*arrowhead*). **B** Cervical CT scan showing the enlarged supraclavicular lymph node (*white arrow*). **C** Chest CT scan shows the enlarged mediastinum lymph nodes (*white arrow*). **D** After three cycles of interferon-α/5-fluorouracil combination therapy, the mediastinum lymph nodes had diminished in size. **E** Residual tumors in the left lobe of the liver disappeared and there was no recurrence in the liver 12 months after hepatectomy

(TAE) was performed. Thirteen months after the treatment, CT scan and angiography showed a huge mass with PVTT extending to the portal trunk in the right lobe of the liver and four intrahepatic metastases in the left lobe of the liver. After receiving TAE for the intrahepatic metastases in the left lobe of the liver from the left hepatic artery and transcatheter arterial infusion (TAI) therapy with cisplatin (30 mg), mitomycin (6 mg), and daunorubicin (20 mg) from the right hepatic artery, he was referred to our hospital for further treatment. On admission, abdominal CT 1 month after TAE and TAI showed a dense accumulation of iodized oil in the intrahepatic metastases in the left lobe and an early enhanced area in the whole right lobe of the liver (Fig. 4A). The PVTT had developed from both the right anterior and posterior branches into the main trunk of the portal vein. Chest CT scan revealed enlarged lymph nodes, 1.0 cm in size, from the mediastinum to the left supraclavicular space (Fig. 4B, C).

Laboratory data were as follows: total bilirubin, 1.0 mg/dl; AST, 42 U/l; ALT, 28 U/l; prothrombin time, 59%; ICG-R15, 20%; AFP, 17920 ng/ml; PIVKA-II, 3873 mAU/ml; and albumin, 3.5 g/dl.

We performed a right lobectomy of the liver to reduce the tumor volumes and removed the PVTT to relieve portal vein obstruction. Following the surgery, the patient was treated with subcutaneous administration of IFN-α and continuous hepatic arterial infusion chemotherapy of 5-FU. The patient underwent open biopsy of the enlarged supraclavicular lymph node 1 month after the surgery, and we histologically confirmed that the swelling lymph node was metastasis from HCC. Immunohistochemical examination showed positive expression of IFNAR2 in the primary liver tumor, PVTT, and metastatic lymph node (Fig. 2). Three months after the initial chemotherapy, tumor markers (both AFP and PIVKA-II) returned to normal range (Fig. 3B), and a CT scan showed that residual tumors in the left lobe of the liver had disappeared and that the enlarged lymph nodes in the mediastinum had diminished in size (Fig. 4D). Twelve months after the surgery, the patient is still alive without tumor recurrence (Fig. 4E).

Discussion

The incidence of lymph node (LN) metastasis in HCC is generally rarer than that in other cancers. The incidence of LN metastasis from HCC is reported to be 25%–42% at autopsy.[12-14] In clinical series, the incidence rate of LN metastasis from HCC is lower than at autopsy, because LN dissection is not routinely performed during hepatectomy for patients with HCC, and LN

metastases occur in patients with far advanced and unresectable HCC. The prognosis of HCC with LN metastases is extremely poor. The cause of death with LN metastases from HCC is usually liver failure due to the progression of liver tumors.[15] Uenishi et al.[5] reported that six HCC patients with LN metastasis at primary surgery died within 424 days owing to tumor progression or liver failure. Furthermore, all patients had intrahepatic metastases, and five had PVTT.

Our two patients had PVTT. The prognosis of HCC patients with PVTT is also extremely poor: median survival time is only 2.7 months.[16] Furthermore, the presence of extrahepatic metastasis is one of the most important negative prognosis factors. According to previous reports, there is no survival benefit of systemic chemotherapy for patients with advanced HCC, and the median survival time is less than 4 months.[17] Previously, we and others reported that chemotherapy with 5-FU combined with systemic administration of INF-α is effective against HCC.[8,11,18] Our recent report showed that arterial infusion chemotherapy combined with systemic administration of IFN-α is very effective for unresectable HCC with PVTT, and the median survival time of the patients who received this combined therapy was 10.2 months.[11] Furthermore, we recently reported a clinical trial in which hepatic resection followed by IFN-α and 5-FU improved the prognosis of HCC patients with PVTT compared with surgery alone.[19] Therefore, in our two cases, we determined that the hepatic arterial infusion of 5-FU combined with systemic administration of IFN-α should be administered as adjuvant treatment after hepatectomy.

Yatsuhashi et al.[20] showed that efficacy of IFN therapy is related to the expression of IFNAR2. In transfected cancer cell lines showing enhanced expression of IFNAR2, the antiproliferative effects of IFN were markedly increased.[21] In a clinical trial of IFN-α/5-FU combined therapy for 55 patients with unresectable HCC with PVTT, we revealed that the efficacy of this combined therapy is significantly related to the expression of IFNAR2.[11] Therefore, we investigated the immunohistochemical expression of IFNAR2 in the primary liver tumor, PVTT, and metastatic lymph node of both these cases. In both cases, the expression of IFNAR2 in the primary liver tumor, PVTT, and metastatic lymph node were positive. These results suggest that IFN-α/5-FU combined therapy is effective not only against the primary liver tumor but also against LN metastasis in which IFNAR2 is expressed.

Generally, lymph node metastasis is considered to be spread hematogenously to distant organs, similar to metastasis to other organs.[22] In this regard, IFN/5-FU therapy may be effective against systemic circulating hematogenously spread metastasis. This suggests that IFN/5-FU might be a useful adjuvant therapy not only following hepatic resection in HCC with good liver function, but also following hepatic transplantation in HCC with poor liver function.

In conclusion, IFN-α/5-FU combined therapy following hepatic surgery may be a promising modality for advanced HCC with PVTT, even with lymph node metastasis.

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