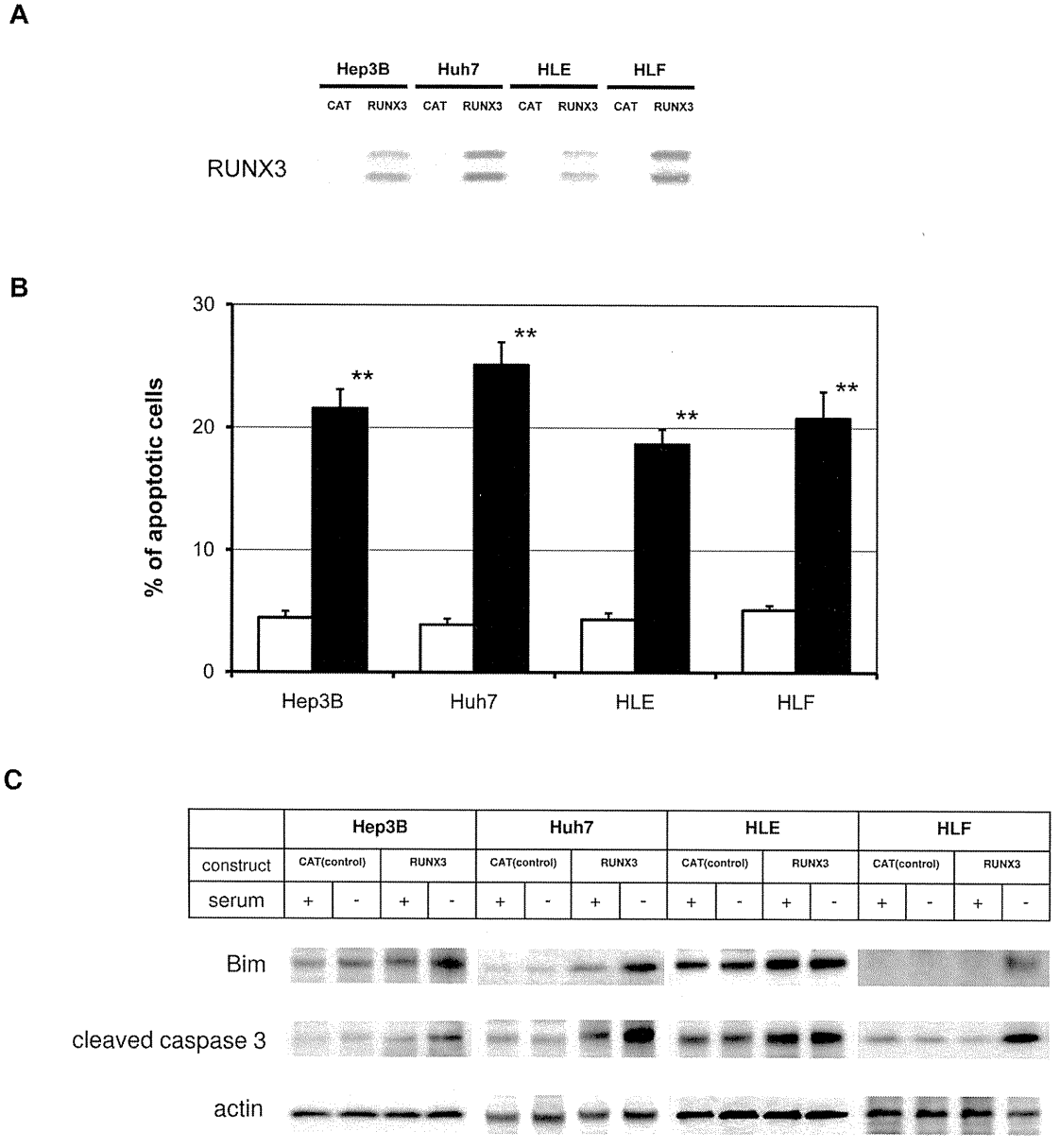


**Figure 6 Effect of transient ectopic RUNX3 expression**. CAT (control) and RUNX3 constructs were introduced into HCC cell lines. After a 48-h incubation period, an immunoblot analysis for RUNX3 expression (A), a DAPI apoptosis detection assay (B), and an immunoblot analysis for Bim and caspase (C) were performed. Shown here are representative blots from more than three independent experiments. All results are expressed as ratios to control CAT-transfected cells (CAT; white bars, RUNX3; black bars). Data represent the mean ± S.E. of more than five independent experiments, each with triplicates. **, $P < 0.01$ (vs. data at 0 h); Student's *t*-test.

reported [23-26]. However, little is known about the role of RUNX3 in HCC tumor suppression. We hypothesized that loss of RUNX3 expression contributes the development of HCC by escaping apoptosis. The results of the present study provide clear evidence that RUNX3 elicits serum starvation-induced apoptosis in HCC cells by activating the Bim-caspase pathway.

Stable expression of RUNX3 protein was established in Hep3B cells (Figure 3A), and they showed apoptosis under serum starved conditions (Figure 3B). This effect was reproducible in the Hep3B, Huh7, HLE, and HLF HCC cell lines transiently expressing RUNX3. The inhibition of cell growth in transient RUNX3-expressing cells was generally lower than that in stable RUNX3-

expressing Hep3B cells, probably due to low transfection efficiency.

Serum starvation-induced apoptosis is caused by caspase activation in ectopic RUNX3-expressing Hep3B cells (Figures 3C and 3D). To explore the signaling molecule responsible for apoptosis, Bim protein expression was induced in serum starved RUNX3-expressing Hep3B cells (Figure 4A). This is the first report demonstrating that RUNX3 enhances Bim expression under serum starved conditions in HCC cells, which appears to be consistent with the important role of Bim in previous studies on other types of cells. Bim expression was induced by the cooperation of RUNX3 and TGF-β in a study of gastric epithelial cells [21,31]. Bim protein also plays an important role in cell death [32]. Bim induces sequential activation of caspase-9 and -3 [32]. The potency of Bim as a cell death inducer is attenuated by Bax and Bcl-2 subfamily proteins [33]. The expression of Bax and Bcl was not affected by RUNX3 expression (Figure 4A). The expression of Bad (data not shown), a Bcl-2 antagonist known as a serum starvation-induced apoptosis initiator [34], increased with serum starvation but was not attenuated by RUNX3 expression (Figure 4A). Bim siRNA was used to evaluate whether Bim expression regulates serum starvation-induced apoptosis in RUNX3-expressing cells. As a result, Bim siRNA successfully knocked down Bim expression in RUNX3-expressing Hep3B cells (Figure 5A). Knockdown of Bim expression abrogated serum starvation-induced apoptosis in RUNX3-expressing Hep3B cells (Figure 5B). Consequently, RUNX3 expression enhanced serum starvation-induced apoptosis through the Bim-caspase pathway in Hep3B cells. This effect was reproducible in the Huh7, HLE, and HLF HCC cell lines transiently expressing RUNX3 (Figure 6).

Serum starvation triggered apoptosis in RUNX3-expressing HCC cells. As this leads to the question of how serum prevents apoptosis in RUNX3-expressing cells, RUNX3-expressing Hep3B cells were treated with TGF-α, EGF, or PDGF (Figure 4C). These growth factors reduced apoptosis in RUNX3-expressing Hep3B cells by activating the PI3/Akt signaling pathway (data not shown), which is consistent with a previous report [34].

RUNX3 induces apoptosis in the presence of TGF-β [21]. In a study of gastric epithelial cells, RUNX3 enhanced Bim expression during TGF-β-induced apoptosis [21,31]. In a study of a gastric and esophageal cancer cell lines, RUNX3 expression made cancer cells sensitive to TGF-β-induced apoptosis [21,35-38]. These reports suggest that TGF-β is required for RUNX3-related apoptosis. In the present study, ectopic RUNX3 expression enhanced serum starvation-induced apoptosis in the absence of TGF-β. This discrepancy may be explained by the autocrine action of TGF-β in Hep3B cells, which have an intact TGF-β signaling pathway [39]. Furthermore, some HCC cell lines, including Hep3B, produce TGF-β [40]. Further study is required to establish whether TGF-β is involved in the enhanced apoptosis of HCC.

It has been reported that p53, Rb, p16, phosphatase, and tensin homolog (PTEN) are altered in HCC. The p53 gene is the most extensively studied gene of solid tumors. Alteration of this gene occurs at a relative low frequency (28-42%) in HCC compared to other solid tumors [11,17,41,42]. The Rb gene is another well-studied tumor suppressor gene in HCC and other solid tumors. Rb mutations are found in only 15% of HCCs [42]. The LOH of chromosome 13q, where Rb gene is located, is more frequent in HCC (25-48%) [43,44]. The p16 gene, also known as the cyclin-dependent kinase inhibitor 2A gene, regulates the Rb pathway and is found in 64% of HCCs [9]. PTEN negatively regulates the PI3K/Akt signaling pathway, which is involved in the regulation of cell survival [45]. Alteration of PTEN was found in ~40% of HCCs [10]. The frequency of alteration of each individual gene was relatively low, while RUNX3 expression was frequently down-regulated in both human HCC cell lines (91%) and tissues (90%).

Alterations in some tumor suppressor genes are due to LOH in HCC [17]. Similar to other tumor suppressor genes, some of the alterations in RUNX3 are due to the LOH of chromosome 1p36, where RUNX3 is located. Perhaps another mechanism for RUNX3 down-regulation is hypermethylation of the RUNX3 promoter region [13-16]. In a previous report, 30-40% of HCCs showed LOH of the RUNX3 gene and 40-80% showed promoter hypermethylation [28]. In agreement with these reports, RUNX3 down-regulation was detected in ~90% of HCC tissue specimens.

## Conclusions

RUNX3 expression elicits serum starvation-induced apoptosis in HCC cells via the Bim-caspase pathway. Because RUNX3 expression is generally suppressed in HCC cell lines and tissues, loss of RUNX3 expression leads to tumorigenesis by escaping apoptosis.

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#### Author details

[1]Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. [2]Department of Molecular Hepatology, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. [3]Department of Gastroenterological Surgery, Transplant, and Surgical Oncology, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan.

**Authors' contributions**

HS conceived the design and drafted the manuscript. YN performed experiments. NT, ST, SN, MU, MM, MI and AT helped performing experiments for YN. SN, YK, KN, KK, HH, JT, HO and TY contributed for the collection of HCC tissues. YN performed immunohistochemical study. KY provides financial supports and participates in the discussion of the results. All authors read and approved the final manusctipt.

**Competing interests**

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

# Effect of Previous Interferon Treatment on Outcome After Curative Treatment for Hepatitis C Virus-Related Hepatocellular Carcinoma

**Hirokazu Miyatake · Yoshiyuki Kobayashi · Yoshiaki Iwasaki · Shin-ichiro Nakamura · Hideki Ohnishi · Kenji Kuwaki · Junichi Toshimori · Hiroaki Hagihara · Kazuhiro Nouso · Kazuhide Yamamoto**

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**Abstract**
*Background and Aims* Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) prevents the development of hepatocellular carcinoma (HCC). The purpose of this study was to clarify the effect of previous IFN treatment before the development of HCC on recurrence and survival in HCV-related HCC patients.
*Methods* Three hundred ninety-five patients who underwent curative treatment for HCV-related HCC were enrolled. Of these, 124 had received IFN treatment before the development of HCC (17 achieved sustained virological response [SVR group] and 107 did not [non-SVR group]), whereas 271 patients had never received IFN treatment (IFN-untreated group). The first and second recurrence and survival rates in these patient groups were statistically analyzed.
*Results* The first HCC recurrence rate was similar among patient groups. In contrast, the second HCC recurrence rate was significantly lower in the SVR group than in the non-SVR group ($p = 0.003$) and the IFN-untreated group ($p = 0.006$). In multivariate analysis, platelet count ($p = 0.033$) and number of tumors ($p = 0.001$) were associated with the first HCC recurrence, while SVR ($p = 0.002$) was the only factor associated with the second HCC recurrence. The survival rate was higher in the SVR group than in non-SVR and IFN-untreated groups, and SVR to previous IFN treatment was an independent factor associated with better survival ($p < 0.001$).
*Conclusions* SVR to previous IFN treatment before the development of HCV-related HCC was associated with lower risk of the second recurrence of HCC and better survival.

**Keywords** Hepatocellular carcinoma · Hepatitis C virus · Previous interferon therapy · Recurrence · Survival

## Introduction

Chronic hepatitis and cirrhosis following hepatitis C virus (HCV) infection are major risk factors for hepatocellular carcinoma (HCC) [1–3]. Particular risk factors for developing HCV-related HCC in patients are advanced stage fibrosis, male gender, older age, heavy drinking, and high serum alanine aminotransferase (ALT) levels [4, 5]. Interferon (IFN) therapy improves hepatic inflammation and inhibits the progression of hepatic fibrosis [6]. Furthermore, treating patients with IFN with chronic HCV infection can prevent the development of HCC, particularly in patients with sustained virological response (SVR) to IFN therapy [7–13]. In contrast, HCC is liable to frequently recur even after curative therapy primarily because of its multicentric occurrence, leading to a poor prognosis [14–19]. The recurrence rate after resection of HCV-related HCC is higher in patients with HCV viremia than in those without it [20]. It has been reported that IFN therapy after resection or ablation of HCC reduces recurrence and improves prognosis in patients with HCV-related HCC [21–28]. However, no complete investigation has been performed of the possible effect of IFN therapy before HCC development on the outcome of curative treatment for

H. Miyatake · Y. Kobayashi · Y. Iwasaki (✉) · S. Nakamura · H. Ohnishi · K. Kuwaki · J. Toshimori · H. Hagihara · K. Nouso · K. Yamamoto
Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama 700-8558, Japan
e-mail: yiwasaki@cc.okayama-u.ac.jp

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HCV-related HCC particularly in relation to the response to IFN treatment. Only a few relevant studies involving limited number of patients with previous IFN therapy are available [29–32].

The purpose of this study was to clarify the effect of previous IFN treatment before the development of HCV-related HCC on recurrence and prognosis after curative treatment of HCC in a large cohort of patients.

## Patients and Methods

### Patients

Between 1995 and 2006, 733 consecutive patients with HCC positive for HCV antibody and HCV RNA were diagnosed at Okayama University Hospital. Three hundred thirty-eight patients who did not receive curative treatment for HCC or undergo IFN therapy after the development of HCC were excluded from the study (Fig. 1). Inclusion criteria were as follows: (1) no evidence of HCC before consulting the Okayama University Hospital, (2) absence of hepatitis B surface antigen, (3) absence of co-existing liver diseases such as autoimmune hepatitis or primary biliary cirrhosis, and (4) absence of a history of alcohol abuse.

HCV infection was diagnosed on the basis of identification of anti-HCV antibodies using the first, second, or third enzyme-linked immunosorbent assays (Ortho Diagnostics, Tokyo, Japan). HCV RNA was identified by reverse transcription-polymerase chain reaction (RT-PCR) [33].

HCC was suspected on the basis of several imaging methods, including abdominal ultrasonography (US), dynamic computed tomography (CT), magnetic resonance imaging, and angiography. Diagnosis of HCC was confirmed by needle biopsy, by surgically resected tumor specimens, or by typical radiological findings on hepatic angiography or dynamic CT.

The study was conducted in accordance with the Helsinki Declaration and approved by the Ethical Committee of the institution.

### Treatment

Of the 395 patients receiving curative treatment of HCC, 103 were treated with surgical resection and 292 with percutaneous tumor ablation (PTA) [34–37], that is, percutaneous ethanol injection therapy (PEIT) ($n = 116$), percutaneous microwave coagulation therapy (PMCT) ($n = 11$), or radiofrequency ablation (RFA) ($n = 165$). There were no patients who underwent liver transplantation or other modes of HCC treatment. The choice between surgical resection and PTA were determined according to the extent of tumor and hepatic functional reserve as assessed by Child's classification [38]. If the liver tumor consisted of fewer than three nodules that were less than 3 cm in diameter, patients were indicated

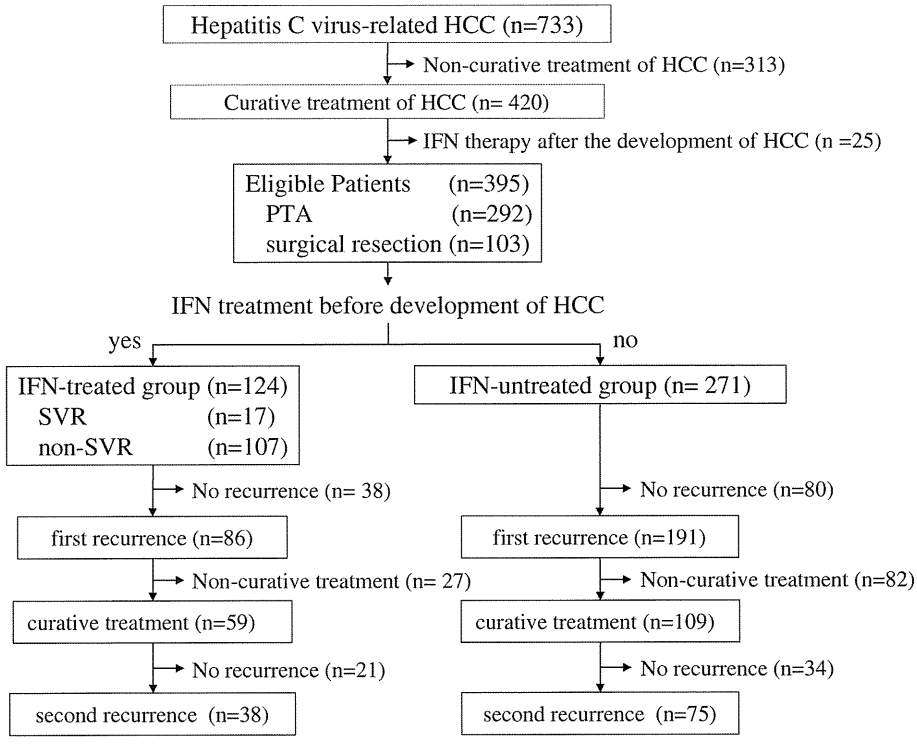


**Fig. 1** Schematic presentation of patients with HCV-related hepatocellular carcinoma (HCC). Patients with HCV-related HCC who were diagnosed at Okayama University Hospital were classified into three groups according to their previous IFN treatment and response to that treatment. One hundred twenty-four patients had received IFN treatment before the development of HCC (IFN-treated group) and the remaining 271 had not (IFN-untreated group). Patients who had undergone IFN treatment before the development of HCC were further classified according to their response into a sustained virological response (SVR) group or a non-SVR group. Patients were regularly screened for HCC

for PTA. When a patient was indicated for both surgery and PTA, the modality of treatment was determined by patient choice after obtaining fully informed consent. PEIT was carried out under US guidance using a 15- or 20-cm-long needle (21 gauge) (Hakko, Chikuma, Japan) [35], PMCT was performed under US guidance using a 15-cm-long guide needle (14 gauge) according to the procedure described previously [37], and RFA was executed under US guidance using a 15- or 20-cm-long guide needle (16 gauge) (Tyco Healthcare Japan, Tokyo, Japan) [36]. PTA was repeated until complete necrosis of all HCC lesions was confirmed by dynamic CT. Treatment of HCC was considered curative, when no viable HCC lesions were detected on dynamic CT 3 months after completion of the treatment.

Of the 395 patients receiving curative treatment for HCC, 124 had received either human lymphoblastoid IFN, recombinant IFN-alpha 2a, or recombinant IFN-alpha 2b monotherapy for chronic HCV infection before the development of HCC (IFN-treated group), whereas 271 had not (IFN-untreated group) (Fig. 1). Patients received 6 million units of IFN by intramuscular injection three times weekly for 24 weeks as outpatients. If patients could not tolerate this dose, the IFN dose was reduced to 3 million units. SVR was defined as HCV RNA (as determined by RT-PCR; detection limit, $10^2$ copies/ml) negativity for over 6 months after the termination of IFN therapy. SVR was achieved in 17 of the 124 patients (SVR group) and the remaining 107 were regarded as non-SVR (non-SVR group) (Fig. 1).

## Follow-up of Patients

Patients attended a monthly medical consultation at the Okayama University Hospital outpatient clinic. Blood biochemical markers, including α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), were measured every 1–2 months; US was performed every 2–3 months, and dynamic CT was performed every 6 months. If HCC recurrence was suspected, further imaging examinations including dynamic CT, magnetic resonance imaging, abdominal angiography, or US-guided tumor biopsy were performed to confirm the diagnosis.

New HCC foci as well as local recurrent nodules at tumor, node, metastasis (TNM) stage I, II, and III, were mainly treated by a second course of PTA; local recurrent nodules at TNM stage IV were treated with transarterial chemoembolization or chemotherapy. Further development of HCC and survival of patients (tumor recurrence rate and survival rate) were analyzed in relation to the time interval after treatment of HCC.

**Table 1** Demographic and clinical characteristics of patients with HCV-related HCC

| Groups | IFN-treated | | IFN-untreated ($n$ = 271) | $p^a$ | $p^b$ |
|---|---|---|---|---|---|
| | SVR ($n$ = 17) | Non-SVR ($n$ = 107) | | | |
| Characteristics | | | | | |
| Sex (men/women), $n$ | 13/4 | 60/47 | 187/84 | 0.049 | 0.112 |
| Age (years) | 63 (52–71) | 65 (46–82) | 67 (33–85) | 0.018 | 0.061 |
| Laboratory data | | | | | |
| Total bilirubin (mg/dl) | 0.74 (0.40–1.29) | 0.85 (0.36–3.28) | 0.91 (0.16–4.13) | 0.194 | 0.171 |
| Albumin (g/dl) | 4.4 (3.7–4.8) | 3.7 (2.5–4.8) | 3.6 (2.2–4.7) | <0.001 | <0.001 |
| Prothrombin time (%) | 93 (70–121) | 85 (47–142) | 85 (40–145) | 0.355 | 0.023 |
| ALT (IU/l) | 22 (10–54) | 55 (12–198) | 60 (14–201) | 0.058 | <0.001 |
| Platelet count ($\times 10^4/\mu l$) | 16.6 (8.4–30.3) | 9.2 (2.8–37.2) | 10.1 (3.2–31.9) | 0.980 | <0.001 |
| Child–Pugh stage (A/B/C), $n$ | 17/0/0 | 87/20/0 | 213/54/4 | 0.236 | 0.049 |
| Tumor-related variables | | | | | |
| Number of tumors (single/multiple), $n$ | 15/2 | 76/31 | 192/79 | 0.603 | 0.136 |
| Size of largest tumor (mm) | 20 (8–40) | 18 (10–53) | 20 (9–74) | 0.033 | 0.942 |
| AFP (ng/ml) | 13 (1.9–25,716) | 24 (1.7–3,480) | 20 (0.6–54,535) | 0.956 | 0.297 |
| DCP (mAU/ml) | 34 (1–35,000) | 46 (10–56,000) | 46 (1–66,700) | 0.294 | 0.195 |
| Initial treatment of HCC | | | | | |
| PTA/surgical resection, $n$ | 6/11 | 79/28 | 207/64 | 0.100 | 0.002 |

Laboratory data and tumor-related variables are at the development of HCC. Continuous variables are given as medians with ranges

*HCV* hepatitis C virus, *HCC* hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation

[a] IFN-treated versus IFN-untreated

[b] SVR versus non-SVR

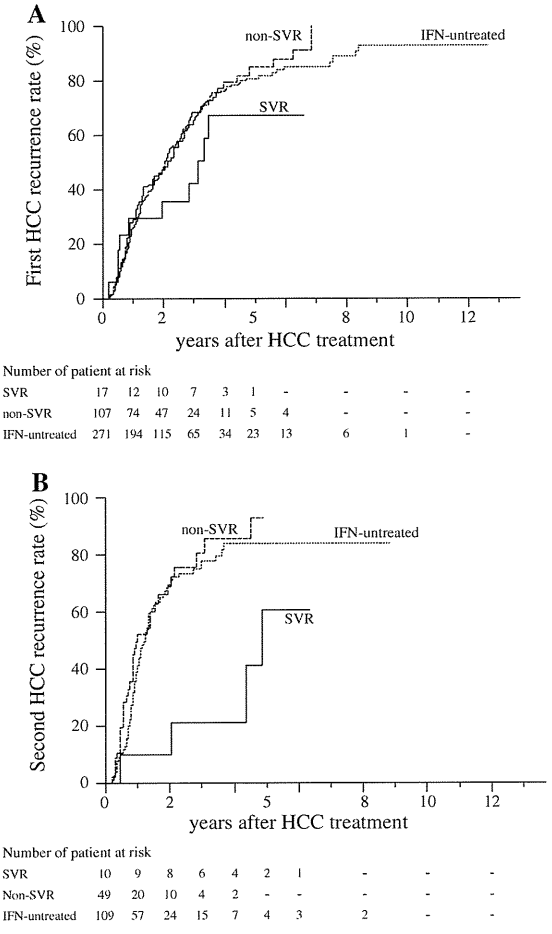


**Fig. 2** Cumulative first (**a**, $n = 395$) and second (**b**, $n = 168$) HCC recurrence rates in patients with curative treatment of HCC according to the previous IFN treatment and response to the treatment. The first HCC recurrence rates were similar among SVR, non-SVR, and IFN-untreated groups (**a**). However, the second HCC recurrence rate in the SVR group at 2 years after HCC treatment was significantly lower than that in the non-SVR group (10 vs. 69%, $p = 0.003$) and the IFN-untreated group (10 vs. 70%, $p = 0.006$) (**b**)

### Statistical Analysis

Statistical analysis was performed using JMP statistical discovery software, version 8.0 (SAS Institute Inc., Cary, NC). Differences between two groups were evaluated using the unpaired Student's $t$ test or the Mann–Whitney $U$ test. The Chi-square test or the Fisher's exact probability test was used to compare categorical data. Cumulative incidence curves were determined with the Kaplan–Meier method, and the differences between patient groups were assessed using the logrank test. Possible risk factors for recurrence of HCC and survival included both IFN-related variables and variables at the development and recurrence of HCC (age, total bilirubin level, albumin level, prothrombin time, ALT level, platelet count, number of tumors, largest tumor size, AFP level, and DCP level). Tumor associated variables, number of tumors and size of largest tumor, were transformed into categorical data consisting of two ordinal numbers by the median value. Variables exhibiting $p$ values less than 0.10 in univariate analysis were subjected to a stepwise Cox proportional hazards regression analysis. A risk ratio with a 95% confidence interval was denoted for each analysis. $p$ values less than 0.05 were considered statistically significant.

## Results

Demographic and clinical characteristics of patients at the development of HCC are shown in Table 1. The patient group comprised 260 men and 135 women (73 men and 51 women in the IFN-treated group), and median age was 58 years (65 years in the IFN-treated group). Of the 395 patients (80%), 317 were classified as Child–Pugh stage A. Significant differences were observed between IFN-treated and untreated patients in sex, age, albumin level, and size of largest tumor. On the other hand, significant differences were observed between IFN-treated patients with SVR and non-SVR in albumin level, prothrombin time, ALT level, platelet count, Child–Pugh stage, and initial treatment of HCC. This indicated better hepatic functional reserve in SVR patients than in non-SVR patients.

The median follow-up period after curative treatment of HCC for patients with and without IFN treatment was 3.8 years and 3.5 years, respectively. In the IFN-treated group, patients underwent IFN therapy 7.2 (0.8–17.4) (median and range) years before development of HCC. Of the 395 patients, 277 (70%) had recurrence of HCC during a median follow-up period of 2.1 (1.8–2.4) years [including 86 of 124 IFN-treated patients (69%)]. Of the 168 patients receiving curative treatment for the first recurrence of HCC, 113 (67%) had a second HCC recurrence during a median follow-up period of 1.3 (1.0–1.4) years [including 38 of 59 IFN-treated patients (64%)] (Fig. 1).

### HCC Recurrence Rates

The rates of the first and second HCC recurrence after curative treatment of primary HCC in each treatment group are shown in Fig. 2. In the IFN-treated group, 86 patients (10 with SVR and 76 with non-SVR) had the first HCC recurrence and 38 (four with SVR and 34 with non-SVR) had the second HCC recurrence during the follow-up period. The average times to the first and second HCC recurrence were 632 and 1,069 days, 661 and 401 days, and 666 and 428 days in SVR, non-SVR, and IFN-untreated groups, respectively. The rates of the first recurrence at 2 years in SVR, non-SVR, and IFN-untreated groups were 36, 47, and 48%, respectively. The differences between these rates were not statistically significant

($p = 0.410$) (Fig. 2a). However, the rates of the second HCC recurrence at 2 years were significantly lower in the SVR group than in the non-SVR group (10 vs. 69%, $p = 0.003$) and in the IFN-untreated group (10 vs. 70%, $p = 0.006$) (Fig. 2b). There was no significant difference in the second HCC recurrence rates between non-SVR and IFN-untreated groups ($p = 0.441$). In multivariate analysis, platelet count ($p = 0.033$) and number of tumors ($p = 0.001$) were independent factors associated with the first recurrence of HCC (Table 2), whereas SVR to previous IFN therapy ($p = 0.002$) was the only factor associated with lower risk for the second recurrence of HCC (Table 3).

### Overall Survival

Survival rates after curative treatment of primary HCC in each group are shown in Fig. 3. A tendency was observed toward a higher survival rate in the IFN-treated group than in the IFN-untreated group but it was not significant ($p = 0.053$) (Fig. 3a). In contrast, survival rates at 5 years were higher in the SVR group (100%) than in non-SVR (73%) and IFN-untreated groups (62%) ($p = 0.004$) (Fig. 3b). No significant difference was observed in the survival rates between non-SVR and IFN-untreated groups ($p = 0.450$). In multivariate analysis, SVR to previous IFN therapy ($p < 0.001$), albumin level ($p = 0.006$), number of tumors ($p = 0.007$), and AFP level ($p = 0.046$) were independent factors associated with overall death after curative treatment of primary HCC (Table 4).

## Discussion

In the present study, we have demonstrated that patients with SVR to previous IFN treatment before development of HCC showed lower risk for the second recurrence of HCC and better survival compared to patients with non-SVR to previous IFN treatment or IFN-untreated patients. Several studies have demonstrated that IFN therapy reduces the risk of HCC development among chronic hepatitis C patients. On the other hand, a few reports are available on the influence of previous IFN therapy before the development of HCC on patient outcomes after curative treatment of HCV-related HCC. It was initially reported that HCV-related HCC patients who received IFN therapy before development of HCC showed lower recurrence rates and better survival rates, independent of response to IFN therapy, compared to those without previous IFN therapy [29, 30]. It has recently been reported that patients showing

**Table 2** Risk factors for the first recurrence of HCC ($n = 395$)

| Variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Odds ratio (95% CI) | $p$ | Odds ratio (95% CI) | $p$ |
| Sex (male) | 1.17 (0.91–1.51) | 0.229 | – | |
| IFN-related variables | | | | |
| IFN-untreated | 1 | | | |
| Non-SVR | 1.07 (0.82–1.39) | 0.623 | – | |
| SVR | 0.68 (0.34–1.22) | 0.209 | – | |
| Variables at the development of HCC | | | | |
| Age (≥60 years) | 1.13 (0.84–1.56) | 0.434 | – | |
| Total bilirubin (≥1.0 mg/dl) | 1.07 (0.83–1.37) | 0.579 | – | |
| Albumin (<3.5 g/dl) | 1.34 (1.04–1.71) | 0.022 | 1.24 (0.95–1.61) | 0.108 |
| Prothrombin time (<70%) | 1.07 (0.79–1.43) | 0.664 | – | |
| ALT (≥40 IU/l) | 1.09 (0.83–1.43) | 0.542 | – | |
| Platelet count(<10 × $10^4$/μl) | 1.37 (1.08–1.75) | 0.009 | 1.34 (1.04–1.75) | 0.026 |
| Tumor-related variables | | | | |
| Number of tumors (multiple vs. single) | 1.66 (1.27–2.15) | <0.001 | 1.63 (1.24–2.14) | 0.001 |
| Size of largest tumor (≥20 mm) | 1.24 (0.98–1.57) | 0.074 | 1.22 (0.94–1.59) | 0.140 |
| AFP (≥100 ng/ml) | 1.45 (1.07–1.92) | 0.016 | 1.30 (0.96–1.74) | 0.093 |
| DCP (≥40 mAU/ml) | 1.33 (1.02–1.75) | 0.034 | 1.11 (0.85–1.44) | 0.448 |
| Initial treatment of HCC | | | | |
| PTA/surgical resection | 1.09 (0.84–1.43) | 0.530 | – | |

*HCC* hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

**Table 3** Risk factors for the second recurrence of HCC ($n = 168$)

| Variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Odds ratio (95% CI) | *p* | Odds ratio (95% CI) | *p* |
| Sex (male) | 1.07 (0.73–1.61) | 0.719 | – | |
| IFN-related variables | | | | |
| IFN-untreated | 1 | | 1 | |
| Non-SVR | 1.17 (0.77–1.74) | 0.447 | 1.09 (0.68–1.72) | 0.718 |
| SVR | 0.27 (0.08–0.65) | 0.002 | 0.10 (0.01–0.50) | 0.002 |
| Variables at the development of HCC | | | | |
| Age (≥60 years) | 1.50 (0.91–2.61) | 0.115 | – | |
| Total bilirubin (≥1.0 mg/dl) | 1.08 (0.72–1.60) | 0.701 | – | |
| Albumin (<3.5 g/dl) | 1.04 (0.68–1.57) | 0.847 | – | |
| Prothrombin time (<70%) | 1.18 (0.70–1.89) | 0.529 | – | |
| ALT (≥40 IU/l) | 1.30 (0.86–2.01) | 0.220 | – | |
| Platelet count ($<10 \times 10^4/\mu l$) | 1.00 (0.69–1.47) | 0.984 | – | |
| Number of tumors (multiple vs. single) | 1.57 (1.04–2.32) | 0.033 | 1.51 (0.93–2.42) | 0.098 |
| Size of largest tumor (≥20 mm) | 0.91 (0.63–1.32) | 0.613 | – | |
| AFP (≥100 ng/ml) | 0.65 (0.38–1.06) | 0.084 | 0.77 (0.39–1.39) | 0.391 |
| DCP (≥40 mAU/ml) | 0.81 (0.54–1.23) | 0.331 | – | |
| Initial treatment of HCC | | | | |
| PTA/surgical resection | 1.12 (0.75–1.69) | 0.595 | – | |
| Variables at the first recurrence of HCC | | | | |
| Age (≥60 years) | 0.97 (0.46–2.39) | 0.950 | | |
| Total bilirubin (≥1.0 mg/dl) | 0.94 (0.59–1.46) | 0.785 | – | |
| Albumin (<3.5 g/dl) | 1.67 (1.06–2.61) | 0.029 | 1.47 (0.90–2.36) | 0.125 |
| Prothrombin time (<70%) | 1.24 (0.60–2.30) | 0.531 | – | |
| ALT (≥40 IU/l) | 1.49 (0.95–2.40) | 0.083 | 1.21 (0.75–2.01) | 0.452 |
| Platelet count ($<10 \times 10^4/\mu l$) | 1.13 (0.74–1.73) | 0.573 | – | |
| Number of tumors (multiple vs. single) | 2.09 (1.37–3.13) | <0.001 | 1.47 (0.91–2.34) | 0.112 |
| Size of largest tumor (≥20 mm) | 0.96 (0.62–1.45) | 0.840 | – | |
| AFP (≥100 ng/ml) | 0.72 (0.32–1.41) | 0.355 | – | |
| DCP (≥40 mAU/ml) | 1.05 (0.67–1.63) | 0.842 | – | |

*HCC* hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

biochemical response, with or without SVR to previous IFN therapy, showed higher tumor-free survival rates after surgery than those without such a response to IFN or those without previous IFN therapy [31, 32]. In these previous reports, a biochemical response as well as SVR to previous IFN therapy was associated with favorable outcome, demonstrating the importance of response to previous IFN therapy for the outcome after surgery of HCV-related HCC.

However, in the present study, patients with non-SVR showed similar recurrence and survival rates as IFN-untreated patients. Furthermore, no difference was observed in the recurrence and survival rates among non-SVR patients with and without biochemical response to previous IFN therapy (data not shown). In fact, only patients with SVR to previous IFN therapy showed better outcome than those with non-SVR or IFN-untreated patients. Therefore, the present data indicate that SVR but not biochemical response without SVR to previous IFN treatment is a predictor of favorable outcome in patients who have developed HCC.

The reason for the difference between the present and previous studies in the outcome of non-SVR patients with biochemical response to previous IFN therapy is currently unknown. In patients with HCV-related chronic hepatitis and cirrhosis, who received IFN therapy and showed normalization of ALT levels, suppression of primary HCC development and better survival rates have been independently demonstrated of eradication of HCV infection by the IFN therapy [10, 11, 13, 39]. However, this

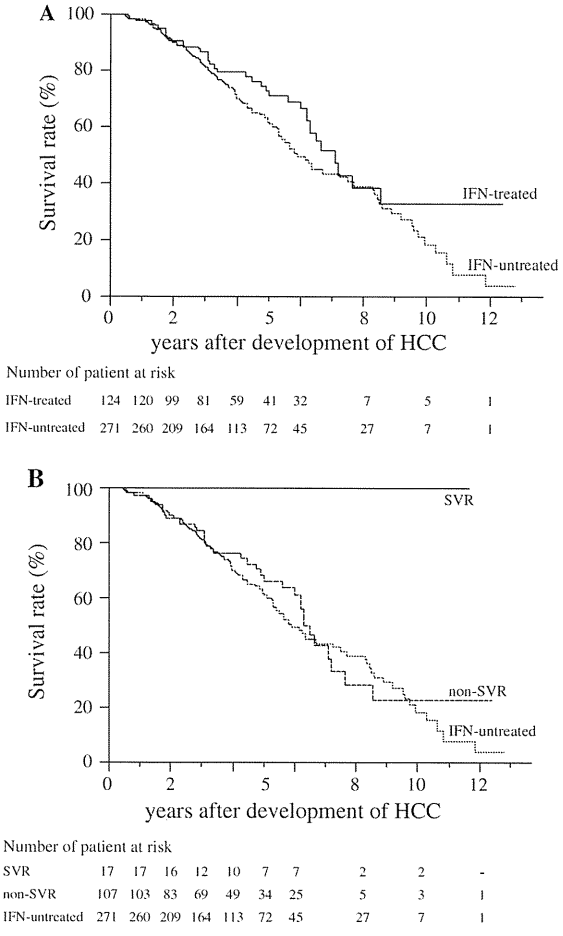


**Fig. 3** Overall survival rates of HCV-related HCC patients ($n = 395$) according to their previous IFN treatment before development of HCC (**a**) and their response to the treatment (**b**). A tendency was observed toward a higher survival rate in the IFN-treated group than in the IFN-untreated group but it was not significant ($p = 0.053$) (**a**). On the other hand, the survival rate of the SVR group was significantly higher than those of non-SVR and IFN-untreated groups ($p = 0.004$) (**b**)

suppression observed for primary carcinogenesis in non-SVR patients with biochemical response to IFN therapy does not appear to be the case for secondary carcinogenesis in the present study. The period after IFN therapy was much longer in the present study than in the previous reports on primary carcinogenesis. The IFN therapy had preceded to the HCC development, that should have required long incubation after the termination of IFN treatment, and in the present study the observation of HCC recurrence and survival started with the curative treatment of the primary HCC. In patients who had sustained biochemical response but had not eradicated HCV infection, we and others demonstrated that platelet count transiently increases following IFN therapy but decrease over the following 3 years after the termination of IFN therapy. On the other hand, in patients with SVR an increase followed by persistence in platelet counts was observed [40, 41]. These observations suggest the progression of fibrosis during a longer incubation period after IFN therapy, even in the non-SVR patients with biochemical response to the therapy. Therefore, the suppressive effect of IFN therapy on development of HCC may not persist beyond the development of primary HCC particularly in these patients.

It has also been demonstrated that HCV core transgenic mice can develop HCC without apparent hepatitis [42]. Therefore, besides active hepatitis, which involves persistent hepatocyte death and regeneration, and should result in both genetic and epigenetic disorders as well as increased oxidative stress, the presence and persistence of HCV infection and viral products such as core protein may themselves play an important role in the development of HCC in non-SVR patients with biochemical response. Thus, patients with SVR who had eradicated HCV infection should have a lower incidence of HCC recurrence and higher survival rates than non-SVR patients with biochemical response.

In the present study, patients with SVR showed a better overall survival rate than other groups. However, although patients with SVR showed lower rates of the second HCC recurrence, this was not the case for the first HCC recurrence. Although both SVR and non-SVR groups have a carcinogenic background during the development of primary HCC, the carcinogenic potential in SVR patients may be gradually attenuated because of the eradication of HCV infection, whereas it may increase in those with non-SVR because of persistence of HCV infection and relapse of hepatitis, finally leading to progression of fibrosis over a longer period. However, a substantial time may be required before differences between patients with and without SVR become apparent, and these differences eventually become significant in the second recurrence of HCC.

It should also be noted that IFN-treated patients enrolled in the present and previous studies are a selected cohort, since the incidence rates of HCC development in patients treated with IFN should be lower than in those untreated with IFN [13]. This is particularly the case for patients with SVR to previous IFN treatment, whose risk for development of HCC is less than one fifth of that for IFN-untreated patients [13]. Reported risk factors for HCC development in patients who received IFN therapy include advanced fibrosis, lower platelet count, advanced age, male gender, and regular drinking [8, 9, 12, 13, 43]. Therefore, in the present study, HCC patients who received IFN therapy before the development of HCC may have demonstrated many of these characteristics, making them more prone to develop HCC than those not developing HCC after IFN therapy and not included in this study. Furthermore, it has been suggested that cirrhotic patients who develop primary

**Table 4** Analysis of factors associated with overall death after curative treatment for primary HCC ($n = 395$)

| Variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Odds ratio (95% CI) | $p$ | Odds ratio (95% CI) | $p$ |
| Sex (male) | 1.02 (0.73–1.44) | 0.911 | – | |
| IFN-related variables | | | | |
| IFN-untreated | 1 | | 1 | |
| Non-SVR | 0.86 (0.59–1.24) | 0.445 | 1.05 (0.71–1.54) | 0.794 |
| SVR | <0.01 (0–0.17) | <0.001 | <0.01 (0–0.26) | <0.001 |
| Variables at the development of HCC | | | | |
| Age (≥60 years) | 1.06 (0.72–1.63) | 0.773 | – | |
| Total bilirubin (≥1.0 mg/dl) | 1.45 (1.04–2.01) | 0.028 | 1.21 (0.82–1.76) | 0.332 |
| Albumin (<3.5 g/dl) | 2.07 (1.49–2.89) | <0.001 | 1.70 (1.16–2.49) | 0.007 |
| Prothrombin time (<70%) | 1.44 (0.99–2.06) | 0.059 | 0.97 (0.65–1.43) | 0.874 |
| ALT (≥40 IU/L) | 1.12 (0.78–1.67) | 0.531 | – | |
| Platelet count ($<10 \times 10^4$ μl) | 1.72 (1.23–2.41) | 0.001 | 1.35 (0.93–1.96) | 0.118 |
| Tumor-related variables | | | | |
| Number of tumors (multiple vs. single) | 1.59 (1.10–2.26) | 0.014 | 1.71 (1.16–2.46) | 0.007 |
| Size of largest tumor (≥20 mm) | 1.15 (0.83–1.60) | 0.395 | – | |
| AFP (≥100 ng/ml) | 1.71 (1.17–2.45) | 0.006 | 1.50 (1.00–2.18) | 0.047 |
| DCP (≥40 mAU/ml) | 1.33 (0.91–1.98) | 0.145 | – | |
| Initial treatment of HCC | | | | |
| PTA/surgical resection | 1.69 (1.16–2.53) | 0.006 | 1.03 (0.68–1.60) | 0.882 |

*HCC* hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

HCC may already be at a "carcinogenic stage" and have a higher potential to develop intrahepatic multicentric carcinogenesis than those without HCC [15]. Patients who have already developed HCC may have background features such as greater age and impaired liver function because of more advanced fibrosis. Therefore, the observed recurrence and survival rates in the present study are those of selected patients who were already at the carcinogenic stage, and are thus biased in comparison to previous observations on primary prevention of HCC development in patients who had received IFN therapy. Recently, Imai et al. reported that an inhibitory effect of IFN therapy on development of HCC in older patients was limited to patients with SVR [44]. This also supports the notion that patients already at a carcinogenic stage or with risk factors associated with HCC development, such as greater age or advanced fibrosis, require eradication of HCV infection in order to achieve a significantly better prognosis.

The present observation highlights the importance of eradication of HCV in order to prevent HCC recurrence and to achieve better survival in this patient group. Plenty of reports are available that demonstrated the favorable effect of IFN therapy on the recurrence of HCC and survival particularly in patients who achieved SVR [21–28]. Therefore, re-treatment with more potent IFN therapies, such as combination therapy of PEGylated IFN plus ribavirin [45], should be recommended for patients who previously underwent IFN treatment without achieving SVR.

The present study has limitations as it is retrospective in nature, and thus, patients enrolled were biased in favor of experience of IFN treatment, and also HCC patients with previous IFN treatment were a selected population from a large cohort of patients who had undergone IFN treatment. Also, information on the histological data that may have influence on the outcome of HCC patients was not available in the present study. Further prospective studies are required to address these issues.

In conclusion, the present study demonstrated that patients with SVR to IFN treatment before the development of HCV-related HCC showed lower second HCC recurrence rates and higher survival rates than those with non-SVR to previous IFN treatment or IFN-untreated patients. Therefore, treatment with potent antiviral therapy is recommended for patients in the latter groups in order to suppress recurrence and improve survival by eradicating HCV infection.

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Hepatology Research

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*Case Report*

# Hepatocellular carcinoma occurring in hepatobiliary fibropolycystic disease

Hideaki Kinugasa, Kazuhiro Nouso, Yoshiyuki Kobayashi, Tetuya Yasunaka, Hideki Onishi, Shin-ichiro Nakamura, Hidenori Shiraha, Hiroki Takayama, Junichi Toshimori, Kenji Kuwaki, Hiroaki Hagihara, Yasuhiro Miyake, Fusao Ikeda, Akinobu Takaki, Haruhiko Kobashi and Kazuhide Yamamoto

*Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan*

Congenital hepatic fibrosis (CHF) and bile duct hamartomas (von Meyenburg complexes) are hepatobiliary fibropolycystic diseases. There have been several reports of liver neoplasias arising in hepatobiliary fibropolycystic diseases. However, most of them were cholangiocarcinomas and cases involving hepatocellular carcinoma (HCC) are rare. A 51-year-old woman was found to have multiple hepatic tumors by ultrasonography and enhanced computed tomography (CT) during a regular work-up for the recurrence of lung cancer and thyroid cancer, which had been surgically removed 4 and 3 years ago, respectively. Nodules were observed at S3, S5, and S6 (2 cm in diameter). All of the nodules were hyperattenuated at the early arterial phase, and the main tumor at S5 showed hypoattenuation at the delayed phase on dynamic CT and magnetic resonance imaging (MRI). HCC was suspected from these findings. She also suffered from multiple small cystic lesions in the liver. The surgically removed liver showed HCC arising in CHF, which is a rare histological finding.

## INTRODUCTION

DUCTAL PLATE MALFORMATION results from the persistence or absence of remodeling of the embryonic ductal plate during ontogenesis.[1] This leads to different hepatobiliary fibropolycystic diseases, such as Caroli's disease and syndrome; autosomal recessive polycystic kidney disease (ARPKD); autosomal dominant polycystic kidney disease (ADPKD); congenital hepatic fibrosis (CHF); and bile duct hamartomas (BDH, also known as von Meyenburg complexes), which were first described by von Meyenburg in 1918.[2] They are all characterized by a variable degree of ectasia of intrahepatic bile ducts and associated with a variable degree of fibrosis. The main difference between CHF and BDH is the approximate size of the bile ducts affected by ductal plate malformation. The main pathological change in CHF is larger bile ducts than that in BDH. Caroli's disease is congenital dilation of the larger intrahepatic bile ducts without fibrosis.[1]

Although most CHF, BDH and Caroli's disease are benign, there have been several reports of liver neoplasias arising in these hepatobiliary fibropolycystic diseases. The prevalence of liver neoplasia has not been fully elucidated, but Summerfield *et al.* reported that carcinomas arised in the liver with a frequency of approximately 1% in CHF, 4% in choledochal cysts, and 7% in Caroli disease.[3] In most cases, an association with cholangiocarcinoma involving mechanical or chemical irritation and chronic inflammation was established.[4,5] Here, we report a rare case of hepatocellular carcinoma (HCC) arising in CHF.

## CASE REPORT

A 51-YEAR-OLD WOMAN WAS found to have hepatic tumors during a regular work-up for the recurrence of lung cancer and thyroid cancer by ultrasonography (US) and contrast-enhanced computed tomography (CT) in 2008. The lung cancer and thyroid cancer were surgically removed in 2004 and 2005,

*Correspondence: Dr Hideaki Kinugasa, Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences 2–5-1 Shikata-cho, kita-ku, Okayama 700–8558, Japan.*
*Email: gyacy14@gmail.com*
*Received 9 November 2010; revised 9 November 2010; accepted 1 December 2010.*

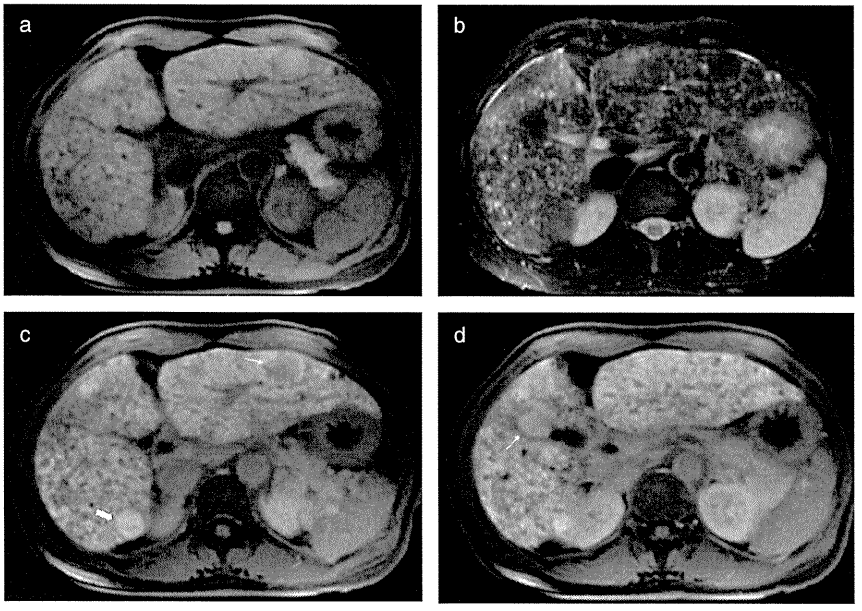


**Figure 1** Magnetic resonance imaging showed multiple small lesions scattered throughout the whole liver, which demonstrated low signal intensity on T1-weighted imaging (a) and high signal intensity in T2-weighted imaging (b). On Gadolinium ethoxybenzyl magnetic resonance imaging, only the nodule at S3 showed perfusion defect (arrow) (c) at the hepatic phase, whereas the nodule at S5 demonstrated iso-signal intensity (arrow) (d), and the nodule at S6 displayed high signal intensity (large arrow) (c).

respectively. She did not have a family history of liver diseases or polycystic kidney. Collateral veins around hilum of spleen was observed by CT; however, no esophageal or gastric varices was detected by esophagogastric-duodenoscopy. Her laboratory findings were as follows: hemoglobin: 9.5 g/dL, hematocrit: 29.4%, white blood cell count: $4.49 \times 10^9$ /L, platelet count: $172 \times 10^9$ /L, prothrombin (PT) activity: 98%, albumin: 3.9 g/dL, total bilirubin: 0.62 mg/dL, ALT: 34 IU/L, γ-glutamyl transpeptidase: 137 IU/L. Her levels of tumor markers such as carcinoembryonic antigen (CEA), carbohydrate antigen19-9 (CA19-9), alpha-fetoprotein (AFP), and des-gamma carboxy prothrombin (DCP) were within the normal range. Tests for hepatitis B virus surface antigen, hepatitis B virus surface antibody, hepatitis B virus core antibody and hepatitis C virus antibodies were negative and no autoantibodies, such as anti-nuclear antibodies (ANA) or anti-mitochondrial antibodies (AMA) were detected. She was not a habitual drinker, and her body mass index (BMI) was 19.4. The detection of multiple small hypoechoic areas of approximately 4 mm in diameter in the liver indicated the presence of multiple cystic lesions; however, no dilation of the common bile duct (CBD) or intrahepatic bile ducts (IHBD) was observed. CT findings of multiple small low-density areas also indicated that the lesions in the liver were cysts. Magnetic resonance imaging (MRI) showed multiple small lesions scattered throughout the liver, and their signal patterns were low signal intensity on T1-weighted imaging (Fig. 1a) and high signal intensity on T2-weighted imaging (Fig. 1b). No communication between these lesions and the intrahepatic bile ducts was observed by magnetic resonance cholangiopancreatography (MRCP). All of the findings were compatible with BDH. The cystic lesions had been pointed out by CT in 2004 and 2005.

Other than the findings of BDH, 2 cm nodules at S3, S5, and S6 were observed by imaging, all of which were found to be hypoechoic on US and enhanced at the early arterial phase on CT (Fig. 2a,b); however, hypoattenuation at the portal phase was only observed in the nodules at S3 and S5 (Fig. 2c,d). MRI of the nodules demonstrated high signal intensity on T1-weighted imaging and low signal intensity on T2-weighted imaging. The enhancement pattern of the nodules produced by Gadolinium ethoxybenzyl magnetic resonance imaging (Gd-EOB-MRI) was the same as that observed on enhanced CT. Only the nodule at S3 showed perfusion defects during the hepatic phase, and the nodule at S5 demonstrated iso-signal intensity, and the nodule at S6 displayed high signal intensity (Fig. 1c,d).

A needle biopsy was performed, and a histological examination revealed that all 3 nodules were well-differentiated HCC and that the background of the liver was BDH which was portal and periportal fibrosis. There were no histological differences between these nodules.

Transcatheter hepatic arterial chemoembolization (TACE) was performed on these nodules prior to local ablation therapy because they were hypervascular. Radiofrequency ablation (RFA) was performed on the

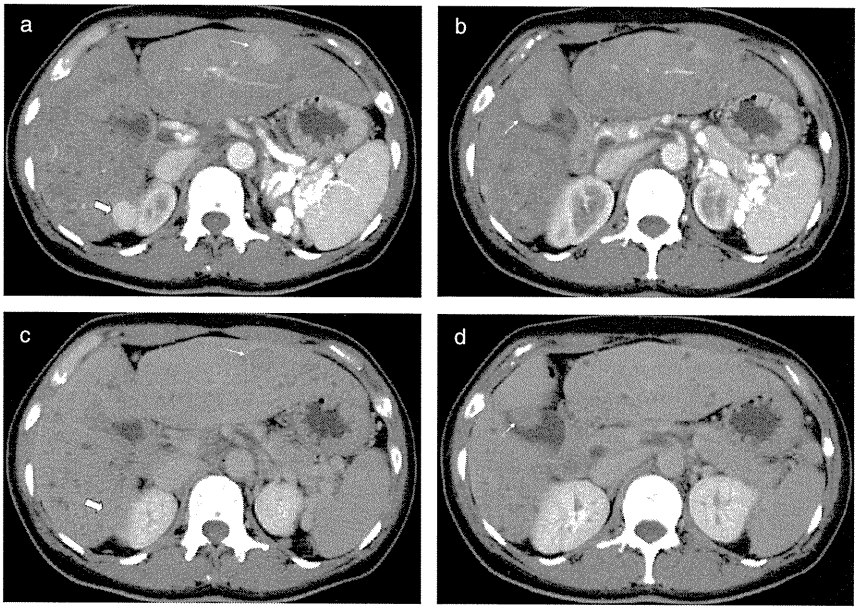


**Figure 2** The nodules at S3 (arrow) (a), S5 (arrow) (b), and S6 (large arrow) (a) were enhanced on computed tomography at the early arterial phase. Hypoattenuation at the portal phase was only observed for the nodules at S3 (arrow) (c) and S5 (arrow) (d). The nodule at S6 displayed isodensity at the portal phase (large arrow) (c).

nodules at S3 and S6; however, surgical resection was chosen for the treatment of the nodule at S5 because it protruded toward the gall bladder, and the risk of visceral damage or rupture of the HCC by RFA was considered to be too great. The removed liver segment contained a poorly demarcated firm, white-yellowish nodule of 2.2 × 2.0 cm in size (Fig. 3a). The histological findings of the tumor included thin-trabecular well-differentiated HCC (Fig. 3b). Portal and periportal fibrosis were detected in the rest of the liver. Normal shaped hepatocytes were surrounded by dense and mature fibrous tissue containing structures lined by a cuboidal biliary epithelium, which was elongated, tortuous, and/or branched (Fig. 3c). The lumen contained inspissated bile. No inflammatory cell infiltration was seen. The background of the liver was diagnosed as CHF by the committee in The Japanese Society of Pathology as well as by pathologists in our institute.

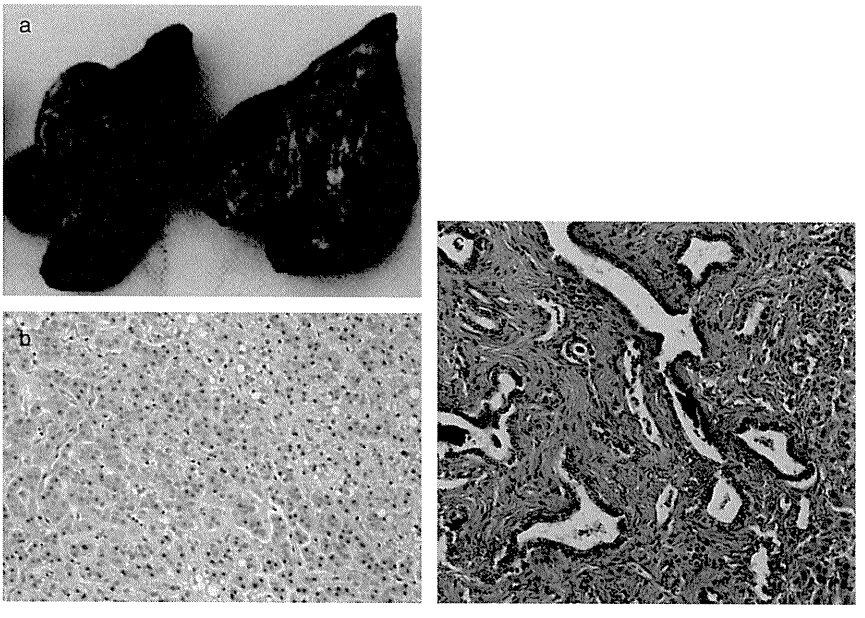


**Figure 3** The removed liver segment contained a poorly demarcated firm (a), white-yellowish nodule of 2.2 × 2.0 cm in size. Histological examination of the tumor found that it was a thin-trabecular well-differentiated HCC (b). Normal shaped hepatocytes were surrounded by dense and mature fibrous tissue containing structures lined by a cuboidal biliary epithelium, which was elongated, tortuous, and/or branched (c).

The amount of tissue obtained by needle biopsy is small, so it is difficult to diagnose whether BDH or CHF properly. A final histological diagnosis was performed by examining the surgically resected liver and was HCC arising in CHF. At 2 years post treatment and the patient was alive without recurrence.

## DISCUSSION

HEPATOBILIARY FIBROPOLYCYSTIC DISEASES do not exist as single entities, but members of a family. BDH, CHF and Caroli's disease belong to hepatobiliary fibropolycystic diseases.

Recently, it has been shown that hepatobiliary fibropolycystic diseases sometimes progress to malignant neoplasia. However, an association with cholangiocarcinoma was established in most cases,[6–8] and HCC combined with hepatobiliary fibropolycystic diseases has rarely been reported (Table 1). In a review of liver neoplasms associated with hepatobiliary fibropolycystic diseases, only four HCC cases without known risk factors were associated with BDH/CHF/Caroli's disease.[9–11]

Bauman ME *et al.* reported a case of HCC arising in CHF.[9] The case was incidentally-found multifocal HCC in an explanted liver with CHF. The renal lesion lacked the multiple large cortical cysts of ADPKD and yet cerebral and multiple splenic artery aneurysms were present. Heinke T *et al.* also reported two other HCC associated with BDH.[10] Ijima M *et al.* reported HCC associated with Caroli's disease recently.[11]

We report herein a case of CHF with HCC without other risk factors for HCC such as hepatitis B virus, hepatitis C virus, alcohol, and nonalcoholic steatohepatitis (NASH). Our case had multiple well-differentiated HCC, indicating multifocal development of HCC rather than intrahepatic metastasis. Two out of four reported cases of HCC arising in hepatobiliary fibropolycystic diseases also demonstrated multiple well-differentiated HCC. Therefore, it is possible that livers of hepatobiliary fibropolycystic diseases bearing HCC are in a hyper carcinogenetic state.

All four reported cases were younger than 60-years-old. Our case was 51-years-old and had multiple HCC, lung cancer, and thyroid cancer. As HCC arising in hepatobiliary fibropolycystic diseases occurs in younger patients than HCC associated with other risk factors such as hepatitis C virus and intrahepatic cholangiocarcinoma (ICC)[12] and the syndromes associated with CHF are commonly inherited in an autosomal recessive manner, unknown somatic mutations might be present in these patients, although patient number is too small to refer.

Recently, Otto EA *et al.* reported that hypomorphic mutations in meckelin (MKS3/TMEM67) caused nephronophisis combined with liver fibrosis.[13] Although we did not examine meckelin mutations, this SNP did not seem to be involved in our case because no family history of kidney disease was present. Further studies are needed to explore SNP that correlate with the pathology of CHF combined with HCC. Although the mechanisum of development of HCC in CHF is not clear, it might be different from that of viral hepatitis related HCC, because liver functions were normal in most of CHF and the accumulation of genetic mutation caused by the repeat of necrosis and regeneration was unlikely.

The prevalence of CHF is not clearly defined; however, it was estimated to be one in 10 000 to 20 000 based on the data of various specific ciliopathies associated with CHF.[14] Nagano Y *et al.* reported that MRI was suitable for imaging diagnosis of BDH.[15] Through the development and spread of imaging modalities including MRI, the likelihood of diagnosing hepatobiliary fibropolycystic diseases is increasing, and more patients with hepatobiliary fibropolycystic diseases will be found. Hence, the actual number of HCC arising in hepatobiliary fibropolycystic diseases might be higher.

**Table 1** List of hepatocellular carcinoma (HCC) with hepatobiliary fibrocystic dieases

| Reference | Year | Hepatic lesion | Grade of differentiation | Sex | Age (years) |
|---|---|---|---|---|---|
| Bauman *et al.*[9] | 1994 | CHF | Well differentiated HCC | M | 31 |
| Heinke *et al.*[10] | 2008 | BDH | Well differentiated HCC | F | 19 |
| Heinke *et al.*[10] | 2008 | BDH | Moderately differentiated HCC | M | 39 |
| Ijima M *et al.*[11] | 2010 | Caroli's disease | Moderately differentiated HCC | M | 29 |
| Present case | 2010 | CHF | Well differentiated HCC | F | 51 |

BDH, Bile duct hamartoma; CHF, Congenital hepatic fibrosis; F, Female; M, Male.

We reported a rare case of HCC occurring in hepatobiliary fibropolycystic disease. When diagnosing HCC without known risk factors, the presence of hepatobiliary fibropolycystic diseases must be considered and vice versa.

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*Original Article*

# Prognostic Model for Hepatocellular Carcinoma with Time-Dependent Factors

Kenji Kuwaki[a]*, Kazuhiro Nouso[a,b], Yoshiyuki Kobayashi[a], Shinichiro Nakamura[a], Yoichi M. Ito[c], Shouta Iwadou[a], Hiroaki Hagihara[a], Tetsuya Yasunaka[a], Junichi Toshimori[a], Hirokazu Miyatake[a], Kenji Miyoshi[a], Hideki Onishi[a], Yasuhiro Miyake[a,b], Bon Shoji[a], Akinobu Takaki[a], Hidenori Shiraha[a], Yoshiaki Iwasaki[a], Haruhiko Kobashi[a], and Kazuhide Yamamoto[a]

*Departments of [a]Gastroenterology and Hepatology, [b]Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan, and [c]Hokkaido Organization for Translational Research, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan*

**The purpose of this study was to build a prognostic model of hepatocellular carcinoma (HCC) using time-dependent covariates to re-evaluate the prognosis at any stage of the disease. The subjects were consecutive HCC patients who were treated at our institute between 1995 and 2007. We constructed time-fixed and time-dependent prognostic models with a training group (n = 336) and compared the prognostic abilities between conventional Cancer of the Liver Italian Program (CLIP) scores, Japan Integrated Staging (JIS) scores, an Okuda classification, and our prognostic models in the testing group (n = 227) with the c-index. The time-dependent prognostic model consisted of main tumor size, tumor number, portal vein invasion, distant metastasis, alpha-fetoprotein, des-gamma-carboxy prothrombin (DCP), bilirubin, and albumin and the weighted scores were set for each factor depending on the hazard ratio for the prognosis. The prognostic index was determined by summing the scores. The c-index values for the CLIP scores, JIS scores, Okuda classification, and our time-dependent model were 0.741, 0.727, 0.609, and 0.870, respectively. These results indicate that our time-dependent model can estimate the prognosis of HCC more precisely than traditional time-fixed models and can be used to re-predict the prognosis of HCC.**

**Key words**: hepatocellular carcinoma, humans, prognosis, proportional hazards models, time factors

Hepatocellular carcinoma (HCC) is the fifth most common cancer and is one of the leading causes of cancer death in the world [1]. In Japan, approximately 35,000 people die of HCC every year, and 90% of patients suffer from persistent infection of hepatitis B virus (HBV) or hepatitis C virus (HCV) [2].

The prognosis is affected by both the tumor severity, as indicated by factors such as size and the number or levels of alpha-fetoproteins (AFP), and the degree of pre-existing liver damage, as indicated by serum albumin or serum bilirubin levels [3]. Many prognostic models of HCC using Cox regression models have been described. As for unification scores to estimate prognosis, the Child-Pugh stage [4], the Cancer of the Liver Italian Program (CLIP) score [5], the Japan Integrated Staging (JIS) score [6], and the Barcelona Clinic and Liver Cancer (BCLC) classification [7] have been reported. However, most of these prognostic models are based on tumor-related

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*Corresponding author. Phone:+81-86-235-7219; Fax:+81-86-225-5991
E-mail:kkuwaki-gi@umin.net (K. Kuwaki)