The membrane was then blocked with 5% bovine serum albumin in TBS with 0.1% Tween 20 (TBS-T) and incubated with MU-3, P11, P16, or rabbit anti-human prothrombin antibody (Abcam plc) overnight at 4°C. The membrane was incubated with HRP-conjugated secondary antibody and developed using an ECL Prime western blotting detection system (GE Healthcare UK Ltd., Buckinghamshire, UK).

Immunohistochemistry. Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase method with labeled streptavidin-biotin (CSA-II, Dako, Kyoto, Japan), according to the manufacturer's instructions. Briefly, 3-μm thick sections were cut from formalin-fixed paraffin-embedded tissues, deparaffinized in xylene and hydrated in phosphate-buffered saline (PBS). The endogenous peroxidase was inactivated by incubation with 0.3% H_2O_2-MeOH. They were blocked with Protein Block (Dako) and incubated with MU-3, P11, or P16, respectively, as the primary antibody. After washing with PBS, the slides were incubated with biotinylated secondary antibody, followed by incubation with HR-streptavidin and visualization with DAB chromogen (3', 3-diaminobenzidine, Dako). Finally, the sections were counterstained with Mayer's hematoxylin.

Statistical analysis. All data were analyzed using STATA version 8 software (Stata Corp., Texas). Scheffe's test was used for comparisons of age, serum alanine aminotransferase (ALT) level, and prothrombin time (%) among groups. Mantel-Haenzel test for linear association was used to assess differences in positivity rates for each serum marker in stage I - IV. Chi-square test and Fisher's exact test were used to compare positivity rates among each group. Bonferroni correction was performed for multiple comparisons. Survival curves were compared by log-rank test. Receiver operating characteristic (ROC) curves were compared using the DeLong mathematical model.[25]

Results

Patient characteristics. Baseline characteristics of the 259 patients (HCC, LC, CH, and obstructive jaundice groups and warfarin users) are listed in Table 1. There were 29 stage I, 47 stage II, 67 stage III, and 33 stage IV HCC patients. Patients with LC included 8 Child-Pugh A, 3 Child-Pugh B, and 2 Child-Pugh C cases. Of the CH patients, 15 had hepatitis C infection, 8 had hepatitis B infection, 9 had alcoholic hepatitis, 13 had autoimmune hepatitis, 1 had drug-induced hepatitis, and 2 had nonalcoholic steatohepatitis. The obstructive jaundice group consisted of 6 patients with pancreatic cancer, 5 with cholangiocellular carcinoma, and 1 with gastric cancer. There were 10 warfarin users who did not have any liver dysfunction. There were no statistically significant differences in the sex ratios, ages and ALT levels among the 5 patient groups. The percent prothrombin time (PT%) was significantly lower in warfarin users than in HCC, LC and CH groups. It was also significantly lower in obstructive jaundice group than in HCC and CH groups.

Serum DCP and NX-DCP levels in patients with liver diseases and in warfarin users. Conventional serum DCP levels were measured by DCP ECLIA with MU-3 in HCC, LC/CH, and obstructive jaundice patients, and in warfarin users (Fig.1A). The serum DCP levels were positive in 71.0% (125/176) of HCC patients, 29.5% (18/61) of LC/CH patients, 58.0% (7/12) of obstructive jaundice patients, and 100% (10/10) of warfarin users. Although the DCP-positive rate in the HCC group was significantly higher than that of the LC/CH group as well as in comparison with normal subjects ($P<0.02$, chi-square test), there were no significant differences between HCC and obstructive jaundice patients or between HCC patients and warfarin users (chi-square test and Fisher's exact test), which is consistent with previous reports,[17,18] indicating that the serum DCP level is a useful marker for the diagnosis of HCC in patients with LC/CH, but cannot be used for patients with jaundice or warfarin users.

We next measured serum NX-DCP levels using the new sandwich ECLIA with the novel DCP monoclonal antibodies p11 and p16. Based on data for healthy volunteers, the cut off value was set at 90 mAU/ml (mean + 2 SD). The serum NX-DCP levels were positive in 66.7% (8/12)

of patients with obstructive jaundice and in 100% (10/10) of warfarin users, but in only 36.4% (64/176) of patients with HCC, suggesting that NX-DCP was a dominant form in patients with vitamin K-deficiency, such as those with jaundice and warfarin users (Fig. 1B). In this context, we calculated the DCP/NX-DCP ratio for each disease group in order to investigate the value of this marker for differentiating HCC patients from warfarin users and obstructive jaundice patients (Fig. 1C). When we set a cutoff value of 1.4 based on ROC analysis, the DCP/NX-DCP ratio was positive in 59.7% (105/176) of HCC patients, 6.6% (4/61) of CH/LC patients, 8.3% (1/12) of obstructive jaundice patients, and 0% (0/10) of warfarin users ($P<0.01$). The positivity rate of HCC patients was significantly higher than that of CH/LC patients, obstructive jaundice patients, and warfarin users ($P<0.001$, chi-square test or Fisher's exact test). The sensitivity and specificity of the DCP/NX-DCP ratio were 59.7% (105/176) and 91.7% (88/96), respectively. In the ROC analysis, the AUC for the DCP/NX-DCP ratio was significantly higher than that for conventional DCP (Fig. 1D). Thus, the DCP/NX-DCP ratio was a superior diagnostic marker for HCC and was able to exclude non-HCC cases with high serum DCP levels due to vitamin K deficiency.

Serum DCP and NX-DCP levels and the DCP/NX-DCP ratio at each stage of HCC. The serum DCP and NX-DCP levels were evaluated for each stage of HCC. The serum DCP levels were positive in 48.3% (14/29) of stage I, 57.4% (27/47) of stage II, 79.1% (53/67) of stage III, and 93.9% (31/33) of stage IV patients, showing a significant stepwise increment of the positivity rate from stage I to IV by Mantel-Haenzel test ($P<0.05$) (Fig. 2A). There was no significant difference in the positivity rate among stages. The rate of NX-DCP positivity was very low (17.2% - 32.4%) among patients with stage I to III disease, as expected, but surprisnlgy, the rate was very high (81.8%) in patients with stage IV disease. The positivity rate in stage IV was significantly higher than that in stage III by chi-square test ($P<0.001$) (Fig. 2B). In the analysis of the DCP/NX-DCP ratios, a ratio equal to or higher than 1.4 was found in 41.4% (12/29) of stage I, 57.4% (27/47) of stage II, 55.2% (37/67) of stage III, and 87.9% (29/33) of stage IV patients,

showing a significant stepwise increment of positivity rate from stage I to IV (P<0.05) (Fig. 2C). There was no significant difference in the ratio among the groups.

Since the positivity of NX-DCP was very high in stage IV HCC (27/33, 81.8%), we next investigated clinical parameters in these patients and compared them between NX-DCP (+) and NX-DCP (-) groups in stage IV. There was no significant difference in the background liver disease, liver functional reserve (Child-Pugh score), prevalence of ascites, serum ALT level, serum total bilirubin level and prothrombin time (%) between the two groups (Fig.3A). However, patients with vascular invasion showed a significantly higher rate of NX-DCP postivity. (P<0.05) (Fig.3B). Moreover, the overall survival time of the NX-DCP (+) group was significantly lower than that of the NX-DCP (-) group (p<0.05) (Fig.3C). These results suggested that NX-DCP may serve as a biomarker for poor prognosis in association with vascular invasion although the number of stage IV patients was not yet many enough.

Western blot analysis of DCP from patients with HCC and warfarin users using MU-3, p16, or P11. To study the antigenicity of DCP from HCC and obstructive jaundice patients and warfarin users to various antibodies, DCP was extracted from sera by prothrombin (II portion)-affinity chromatographic methods, and western blot analysis was performed using the anti-DCP monoclonal antibodies MU-3, P16 or P11 (Fig.4). As expected, MU-3 strongly reacted with DCP from a warfarin user, an HCC patient, and an obstructive jaundice patient (Fig. 4A), but not with prothrombin. In contrast, P11 strongly reacted with DCP from a warfarin user and an obstructive patient, but very faintly with DCP from a patient with HCC (Fig.4B). Similarly, P16 reacted with DCP from a warfarin user and an obstructive jaundice patient, but very faintly with DCP from an HCC patient (Fig.4C). P11 and P16 did not react with prothrombin. These results clearly indicate that the antigenicity of DCP in HCC patients is distinct from that in vitamin K-deficient patients such those who are warfarin users or who have obstructive jaundice.

Immunohistochemistry for DCP. In order to investigate the expression of DCP in HCC tissues

and autopsied normal liver tissues in warfarin users, immunohistochemical staining was performed using MU-3, P11, or P16 in 3 surgically-obtained HCC tissue samples and 2 normal liver tissue samples from warfarin users. Representative staining patterns are shown in Figure 5. The cancer cells in the HCC tissue from an HCC patient were strongly stained by MU-3 although non-cancerous lesions were not. A pre-incubation test using excess amount of DCP protein showed no apparent staning signal. In contrast, the cancer cells were stained very faintly by P11 and P16 (Fig. 5A). The serum DCP and NX-DCP levels in this patient were 4981 mAU/ml and 581 mAU/ml, respectively. On the other hand, normal liver cells in the liver tissue from an autopsied warfarin user were intensely stained by P11 and P16 in addition to MU-3 (Fig. 5B). Pre-incubation testing showed no aparent staining signal. The serum DCP and NX-DCP levels in this patient were 20597 mAU/ml and 17823 mAU/ml, respectively. Essentially, similar results were obtained in the remaining 2 HCC samples and 1 normal liver sample from warfarin user. The serum DCP and NX-DCP levels in one of these HCC patients was 3153 and 354 mAU/ml, respectively, and in the other, 4591 and 646 mAU/ml respectively, whereas the levels were 18696 and 17085 mAU/ml respectively, in the warfarin user. These results of immunohistochemistry were consistent with the data for serum DCP and NX-DCP concentrations in HCC patients and warfarin users.

Discussion

The present study demonstrated that NX-DCP was predomoinantly increased in the serum of patients with vitamin K-deficiency such as warfarin users and obstructive jaundice patients, but essentially not in HCC patients. When the DCP/NX-DCP ratio was evaluated (cutoff 1.4), the positivity rate in HCC patients (59.7%) was significantly higher than in LC/CH patients (6.6%), obstructive jaundice patients (8.3%), and warfarin users (0%). Moreover, the ROC analysis revealed that the AUC of the DCP/NX-DCP ratio was significantly higher than that of conventional DCP, suggesting superiority of the former for the diagnosis of HCC. Thus, it was evident that the DCP/NX-DCP ratio is very useful for the diagnosis of HCC. In the ROC analysis

of DCP/NX-DCP ratio, the sensitivity for the diagnosis of HCC was slightly lower than that of conventional DCP at the cuttoff level of 1.4; however, the specificity (91.7%) was much higher than that of conventional DCP (61.5%). The advantage of using the DCP/NX-DCP ratio is that it makes it possible to make differentially diagnose HCC from vitamin K-deficiency with very high specificity. While, a potential disadvantage may be slightly lower sensitivity compared with that of conventional DCP. Therefore, for the screening of HCC in patients at high risk for HCC, such as those with LC/CH, it would be useful to evalutate both the conventional DCP and the DCP/NX-DCP ratio as markers; i.e., to pick up HCC-suspected patients by using the DCP value, and to exclude non-HCC through use of the DCP/NX-DCP ratio. However, it would be better to assess the DCP/NX-DCP ratio in a greater number of patients in future.

Since the DCP/NX-DCP ratio gradually increased as the stage of HCC advanced, and was highly positive (87.9%) in stage IV patients, the ratio would also be useful for the diagnosis of advanced HCC, particularly in stage IV patients. Serum NX-DCP was negative in most HCC patients at stage I to III, but highly positive in stage IV patients, and in particular, those with far advanced HCC with vascular (portal vein) invasion. These results demonstrate that NX-DCP, which appeared in patients with vitamin K-deficiency, was also detected in far advanced HCC patients with vascular invasion, and was associated with a poor prognosis. Recently it was reported that sorafenib induces marked elevation of the serum DCP level in patients with HCC. It was surmised that sorafenib caused vascular regression and subsequently induced DCP production through hypoxia in the HCC tissue.[26,27] We also found that sorafenib produced an apparent elevation in serum DCP levels in patients with HCC, and that the elevated DCP in these patients predominantly consisted of NX-DCP (data not shown). In this context, in the far advanced patients with stage IV HCC, the vascular invasion induced ischemia in the HCC tissue and promoted NX-DCP production, which was closely associated with a poor prognosis. Thus, it was indicated that NX-DCP is a good marker for a poor prognosis in HCC patients.

We evaluated not only the DCP/NX-DCP ratio but also the difference between DCP and NX-DCP (DCP – NX-DCP) for the diagnosis of HCC (data not shown). The ROC analysis revealed

that the AUC of the difference was significantly higher than that for conventional DCP, but was smaller than that for the DCP-NX/DCP ratio. Therefore, we performed subsequent analyses using the DCP/NX-DCP ratio.

Western blot analysis and immunohistochemistry revealed that the antigenicity of DCP in warfarin users differed from that in HCC patients. Since MU-3 recognizes acarboxy 19, 20-Glu residues of the DCP molecule, our results indicate that DCPs from HCC and warfarin users had in common an acarboxy 19, 20-Glu residues. On the other hand, the peptide epitope analysis revealed that P11 recognizes amino acid residues 1 – 5 of the Gla domain (unpublished data). Accordingly, it is surmised that the different reactivity of DCPs between HCC patients and vitamin K-deficient patients is ascribed to the degree of carboxylation of DCP.

In conclusion, the DCP/NX-DCP ratio is very useful for the diagnosis of HCC. In particular, it was useful for discriminating false positive cases, such as obstructive jaundice patients and warfarin users, from HCC patients. Serum NX-DCP levels frequently increased in stage IV HCC patients, particularly those with vascular invasion, and it served as a reliable biomarker for a poor prognosis. DCP from HCC patients was distinct from DCP in vitamin K-deficient patients in terms of antigenicity.

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Figure legends

Figure 1. Serum DCP and NX-DCP levels and DCP/NX-DCP ratio in patients with hepatocellular cancer (HCC) and non-HCC, and the ROC curve for DCP and the DCP/NX-DCP ratio. (A) The positivity rates for conventional serum DCP were high in HCC and obstructive jaundice patients, and in warfarin users. (B) The positivity rates for serum NX-DCP were high in obstructive jaundice patients and warfarin users, but relatively low in HCC patients. (C) The incidence of a DCP/NX-DCP ratio ≥1.4 in HCC patients was significantly higher than the incidence in any of the other groups ($P<0.001$, chi-square test or Fisher's exact test). (D) ROC analysis showed that the AUC of the DCP/NX-DCP ratio was significantly higher than the AUC for DCP, indicating superiority of the former as a diagnostic marker. * $P<0.001$, ** $P\leq0.003$, NS denotes not statistically significant.

Figure 2. Serum DCP and NX-DCP levels, the DCP/NX-DCP ratio in each stage of hepatocellular cancer. (A) The positivity rates for serum DCP showed a significant stepwise increment from stage I to IV (Mantel-Haenzel test, $P<0.05$). However, there was no significant difference among stages. (B) The positivity rates for serum NX-DCP were very low in stage I to III, but very high in stage IV. There were significant differences between stage IV and the other stages (chi-square test, $P<0.001$). (C) The rate of patients with a DCP/NX-DCP ratio ≥1.4 showed a significant stepwise increment from stage I to IV ($P<0.05$). However, there was no sigficant difference among stages.

Figure 3. NX-DCP positivity, clinical parameters, and overall survival of HCC stage IV patients. (A) There was a significant correlation between NX-DCP positivity and vascular invasion in stage IV patients with HCC. (B) The serum NX-DCP levels in patients with vascular invasion were significantly higher than those without vascular invasion. (C) The overall survival time of the NX-DCP (+) group was significantly lower than that of the NX-DCP (-) group in stage IV (log-rank test, $P=0.035$). * $P<0.001$. NS denotes not statistically significant.

Figure 4. Western blot analysis of DCP from a hepatocellular cancer patient, a warfarin user, and an obstructive jaundice patient. Western blot analysis was performed as described in Materials and Methods. (A) The anti-DCP monoclonal antibody MU-3 detected strong bands at 60 kDa, corresponding to DCP protein, but no band for prothrombin protein. (B) P11 detected a strong band at 60 kDa in the warfarin user and obstructive jaundice patient, but a very faint band for DCP protein in the HCC case, and no band for prothrombin protein. (C) Similarly, P16 detected strong bands at 60 kDa in the warfarin user and obstructive jaundice patient, but a very faint band from the HCC patient, and no band for prothrombin protein. The bands at the bottom of each figure were detected by rabbit anti-human prothrombin antibody.

Figure 5. Immunohistochemistry for DCP in HCC tissue and autopsied normal liver tissue from warfarin user using the anti-DCP monoclonal antibody MU-3, P11, or P16. (A) The cancer cells in the HCC tissue were strongly stained by MU-3 but very faintly by P11 and P16. The pre-incubation test using excess amount of DCP protein showed no apparent staining signal. (B) The normal liver cells in the warfarin user were intensely stained by P11 and P16 as well as MU-3. The pre-incubation test showed no apparent staining signal.

Table 1. Characteristics of Subjetcts

	No. of Subjects	Sex (M/F)	Age (Mean ± SD)	ALT (IU/L) (Mean ± SD)	PT(%) (Mean ± SD)
HCC	**176**	**122/54**	**71.2 ± 9.0**	**44.9 ± 37.1**	**96.6 ± 14.4**
Stage I	29	20/9	72.2 ± 7.6	46.5 ± 34.2	90.2 ± 22.6
Stage II	47	28/19	70.6 ± 9.8	42.3 ± 32.4	98.0 ± 14.9
Stage III	67	51/16	71.9 ± 8.9	37.7 ± 20.3	95.4 ± 12.8
Stage IV	33	23/10	69.6 ± 9.1	56.2 ± 55.7	84.9 ± 24.6
Liver cirrhosis	**13**	**7/6**	**64.9 ± 7.6**	**46.1 ± 29.0**	**87.2 ± 16.0**
Child-Pugh A	8	4/4	64.9 ± 8.4	43.8 ± 18.3	96.2 ± 8.3
Child-Pugh B	3	2/1	63.7 ± 3.2	26.7 ± 3.21	82.6 ± 0.57
Child-Pugh C	2	1/1	67.0 ± 12.7	35.5 ± 9.19	59.6 ± 2.83
Chronic hepatitis	**48**	**23/25**	**60.1 ± 13.0**	**38.5 ± 16.1**	**100.8 ± 22.6**
HCV	15	7/8	66.6 ± 7.4	33.4 ± 21.4	102.9 ± 13.8
HBV	8	4/4	51.5 ± 15.8	25.1 ± 92.1	106.0 ± 12.8
Alcoholic	9	7/2	54.4 ± 15.7	39.3 ± 32.5	85.0 ± 38.2
Autoimmune	13	3/10	61.4 ± 10.6	39.0 ± 41.2	100.5 ± 21.6
Drug induced	1	1/0	62.2 ± 7.2	137.0	128.5
NASH	2	1/1	61.0 ± 28.3	24.0 ± 5.66	117.9 ± 15.1
Obstructive jaundice	**12**	**9/3**	**61.4 ± 16.1**	**51.7 ± 47.3**	**72.3 ± 26.1†**
Pancreatic cancer	6	3/3	73.8 ± 4.3	58.8 ± 66.1	65.8 ± 13.1
CCC	5	5/0	71.0 ± 10.9	35.0 ± 25.1	66.1 ± 22.7
Gastric cancer	1	0/1	84.0	77.0	136
Patients receiving warfarin	**10**	**3/7**	**68.3 ± 10.8**	**23.0 ± 12.0**	**47.7 ± 23.7‡**
Healthy volumteers	**30**	**19/11**	**66.6 ± 11.2**	**21.1 ± 11.2**	**116.0 ± 13.8**

†Significantly lower in obstructive jaundice group than in HCC and chronic hepatitis groups ($P<0.05$).
‡Significantly lower in warfarin users than in HCC, liver cirrhosis and chronic hepatitis groups ($P<0.05$).
NASH, nonalcoholic steatohepatitis; CCC, cholangiocellular carcinoma; ALT, alanine aminotransferase; PT, prothrombin time.

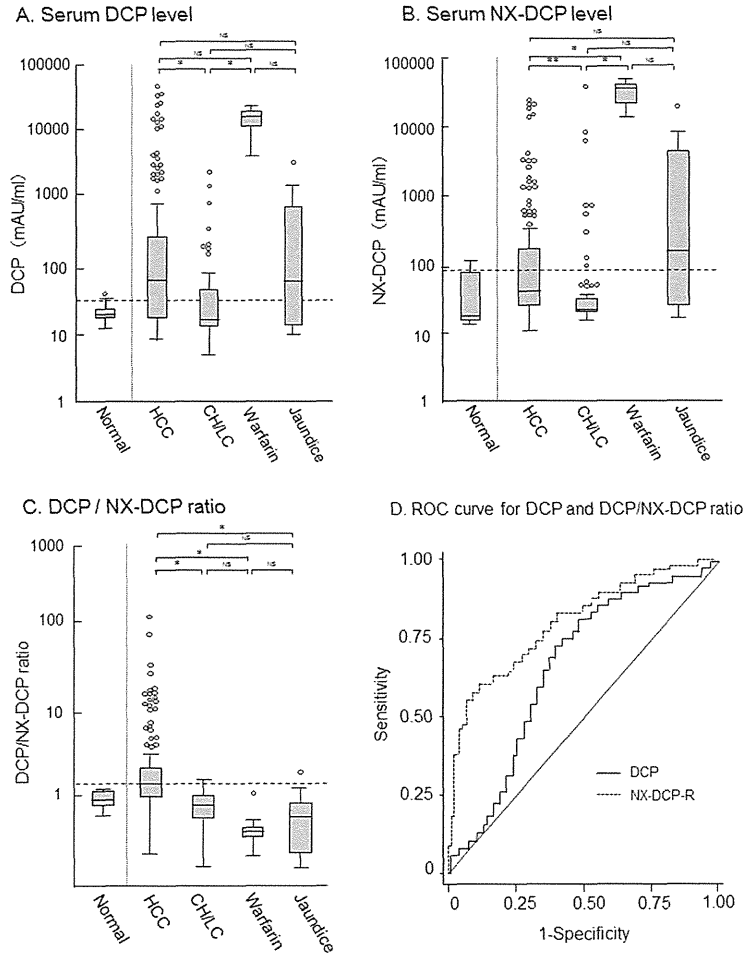

Figure 1.

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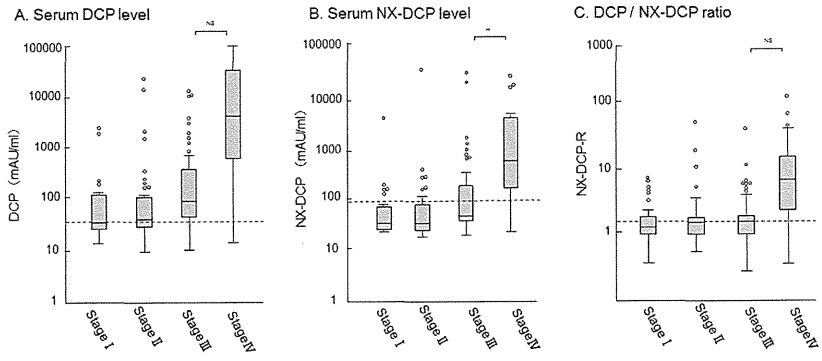


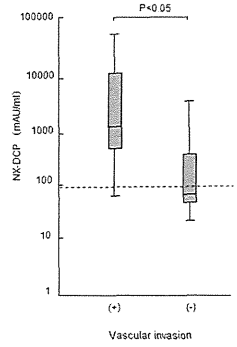
Figure 2.

275x190mm (96 x 96 DPI)

A. NX-DCP positivity and clinical parameters

	NX-DCP (+)	NX-DCP (-)	P-value
Background liver disease			
HBV	5	2	
HCV	13	5	0.99
others	6	2	
Child-Pugh classification			
A	11	6	
B	7	2	0.53
C	6	1	
Ascites			
(+)	12	4	0.65
(-)	11	6	
ALT(IU/L)			
35≤	11	5	0.71
<35	13	4	
Total bilirubin (mg/dL)			
≥2.0	17	6	>0.99
2.0<	7	3	
PT(%)			
≥70	21	7	0.60
70<	3	2	
Vascular invasion			
(+)	16	2	<0.05
(-)	8	7	

B. NX-DCP and vascular invasion



C. Overall Survival

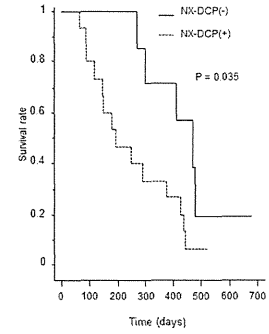


Figure 3.

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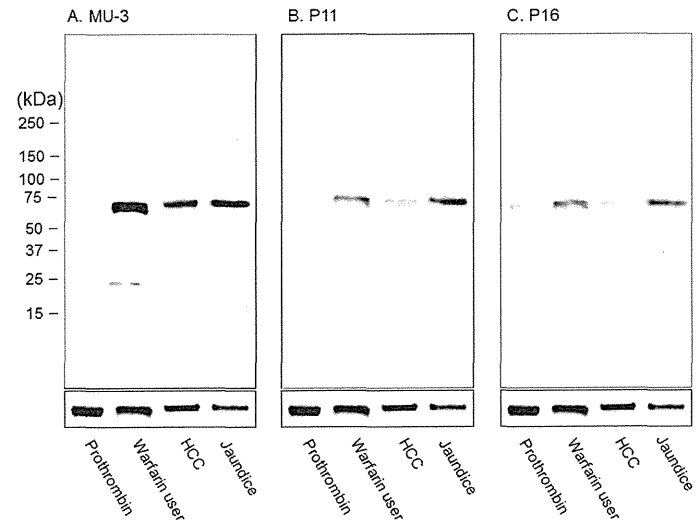

Figure 4

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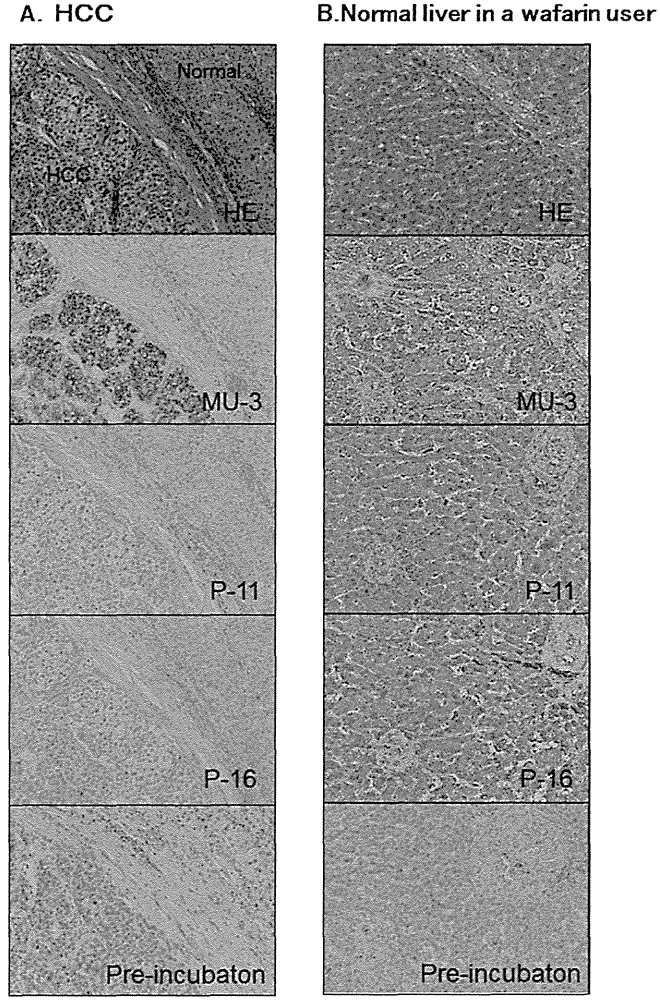

Figure 5

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ORIGINAL ARTICLE

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

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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

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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

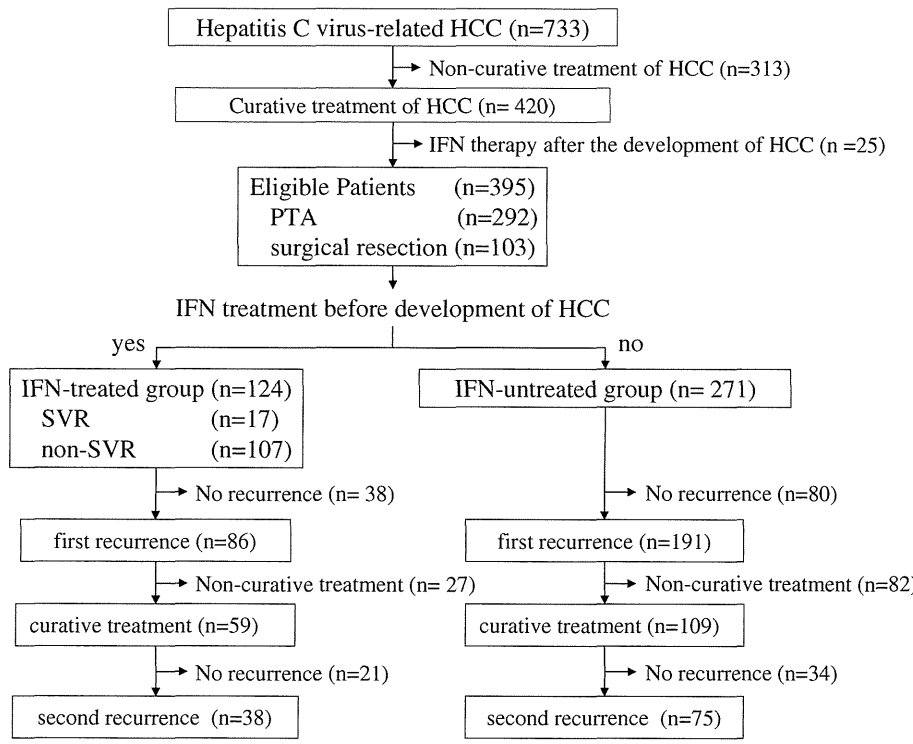

Fig. 1 Schematic presentation of patients with HCV-related hepatocellular carcinoma (HCC). Patients with HCV-related HCC who were diagnosed at Okayama University Hospital were classified into three groups according to their previous IFN treatment and response to that treatment. One hundred twenty-four 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