TABLE 2 Odds Ratio for Massive Blood Loss (>1000mL) Evaluated by Univariate Analysis

Factors	Odds ratio 95%	Confidence interval	p value
Age (per 1 year)	0.928	0.870-0.991	0.0248
Tumor size (>4cm)	4.857	1.636-14.424	0.0044
Portal invasion	11.429	1.908-68.451	0.0076
Hepatic vein invasion	14.536	4.396-48.067	<0.0001
Major hepatectomy	31.154	3.364-288.477	0.0248
Operation time (per 1 min)	1.013	1.006-1.020	0.0003

TABLE 3 Adjusted Odds Ratio for Massive Blood Loss (>1000mL) Evaluated by Multivariate Analysis of Preoperative Factors

Factors	Adjusted odds ratio	95% Confidence interval	p value
Age	0.964	0.883-1.053	0.421
Tumor size (>4cm)	1.707	0.386-7.547	0.481
Portal invasion	11.798	1.335-104.252	0.0264
Hepatic vein invasion	12.274	2.885-52.224	0.0007

patients, tumor thrombus at the bifurcation of the right and left portal veins was removed. Of the six patients who underwent major hepatectomy, five were younger than the mean age for all subjects. Six patients who underwent major hepatectomy had at least one of three tumor-related risk factors (large tumor, portal involvement and/or hepatic vein involvement). Proportions of patients with a large tumor (>4cm) or portal involvement were significantly higher in major hepatectomy patients than in minor hepatectomy patients ($p=0.0240$ and $p<0.0001$ respectively). The proportion of patients with hepatic vein involvement tended to be higher in the major hepatectomy group than in the minor hepatectomy group ($p=0.0807$). Thus, major hepatectomy typically was performed in young patients with advanced HCC. Proportions of patients with portal involvement or hepatic vein involvement were significantly higher in cases with a long operation time (≥360 min) than in cases with a shorter operation ($p=0.0023$ and $p=0.0026$ respectively). The proportion of patients with large tumors tended to be higher in cases with a long operation time ($p=0.0697$).

By univariate analysis, the OR for massive blood loss (>1000mL) increased as age decreased (**Table 2**). Large tumor (>4cm), major hepatectomy, portal involvement, hepatic vein involvement, and prolonged operation time were significant risk factors for massive blood loss. We next calculated an adjusted OR using only preoperative variables to predict risk for massive blood loss before surgery. Multivariate analysis identified hepatic vein involvement and portal vein involvement as independent risk factors for massive blood loss (**Table 3**).

Although blood loss exceeded 1000mL in 4 patients in group B, the operation could be performed safely using only autologous blood transfusion without homologous transfusion. Of these 4 patients, 3 had hepatic vein involvement. In the other 13 group B patients, blood loss was less than 1000mL.

DISCUSSION

Youths, large tumors (>4cm), portal involvement, hepatic vein involvement, major hepatectomy, and prolonged operation time were risk factors for massive blood loss by our present univariate analysis, while portal involvement and hepatic vein involvement were independent risk factors for massive blood loss by multivariate analysis including only preoperative factors. Several previous studies identified factors affecting blood loss during liver resection as tumor size, number of tumors, tumor stage, macroscopic vascular invasion, major hepatectomy, central venous pressure (CVP), and operation time (3,20-23).

Although young patients had a significantly greater risk by univariate analysis, most patients who underwent major hepatectomy were young patients with portal involvement and/or hepatic vein involvement. Thus, amount of blood loss was affected by type of operation and advanced HCC stage rather than age itself. Proportions of patients with large tumors, portal involvement, or hepatic vein involvement were higher in major hepatectomy patients than in minor hepatectomy patients, and higher in association with long than short operations. Accordingly, prolonged major hepatectomy typically was performed for large, advanced HCC with portal involvement and/or hepatic vein involvement, resulting in massive blood loss.

By multivariate analysis using preoperative factors, portal involvement and hepatic vein involvement were independent risk factors for massive blood loss. In two patients, portal thrombectomy, a procedure with serious risk of hemorrhage, was required by portal tumor thrombus situated at the bifurcation of the right and left portal veins. Massive bleeding usually occurs from the major hepatic veins (right, middle, and left hepatic veins), inferior vena cava (IVC), and direct tributaries of the major hepatic veins or IVC. In some studies, maintenance of a low CVP (below 5 cm H2O) was effective in reducing blood loss during hepatectomy (20,23-26), although in the experiences of Hasegawa *et al.* (27) decreasing CVP by hypoventilation did not reduce blood loss during liver resection. Extrahepatic control of the hepatic veins and meticulous ligation of small tributaries entering the hepatic veins and IVC is important for preventing bleeding during parenchymal dissection.

Kajikawa *et al.* (12) performed transfusion when blood loss exceeded 2000mL in patients with cirrhosis underwent liver resection, while Wu *et al.* (3) found that no blood transfusion was needed in some patients even when estimated blood loss exceeded 2000mL. Miyagawa *et al.* (28) administered transfusions in liver resection when estimated blood loss exceeded 1500mL. In our present study, mean blood loss in group C was 1432±773mL, with blood loss exceeding 1000mL in 11 of 16 patients; the largest blood loss not requiring homologous blood transfusion was 1800mL. Thus, need for transfusion was likely when blood loss

exceeded 1000mL. In group B, blood loss exceeded 1000mL in 4 of 17 patients. Assuming that blood transfusion would be necessary only when blood loss exceeded 1000mL, autologous blood transfusion was not necessary in the other 13 patients. In addition, autologous blood transfusion might not have been necessary in all of the 4 patients who received it, since the volume lost ranged from 1045 to 1855mL. Preoperative storage of autologous blood therefore is not necessary for most patients, although autologous blood should be useful for patients with HCC who have risk factors for massive blood loss. In this study, portal involvement and hepatic vein involvement were independent risk factors for such loss. In patients with major venous involvement, autologous blood is useful in avoiding homologous blood transfusion.

In conclusion, patients with cirrhosis who have portal and/or hepatic vein tumor involvement are at a risk for massive blood loss during liver resection for HCC. When specific contraindications are not present, autologous blood storage is indicated in such patients.

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SURGERY TODAY

Surgical Treatment for Hepatocellular Carcinoma Detected After Successful Interferon Therapy

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Abstract

Purpose. Interferon therapy suppresses the development of hepatocellular carcinoma (HCC) and tumor recurrence after a resection of HCC in patients with chronic hepatitis C. However, the value of a liver resection and which method is best for the treatment of HCC detected after successful interferon therapy remains to be clarified. The risk factors for tumor recurrence after a liver resection for HCC detected after successful interferon therapy were investigated to determine the appropriate operative method for such HCC.

Methods. Risk factors including the clinicopathologic findings and the operative methods for tumor recurrence were evaluated by univariate and multivariate analyses in 24 patients who underwent liver resection for HCC detected after successful interferon therapy (sustained viral response or biochemical response).

Results. According to a univariate analysis, large tumor (>2 cm, $P = 0.0326$), multiple tumors ($P = 0.0372$), nonanatomic resection ($P = 0.0103$), and positive surgical margin (<5 mm of a free surgical margin, $P = 0.0245$) were possible risk factors for short tumor-free survival time after surgery. A multivariate analysis showed that large tumor ($P = 0.0407$), nonanatomic resection ($P = 0.0215$), and positive surgical margin ($P = 0.0253$) were independent risk factors for a short tumor-free survival time after surgery.

Conclusion. An anatomic resection with an appropriate surgical margin (≥5 mm of a free surgical margin) is recommended for patients with HCC detected after successful interferon therapy.

Key words Hepatocellular carcinoma · Interferon therapy · Liver resection · Anatomic resection · Chronic hepatitis C

Introduction

Hepatitis C virus (HCV) is one of the most common causes of hepatocellular carcinoma (HCC). The results of treatment for HCV-related HCC are still unsatisfactory because of a high rate of HCC recurrence and the progression of underlying chronic hepatitis and cirrhosis.[1–3] Recently, interferon (IFN) therapy has been shown to improve the liver function and histology, while suppressing the development of HCC in patients with chronic hepatitis C.[4–11] Previous studies have indicated that HCC is less likely to develop in patients in whom IFN effectively normalized the serum alanine aminotransferase (ALT) activity, even when HCV RNA did not disappear.[9,10] In addition, active hepatitis is a risk factor for tumor recurrence after a liver resection for HCV-related HCC[1,2] and postoperative IFN therapy suppresses recurrences while prolonging the survival time after treatment for HCC.[12–15]

Although HCCs can even develop in patients successfully treated with IFN,[16] we previously reported the tumor-free survival rate to be significantly higher in patients in whom IFN therapy was effective than in patients in whom IFN therapy was not effective and in patients who did not undergo IFN therapy.[17,18] Therefore, a liver resection offers hope for the low incidence of postoperative recurrences in HCC patients when previous IFN therapy has controlled their active hepatitis associated with HCV. However, the value of a liver resection for HCC detected after successful IFN therapy is still unclear. In this study, the risk factors for tumor recurrence after a liver resection for HCC detected after successful IFN therapy were investigated to determine the appropriate operative method for such HCC.

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Patients and Methods

Patients

The subjects in this study consisted of 24 patients who underwent a curative liver resection for HCC detected after successful IFN therapy. HCC was detected from 3 months to 13 years (mean, 4 years 5 months) after the end of IFN therapy. Curative surgery was defined as a complete removal of all macroscopic tumor masses and no histologic evidence of any tumor cells along the parenchymal transection line. Before the detection of HCC, IFN-α was administered in 10 patients, IFN-α2a in 1 patient, IFN-α2b in 11 patients, and IFN-β in 2 patients. The response to IFN therapy was classified based on the changes in the HCV RNA levels and the serum ALT activity during and immediately after IFN administration, and for at least 1 year after IFN therapy. Nineteen patients obtained a sustained viral response, which was defined as return of the ALT activity to within the reference range and no detectable serum HCV RNA for at least 1 year after IFN therapy. A biochemical response, which was defined as a normalized ALT activity for at least 1 year after IFN therapy with or without a transient disappearance of serum HCV RNA, was demonstrated in 5 patients. A hemihepatectomy, bi-segmentectomy, and segmentectomy, are all assumed to be anatomic resections. The median follow-up from the operation until the detection of HCC recurrence or study endpoint (September 30, 2006) was 678 days (range, 125–4580).

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

Detection of recurrences

The serum concentration of α-fetoprotein and protein induced by the absence of both vitamin K and antagonist II were measured 1 month after surgery and then every 3 months thereafter. Ultrasonography, computed tomography, chest radiography, or some combination of these tests were done 1 month after surgery and then every 3 months thereafter. When a recurrence of the HCC was strongly suspected based on the findings of tumor markers or imaging, selective hepatic angiography, ultrasound-guided biopsy, or both was conducted to establish a definitive diagnosis.

Histology

The guidelines of the Liver Cancer Study Group of Japan[19] were used to categorize the histologic findings. The tumor number was determined by macroscopic and microscopic examinations. The histologic grade of differentiation (well, moderate, or poor) of HCC was determined according to a modification of Edmondson and Steiner.[20] The grade (grade of active hepatitis) and stage (degree of hepatic fibrosis) in the noncancerous hepatic tissue specimens were determined based on the score of the histologic activity index,[21] which was determined by four events, i.e., periportal necrosis with or without bridging necrosis, intralobular degeneration with focal necrosis, portal inflammation, and fibrosis.

Statistics

The survival rates were calculated by the Kaplan–Meier method and then were compared with the log-rank test. The tumor-free survival time was measured from the date of resection until the detection of a recurrent tumor or the end point of this study (September 30, 2006) in patients who did not develop a recurrence. Cox's proportional hazard model with a stepwise variable selection was used for a multivariate analysis. A P value of less than 0.05 was considered to be significant. The variables were selected based on the findings of previous studies or our own clinical experience. The variables chosen were age (≥65 or <65), gender, history of alcohol abuse (intake of at least 86 g of ethanol daily for at least 10 years),[22] a history of blood transfusion, anti-hepatitis B core antibody (positive or negative), total bilirubin (≥1 or <1 mg/dl), albumin concentration (≥4.0 or <4.0 g/dl), indocyanine green retention rate at 15 min ($ICGR_{15}$, ≥10 or <10%), a platelet count (≥10×10^4 or <10×10^4/μl), serum α-fetoprotein concentration (>20 or ≤20 ng/ml), the largest diameter of the main tumor (>2 or ≤2 cm), the degree of differentiation of the main tumor (well-, moderately vs. poorly differentiated HCC), the number of tumors (single or multiple including intrahepatic metastasis), microscopic portal invasion, the grading score (0, 1, or 2 to 4), the staging score (0 to 2 or 3, 4), the operative methods (anatomic or nonanatomic resection), and the surgical margin. When the surgical free margin based on a pathologic examination was less than 5 mm, it was defined as a positive surgical margin. The statistical analysis was performed using the StatView program (SAS Institute, Cary, NC, USA).

Results

Of the 24 patients, HCC recurred in 8 patients. In 5 of the 8 patients, multiple recurrent tumors were detected in the remnant liver within 3 years after the operation. In 3 other patients, a solitary recurrent tumor was detected in the remnant liver. In 2 of the 3 patients, the recurrent tumor was treated with microwave coagula-

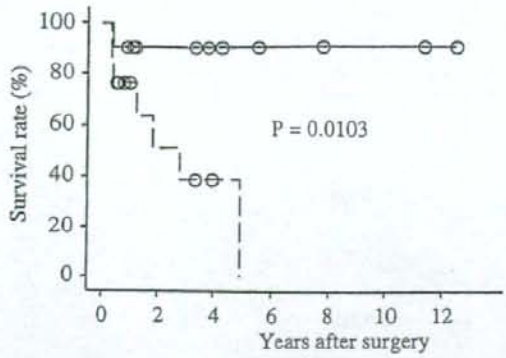

Fig. 1. Tumor-free survival rate after a resection of hepatocellular carcinoma in patients who underwent an anatomic resection or a nonanatomic resection. *Continuous line*, anatomic resection (n = 11); *dotted line*, nonanatomic resection (n = 13)

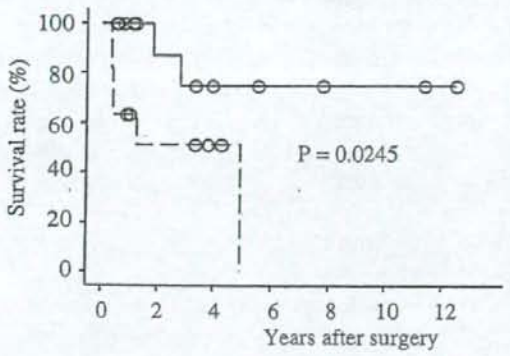

Fig. 2. Tumor-free survival rate after a resection of hepatocellular carcinoma in patients with a positive or negative surgical margin. *Continuous line*, negative surgical margin (n = 13); *dotted line*, positive surgical margin (n = 11)

tion therapy or radiofrequency ablation therapy and the patients are still alive without HCC recurrence (6 years 4 months after the therapy, 1 month after the therapy, respectively). In the other patient, a second liver resection was performed and the patient is alive without HCC recurrence (2 years 6 months after the second operation). Based on a univariate analysis, large size tumor (P = 0.0326), multiple tumors (P = 0.0372), nonanatomic resection (P = 0.0103), and positive surgical margin (P = 0.0245) were possible risk factors for a short tumor-free survival time after the operation (Table 1). The tumor-free survival rates in patients who underwent an anatomic resection or nonanatomic resection and those in patients with positive or negative surgical margin are shown in Figs. 1 and 2. A multivariate analysis showed a large tumor (risk ratio, 0.015; 95% confidence interval, 0.000257–0.836; P = 0.0407), a nonanatomic resection (0.0396, 0.00252–0.622, P = 0.0215), and a positive surgical margin (0.067, 0.006–0.716, P = 0.0253) to all be independent risk factors for a short tumor-free survival time after the operation (Table 2). In the 8 patients with HCC recurrence, 7 patients had a larger tumor (>2 cm) and 5 patients had multiple tumors. Seven patients underwent a nonanatomic resection while another patient with portal invasion in the left portal vein underwent an extended left lobectomy. The surgical margin was positive in 6 of the 8 patients.

Discussion

Our previous study showed that an advanced age, HCV viremia, elevated aspartate aminotransferase (AST) activity, elevated ALT activity, large tumor size, multiple tumors, moderately or poorly differentiated HCC, portal invasion, and high-grade score (active hepatitis) were possible risk factors for HCC recurrence after a liver resection for HCV-related HCC.[3] Some recurrences are thought to result from intrahepatic metastases originating from the primary cancer and some from multicentric (multifocal) carcinogenesis after surgery.[23–25] Studies of the risk factors for multicentric occurrence of HCC or recurrence after resection of HCC suggest that chronic active hepatitis and hepatic fibrosis are important factors in multicentric carcinogenesis after surgery.[2,3,26,27] It is thus important to consider the potential for hepatic carcinogenesis that is related to active hepatitis and hepatic fibrosis to determine the appropriate treatment for HCC patients with chronic hepatitis C.[28] On the other hand, many studies have shown that IFN therapy improves active hepatitis and hepatic fibrosis, thus resulting in a decreased incidence of HCC development.[4–10] Several studies have also confirmed that IFN therapy suppresses the incidence of HCC recurrence after treatment for HCV-related HCC.[12,13,15] Although HCC develops even in patients who were treated successfully with IFN, the prognosis after treatment for such HCC is better than in patients with active hepatitis caused by HCV.[14,17,20] These findings indicate that successful IFN treatment for HCV suppresses the potential for carcinogenesis while decreasing the development of multicentric carcinogenesis after surgery for primary HCC.

In this study, 8 patients had HCC recurrence after the operation. Of the 8 patients, 7 had a large-sized tumor (>2 cm) and 5 had multiple tumors. Seven patients underwent a nonanatomic resection and the surgical margin was positive in 6 patients. A univariate analysis showed the risk factors for recurrence include tumor

Table 1. Tumor-free survival rate after a resection of hepatocellular carcinoma

Variable (*n*)	Survival rate (%)			*P* value
	4 years	8 years	12 years	
Age (years)				
≥65 (10)	67	67	67	0.145
<65 (14)	70	0	0	
Gender				
Male (18)	72	54	54	0.209
Female (6)	33	—	—	
Alcohol abuse				
Yes (4)	51	51	—	0.771
No (20)	68	52	52	
Blood transfusion				
Yes (6)	67	—	—	0.925
No (18)	58	47	47	
Anti-hepatitis B core antibody				
Positive (13)	73	73	73	0.300
Negative (11)	54	—	—	
Total bilirubin (mg/dl)				
≥1.0 (11)	76	—	—	0.255
<1.0 (13)	55	37	37	
Albumin (g/l)				
≥4.0 (16)	81	55	—	0.0884
<4.0 (8)	38	38	38	
$ICGR_{15}$ (%)				
≥10 (17)	62	62	62	0.889
<10 (7)	67	—	—	
Platelet count ($\times 10^4/mm^3$)				
≥10.0 (21)	73	58	58	0.358
<10.0 (3)	—	—	—	
α-Fetoprotein (ng/ml)				
>20.0 (12)	67	—	—	0.958
≤20.0 (12)	63	63	63	
Tumor size (cm)				
≥2.0 (15)	37	37	—	0.0326
<2.0 (9)	100	67	67	
Differentiation of tumor				
Well or moderately (11)	77	63	63	0.0918
Poorly (13)	52	—	—	
Tumor number				
Single (14)	73	73	73	0.0372
Multiple (10)	47	—	—	
Microscopic portal invasion				
Yes (11)	73	—	—	0.922
No (13)	63	42	42	
Grading score				
0, 1 (11)	82	—	—	0.428
2–4 (13)	57	43	43	
Staging score				
0–2 (11)	76	—	—	0.581
3, 4 (13)	56	56	56	
Operative method				
Anatomic (11)	91	91	91	0.0103
Nonanatomic (13)	38	0	0	
Surgical margin				
Positive (11)	51	0	0	0.0245
Negative (13)	75	75	75	

$ICGR_{15}$, indocyanine green retention rate at 15 min

Table 2. Independent risk factors for short tumor-free survival time after liver resection for hepatocellular carcinoma (multivariate analysis)

Variable	Risk ratio	95% confidence interval	*P*
Large tumor (>2 cm)	0.015	0.000257–0.836	0.0407
Nonanatomic resection	0.0396	0.00252–0.622	0.0215
Positive surgical margin	0.067	0.006–0.716	0.067

factors such as large HCC and multiple tumors as well as operative factors such as a nonanatomic resection and a positive surgical margin. A multivariate analysis showed a large tumor, a nonanatomic resection, and a positive surgical margin to be independent risk factor for short tumor-free survival time after the operation. The hepatitis activity was not a risk factor in patients who were treated successfully with IFN therapy. In this study, multiple recurrent tumors were detected in the remnant liver within 3 years after the operation in 5 of 8 patients with an HCC recurrence; the tumors were thought to be intrahepatic metastases originating from the primary tumor in at least 5 of the 8 patients.[23,25] These findings indicate that HCC recurrences after surgery are mainly due to intrahepatic metastases that had not been resected in such patients; successful IFN therapy might thus have decreased the incidence of multicentric carcinogenesis after the resection of the primary HCC. Anatomic resections along the portal system can remove occult intrahepatic metastases, which spread mainly through the portal vein. On the other hand, a nonanatomic resection and ablation therapy including microwave coagulation therapy and radiofrequency ablation therapy are thus considered to be disadvantageous from the stand point of eradiating such intrahepatic metastases.[30] A liver resection with an appropriate surgical margin also can remove small metastatic lesions surrounding the main tumor. Therefore, an anatomic resection with a sufficient surgical margin is recommended in HCC patients who were treated successfully by IFN therapy. Recently, the value of an anatomic resection for HCC has been reported.[30] The value of an anatomic resection should be emphasized especially in patients with HCC detected after successful IFN therapy.

On the other hand, two patients who underwent microwave coagulation therapy or a second liver resection for a solitary recurrent tumor have survived without any HCC recurrence for a long time after treatment for their first recurrence. In such patients, successful IFN therapy possibly suppressed the risk of carcinogenesis, thus inducing a prolonged tumor-free survival time after treatment for their first recurrence. Similar findings have been reported by Shiratori et al.[15]

In conclusion, an anatomic resection with an appropriate surgical margin is recommended for patients with HCC detected after successful IFN therapy. However, the number of patients in this study was too small to yield any definitive conclusions.

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Original Paper

A Histopathological Study on Combined Hepatocellular and Cholangiocarcinoma: Cholangiocarcinoma Component is Originated from Hepatocellular Carcinoma

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KEY WORDS:
Combined hepatocellular and cholangiocarcinoma; Hepatic neoplasm; Ki-67 labeling index; Metaplasia

ABBREVIATIONS:
Alpha-Fetoprotein (AFP); Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II); Carcinoembryonic Antigen (CEA); Carbohydrate Antigen (CA19-9); Hepatitis B virus surface Antigen (HBsAg); Hepatitis C Virus Antibody (HCVAb)

ABSTRACT

Background/Aims: Combined hepatocellular and cholangiocarcinoma of the liver is relatively infrequent, and its pathogenesis remains obscure. The aim of this study is to investigate its clinical and pathological features and proliferative activity.

Methodology: In this study, we investigated the histopathological features, Ki-67 labeling index, and p53 immunohistochemistry of 18 surgically resected cases of combined hepatocellular and cholangiocarcinoma among 1102 consecutive cases of surgically resected primary liver cancers. All tumors were compatible with the WHO definition of this tumor.
Microscopically, we classified the cases into the following three categories according to the arrangement of the hepatocellular carcinoma and cholangiocarcinoma components; 1) Type I in which hepatocellular carcinoma and cholangiocarcinoma formed nodules that could easily be distinguished from each other, 2) Type II in which the both components were finely mixed, so that the two components were almost indistinguishable, and 3) Type III in which the tumors had lobular structures with hepatocellular carcinomas existing centrally and cholangiocarcinomas existing peripherally.

Results: Microscopically, the tumors were classified into type I 7 tumors, type II 5 tumors, and type III 6 tumors. In one case of type I, well differentiated hepatocellular carcinoma demonstrated cholangiocarcinoma in "nodules-in-nodules" fashion. The average of Ki-67 labeling index of hepatocellular carcinoma component of combined hepatocellular and cholangiocarcinoma was 4.4±3.4% and the index of cholangiocarcinoma component was 11.0±8.5%, which is significantly higher than that of the hepatocellular carcinoma component. On p53 immunohistochemistry, 5 of 18 cases (29.4%) were positive. In one case, the cholangiocarcinoma component was positive for p53, but the hepatocellular carcinoma component was negative. In the other 4 cases, both the hepatocellular carcinoma and cholangiocarcinoma components were positive.

Conclusions: Microscopically, type III seems to be a feature of metaplasia or proliferation of bipotential progenitor cells. Metaplasia of hepatocellular carcinoma to intrahepatic cholangiocarcinoma is assumed to be one of the pathogenic pathways of combined hepatocellular and cholangiocarcinoma.

INTRODUCTION

Primary liver cancers can be classified either into hepatocellular carcinoma, originating from the hepatocytes, or intrahepatic cholangiocarcinoma, originating from the intrahepatic bile duct epithelium. However, there are occasional cases that present both hepatocellular carcinoma and intrahepatic cholangiocarcinoma in the same liver. Such tumors are designated as combined hepatocellular and cholangiocarcinoma. But histological criteria for the combined hepatocellular and cholangiocarcinoma have not been universally agreed upon. Allen *et al.* (1) subclassified combined hepatocellular and cholangiocarcinoma into 1: collision type, 2: combined type, and 3: mixed type. However, from the clinicopathological features, it would be better to exclude the collision type from combined hepatocellular and cholangiocarcinoma. In the World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of the Digestive System, combined hepatocellular and cholangiocarcinoma is diagnosed when lesions containing unequivocal elements of both hepatocellular and cholangiocar-

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cinoma that are intimately admixed (2).

In 1985, Goodman *et al.* (3) classified the combined hepatocellular and cholangiocarcinoma into the following three categories: 1) Hepatocellular carcinoma and intrahepatic cholangiocarcinoma could be clearly distinguished. 2) Cholangiocarcinoma lesions with tubular pattern were contiguous to the hepatocellular carcinoma lesions with a trabecular or solid pattern and which shares hepatocellular carcinoma and cholangiocarcinoma transitional features. 3) Hepatocellular carcinoma and cholangiocarcinoma are almost indistinguishable, the tumor could be interpreted as either intrahepatic cholangiocarcinoma or poorly differentiated hepatocellular carcinoma, and these were therefore considered as an intermediate type between hepatocellular carcinoma and intrahepatic cholangiocarcinoma cases.

Combined hepatocellular and cholangiocarcinoma is relatively infrequent, and its clinicopathological features are still obscure. In this study, we investigated the clinicopathological features of 18 surgically resected combined hepatocellular and cholangiocarcinoma classified into Allen type 2 and 3. Also, we evaluated the proliferative activity using the immunostaining of Ki-67 between the hepatocellular carcinoma component and cholangiocarcinoma component.

METHODOLOGY

Among 1102 consecutive cases of primary liver cancers surgically resected at Osaka City University Hospital and Osaka University Hospital, between January 1986 and December 2000, 18 cases were combined hepatocellular and cholangiocarcinoma, 1036 cases were hepatocellular carcinoma, 45 cases were intrahepatic cholangiocarcinoma. The 18 cases of combined hepatocellular and cholangiocarcinoma were examined in this study. The resected liver specimens were fixed in 10% neutralized buffered formalin immediately after hepatectomy, cut into slices, embedded in paraffin, prepared into 4-μm sections and were routinely stained with hematoxylin-eosin, diastase digested periodic acid-Schiff (PAS), and alcian blue. In addition, all 18 cases were immunohistochemically examined for cytokeratin 7 (OV-TL 12/30, DAKO, Glostrup, Denmark), cytokeratin 19 (RCK108, DAKO, Glostrup, Denmark), alpha fetoprotein (rabbit polyclonal antibody, DAKO, Glostrup, Denmark), fibrinogen (rabbit polyclonal antibody, DAKO, Glostrup, Denmark), p53 (DO-7, DAKO, Glostrup, Denmark), and Ki-67 antigen (rabbit polyclonal antibody, DAKO, Glostrup, Denmark). Methods of immunohistochemistry, other than Ki-67, were the labeled streptoavidin-biotin method. The method of immunohistochemistry of Ki-67 was the enhanced polymer one-step staining method.

We identified the hepatocellular carcinoma component based on the following criteria. 1) The trabecular structure surrounded by sinusoid-like vessels. 2) Eosinophilic granularity of the cytoplasm. 3) Bile production. In some cases the immunohistochemical staining of alpha-fetoprotein or fibrinogen was required to help in recognizing the hepatocellular carcinoma component. On the other hand, the cholangiocarcinoma component was defined as follows: 1) Tumor cells were cuboidal or columnar, and had rather basophilic cytoplasm. 2) The nuclei were rather oval or spindle shaped 3) Tumor cells were positive in either alcian blue stain or diastase digested PAS stain (**Figure 1**).

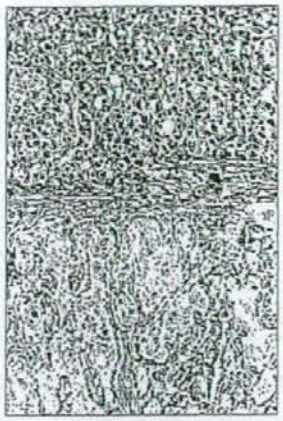

FIGURE 1
The microscopic finding of a combined hepatocellular and cholangiocarcinoma. The upper side of the figure shows the hepatocellular carcinoma component of the combined hepatocellular and cholangiocarcinoma. It shows the trabecular structure surrounded by sinusoid-like vessels, and the eosinophilic granularity of the cytoplasm. The lower side of the figure shows the cholangiocarcinoma component. Tumor cells are cuboidal or columnar, and have rather basophilic cytoplasm (HE).

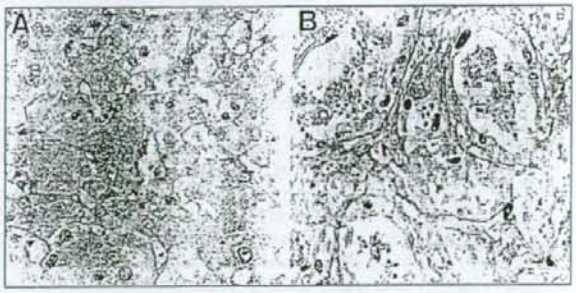

FIGURE 2 Immunohistochemistry for Ki-67. **(A)** The area of a hepatocellular carcinoma component. The Ki-67 labeling index was 6.7% in this area. **(B)** The area of a cholangiocarcinoma component. The Ki-67 labeling index was 13.5% in this area.

For the assessment of the Ki-67 labeling index in each case, the sections were counted at high power magnifications; 1000 or more nuclei were counted in the hepatocellular carcinoma components and cholangiocarcinoma components, and the number of cells showing positive nuclear staining, regardless of staining intensity, was recorded (**Figure 2**). Two independent observers without prior knowledge evaluated the Ki-67 labeling index. Necrotic areas were not included in the counting. For comparison with the combined hepatocellular and cholangiocarcinoma, 11 cases of moderately differentiated hepatocellular carcinoma and 8 cases of intrahepatic cholangiocarcinoma were also stained with Ki-67 and counted for the number of cells showing positive nuclear staining in the same way.

The clinical backgrounds regarding the average age, male: female ratio, hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (HCVAb) positivity, alpha-fetoprotein level, and the presence of chronic liver diseases were analyzed. Disease-free survival was mea-

sured from the date of hepatic resection to the date when recurrent disease was diagnosed or absence of detectable tumor, to the date of death or the date of last follow-up.

The Student's *t*-test was used for the statistical analysis of categorical data. A level of $P<0.05$ was considered statistically significant.

RESULTS

Clinical Features and Prognosis

Of 1102 patients with primary liver cancers, 1036 (94.0%) patients had hepatocellular carcinoma, 45 (4.0%) had intrahepatic cholangiocarcinoma, 3 (0.3%) had both hepatocellular carcinoma and intrahepatic cholangiocarcinoma independently, and 18 (1.6%) had combined hepatocellular and cholangiocarcinoma. The average patient age was 61.0 years for hepatocellular carcinoma, 60.8 years for intrahepatic cholangiocarcinoma, 62.3 years for collision type hepatocellular carcinoma and intrahepatic cholangiocarcinoma, and 58.2 years for combined hepatocellular and cholangiocarcinoma.

TABLE 1 Clinicopathological Findings of Combined Hepatocellular and Cholangiocarcinoma

Case	Age	Sex	AFP (ng/mL)	PIVKAII (AU/mL)	CEA (ng/mL)	CA19-9 (U/mL)	HBsAg	HCV
1	56	M	8.5	1.18	5.1	356	+	+
2	61	F	526.5	0.07	2.4	23	–	+
3	66	F	49.1	NA	11.8	NA	–	+
4	30	M	1300	0.09	NA	NA	+	–
5	61	M	13.6	0.06>	NA	NA	–	+
6	58	M	3.9	0.06>	NA	NA	–	–
7	41	M	5	NA	NA	NA	–	–
8	56	M	5	NA	20	NA	–	–
9	57	M	5	4.037	4.3	26	–	–
10	47	F	5	0.0625	1.4	504	+	–
11	65	M	6	NA	15.6	NA	–	NA
12	64	M	6	NA	NA	NA	–	NA
13	61	M	117	359	27	50	–	–
14	52	M	5	69	13	NA	+	–
15	74	M	3297	9408	3	37	–	–
16	58	M	6	71	5	NA	–	+
17	72	F	1190	1321	NA	NA	–	+
18	69	M	75	25	4.5	NA	–	+

TABLE 2 Histopathology and Ki-67 Labeling Index of Combined Hepatocellular and Cholangiocarcinoma

Case	Non-tumor liver	Predominant part of tumor	Type of arrangement	Diameter (cm)	Ki-67 Labeling Index HCC (%)	Ki-67 Labeling Index CC (%)
1	CH	HCC	3	9	2.79	7.2
2	CH	HCC	3	5.7	3.7	9.77
3	Cirrhosis	CC	2	3	12.66	3.79
4	NL	HCC	2	3.8	1.61	1.58
5	CH	CC	3	5	2.38	9.95
6	NL	HCC	1	17	7.66	34.07
7	NL	UNDIF	2	6.5	NA	NA
8	Cirrhosis	CC	3	2.4	2.1	7.23
9	CH	HCC	2	5	0.85	12.15
10	CH	CC	1	5.6	NA	NA
11	NL	CC	1	12.5	2.98	5.99
12	Cirrhosis	HCC	1	6.8	1.07	2.71
13	NL	UNDIF	2	9	5.61	5.61
14	CH	CC	3	6.9	2.77	4.6
15	CH	HCC	1	6.8	NA	NA
16	CH	HCC	3	4.3	NA	NA
17	Cirrhosis	HCC	1	3.7	8.9	15.71
18	Cirrhosis	CC	1	3.1	6.72	13.5

NA: not available; CH: chronic hepatitis; HCC: part of hepatocellular carcinoma; CC: part of cholangiocarcinoma; UNDIF: part of undifferentiated carcinoma; NL: normal liver.

Clinicopathological findings of the 18 cases are summarized in **Table 1**. The male to female ratio was 5.2:1 for hepatocellular carcinoma, 2.5:1 for intrahepatic cholangiocarcinoma, all three were male for collision type hepatocellular carcinoma and intrahepatic cholangiocarcinoma, and 3.5:1 for combined hepatocellular and cholangiocarcinoma.

Among 18 cases of combined hepatocellular and cholangiocarcinoma, the serum alpha-fetoprotein (AFP) levels were found to be abnormally high (>20ng/mL) in 7 (38.9%). The serum protein induced by vitamin K absence or antagonist-II (PIVKA-II) levels were examined in 13 patients, and 8 (61.5%) were abnormal (0.1>AU/mL). The serum carcinoembryonic antigen (CEA) levels were examined in 12 patients and 11 (91.7%) were abnormal (>2.4ng/mL). The serum carbohydrate antigen (CA19-9) levels were examined in 6 patients, and 4 (67%) were abnormally high (normal ranges <37 U/mL).

Hepatitis B virus surface antigen (HBsAg) was positive in 4 (22.2%) out of 18 cases. Hepatitis C virus antibody (HCVAb) was positive in 7 (43.8 %) out of 16 cases examined. Both HBsAg and HCVAb were positive in 1 (6.3%) and both were negative in 6 (37.5%) out of 16 cases examined. Hepatitis B virus antigen was positive in 174 (18.5%) cases out of 940 cases of hepatocellular carcinoma and 3 (6.5%) cases out of 46 cases in intrahepatic cholangiocarcinoma. HCVAb was positive in 551 (68.3%) cases out of 807 cases in hepatocellular carcinoma and 8 (19.5%) cases out of 41 cases in intrahepatic cholangiocarcinoma.

Fourteen cases of combined hepatocellular and cholangiocarcinoma were followed after hepatic resections. The 1- and 3-year survival rates after operation were 73.3% and 33.3%, respectively.

At the time of hepatectomy, regional lymphadenectomy was done in 4 cases and metastatic tumors in the lymph node were found in 2 cases, in which the metastatic tumors were intrahepatic cholangiocarcinoma. Recurrence was found in 9 cases, in which 6 cases revealed recurrent tumors in the liver, but we could not perform a pathological examination in all 6 cases. The laboratory data suggested that the recurrent tumors were intrahepatic cholangiocarcinoma in 2 cases, hepatocellular carcinoma in 2 cases, and unknown in the remaining 2 cases. Distant metastasis was found in 4 cases (Lung; 2 cases, paraaortic lymph node; 1 case, bone; 2 case, skin; 1 case).

Gross Findings

Ten cases were the hepatocellular carcinoma predominant type, which resembled hepatocellular carcino-

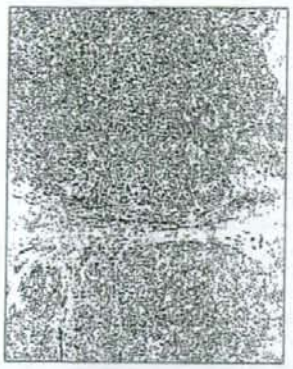

FIGURE 3A
Low power view of type III. The tumor exhibits lobular structures mimicking hepatic lobular structure (HE).

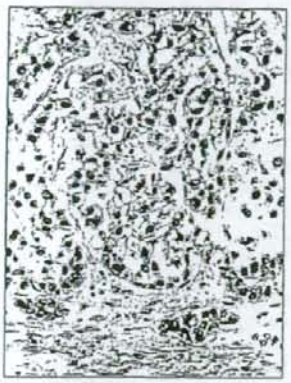

FIGURE 3B
High power view of type III. In the central area of the lobular structures hepatocellular carcinoma components exist and in the periphery cholangiocarcinoma components exist (HE).

ma with a greenish or yellowish color, and were associated with varying degrees of hemorrhage or necrosis and the tumors had demarcated fibrous capsules. The remaining 8 cases were the cholangiocarcinoma predominant type, which resembled intrahepatic cholangiocarcinoma and was gray in color with irregular margins. The largest diameter of the tumors ranged from 2.4 to 17cm (average: 6.5±3.6cm).

Histopathological Findings

Histopathological findings of the 18 combined hepatocellular carcinoma are summarized in **Table 2**. Among the 18 combined hepatocellular and cholangiocarcinomas, 5 cases (27.8%) were associated with liver cirrhosis, and 8 cases (44.4%) were associated with chronic hepatitis. In the remaining 5 cases (27.8%), there was no significant change in non-tumorous liver, in which 1 case was healthy carrier of HBV.

The histological type of the hepatocellular carcinoma component was trabecular type in 15 cases, scirrhous type in one case, compact type in one case, and pseudoglandular type in one case. Differentiation of the hepatocellular components was well-differentiated type in 1 case, moderately differentiated type in 6 cases, and poorly differentiated type in 11 cases.

The cholangiocarcinoma components were well to moderately differentiated tubular adenocarcinoma. Nine (50%) cases were predominantly hepatocellular carcinoma, and 7 (39%) were predominantly cholangiocarcinoma. The remaining 2 (11%) cases were predominantly undifferentiated type. In all cases the areas showing a glandular pattern were depicted as positive in either alcian blue stain or diastase digested PAS stain.

Microscopically, we classified the tumors into the following three categories according to the arrangement of the hepatocellular carcinoma and cholangiocarcinoma components. Type I was the tumor in which hepatocellular carcinoma and cholangiocarcinoma formed nodules that could easily be distinguished from each other. Seven cases were classified into type I. Type II was the tumor in which the both components were finely mixed, so that the two components were almost indistinguishable. Five cases were classified into type II. Type III was the tumor in which the tumors had lobular structures with hepatocellular carcinoma existing centrally and cholangiocarcinomas existing peripherally (**Figure 3A, 3B**). Six cases were classified into type III.

In one case of type I, two nodules were presented and surgically resected. In this case, one nodule was hepatocellular carcinoma and the other was combined hepato-

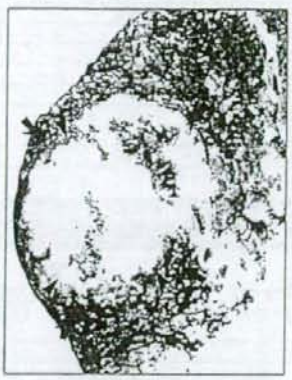

FIGURE 4A
The macroscopic finding of a combined hepatocellular and cholangiocarcinoma in "nodules-in-nodules" fashion. A well-differentiated hepatocellular carcinoma (arrows) 3.1cm in the maximal diameter contains a cholangiocarcinoma (arrow heads) 1.8cm in the maximal diameter.

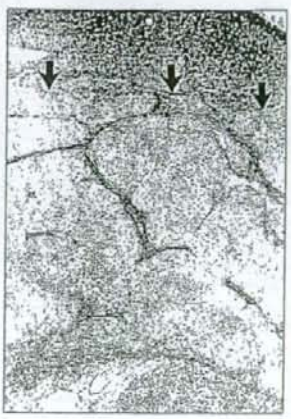

FIGURE 4B
The microscopic finding of **Figure 3B**. In the lower area a cholangiocarcinoma component exists in a well-differentiated hepatocellular carcinoma component (arrows) in "nodules-in-nodules" fashion. The hepatocellular carcinoma component shows clear cell change (HE).

cellular and cholangiocarcinoma, in which well differentiated hepatocellular carcinoma contained cholangiocarcinoma in "nodules-in-nodules" fashion (**Figure 4A, 4B**).

The Result of Immunohistochemical Staining of Ki-67

The average Ki-67 labeling index of the hepatocellular carcinoma component of combined hepatocellular and cholangiocarcinoma was 4.4±3.4% and the index of the cholangiocarcinoma component was 11.0±8.5%, significantly higher than that of the hepatocellular carcinoma component ($p<0.05$). As the control, we counted the Ki-67 labeling index of 11 cases of moderately differentiated hepatocellular carcinoma, and 8 cases of intrahepatic cholangiocarcinoma. The average Ki-67 labeling index of the ordinary hepatocellular carcinoma was 3.9±2.2%, and the index of the intrahepatic cholangiocarcinoma was 12.7±5.6%. In each case, the Ki-67 labeling index of the cholangiocarcinoma component was higher than the hepatocellular carcinoma component (**Figure 5**).

p53 Immunohistochemical Staining

In the p53 immunohistochemistry, 5 of 18 cases (29.4%) were positive.

In one case, the cholangiocarcinoma component was positive for p53, but the hepatocellular carcinoma component was negative. In another case, both the hepatocellular carcinoma and cholangiocarcinoma component were positive. In the remaining 3 cases, the staining of the cholangiocarcinoma component was stronger than that of hepatocellular carcinoma.

DISCUSSION

In this study, of 1102 patients with primary liver cancers, 3 (0.3%) patients had both hepatocellular carcinoma and intrahepatic cholangiocarcinoma independently, and 18 (1.6%) patients had combined hepatocellular and cholangiocarcinoma. These were relatively lower than in the study of autopsy cases and other studies of surgical cases (4-9).

The mean age of patients in the present series was 56.7 years. Regarding the background of the liver, the positivity of the virus markers, hepatitis B virus surface antigen (HBsAg) and hepatitis C virus antigen, was 22.2% and 43.8% in combined hepatocellular and cholangiocarcinoma. The positivity of the HBsAg in the combined hepatocellular and cholangiocarcinoma was significantly higher than intrahepatic cholangiocarcinoma. However, the positivity of the HCVAb in the combined carcinoma was between ordinary hepatocellular carcinoma and intrahepatic cholangiocarcinoma.

Among 18 cases of combined hepatocellular and cholangiocarcinomas, in 5 cases (27.8%) there was no chronic liver disease in non-tumorous liver. In the report of the Liver Cancer Study Group of Japan, chronic liver disease was not identified in non-tumorous liver in 7.2% of hepatocellular carcinoma, and in 72.0% of intrahepatic cholangiocarcinoma. Then, the background of combined hepatocellular and cholangiocarcinoma was more similar to hepatocellular carcinoma than to cholangiocarcinoma

In our study, the 1- and 3-year survival rates after operation were 73.3% and 33.3%, respectively. The immunoreactivity of the p53 protein was 27.8% (5/18), and it was consistent with the previously reported values in Japanese poorly differentiated hepatocellular carcinoma and was lower than that of Japanese intrahepatic cholangiocarcinomas (10-15). Regarding the combined hepatocellular and cholangiocarcinoma, Maeda *et al.* (6) reported that only three of 29 cases were positive for p53 in combined hepatocellular and cholangiocarcinoma, and they were positive for the cholangiocarcinoma component.

In the previous reports about the Ki-67 labeling index of the Japanese primary liver cancer, the Ki-67 labeling index of hepatocellular carcinoma was 6-15.3%, and that of intrahepatic cholangiocarcinoma was 14-28.9% (13,15-18). In this study, the Ki-67 labeling indices of the intrahepatic cholangiocarcinomas and the cholangiocarcinoma part of the combined hepatocellular and cholangiocarcinomas were higher than those of the ordinary hepatocellular carcinomas and the hepatocellular carcinoma part of the combined hepatocellular and cholangiocarcinomas. Compared with the ordinary hepatocellular carcinoma and intrahepatic cholangiocarcinoma, the proliferative activity of the cholangiocarcinoma component in the combined hepatocellular and cholangiocarcinoma was similar to that of intrahepatic cholangiocarcinoma, and the activity of the hepatocellular carcinoma component was similar to that of ordinary hepatocellular carcinoma. This suggested that cholangiocarcinoma components might grow more rapidly than hepatocellular carcinoma parts, and this fact seemed to influence the survival rate of the combined hepatocellular and cholangiocarcinoma, which was similar to that of intrahepatic cholangiocarcinoma.

With regard to the pathogenesis of combined hepatocellular and cholangiocarcinoma, there are three

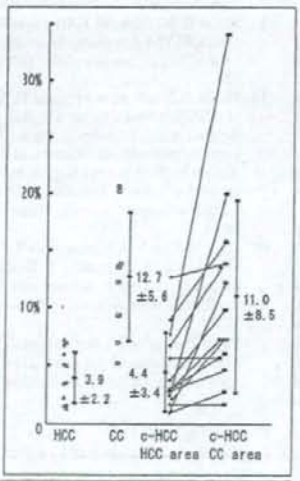

FIGURE 5
Comparison of the Ki-67 labeling indices of ordinary hepatocellular carcinomas, ordinary intrahepatic cholangiocarcinomas, hepatocellular carcinoma components, and cholangiocarcinoma components of combined hepatocellular and cholangiocarcinomas (mean±SD).
HCC: hepatocellular carcinoma;
CC: intrahepatic cholangiocarcinoma;
c-HCC: combined hepatocellular and cholangiocarcinoma.

hypotheses (19); 1) hepatocellular carcinoma and intrahepatic cholangiocarcinoma might have developed in the same liver coincidentally, 2) at first, either hepatocellular carcinoma or intrahepatic cholangiocarcinoma arises, and then is transformed to the other; 3) cancer arises from an intermediate cell between the hepatocytes and bile duct epithelium (stem cell), and then differentiates completely or incompletely into both components. From the standpoint of gene analyses, Imai *et al.* (7) showed the same mutational pattern in the hepatocellular carcinoma component and the cholangiocarcinoma component of the combined hepatocellular and cholangiocarcinoma, which was classified into Allen's type 2 and 3, and indicated the same origin of both components. Fujii *et al.* (20) reported that the majority of tumors classified into Allen's type 2 and 3 were derived from a single clone which shows bi-directional phenotypic diversity. This report supported the transformation hypothesis and the stem cell hypothesis. Yano *et al.* established a cell line designated as KYN-1, from AFP producing hepatocellular carcinoma cells, and reported that the cells, which were transplanted to nude mice, developed into adenocarcinoma within a few months (21).

In this study 6 out of 18 cases were microscopically classified type III, which exhibited lobular structures mimicking the hepatic lobular structure. Type III seems to be a feature of metaplasia or proliferation of bipotential progenitor cells. The background of the combined hepatocellular and cholangiocarcinoma is the middle of that of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. The cholangiocarcinoma component was more rapid in growth potential than the hepatocellular carcinoma component. In one case, we found that well-differentiated hepatocellular carcinoma contained cholangiocarcinoma in "nodules-in-nodules" fashion.

Therefore, it suggested that metaplasia of the hepatocellular carcinoma to the cholangiocarcinoma is one of the pathways in genesis of the combined hepatocellular and cholangiocarcinoma.

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FOOTNOTE

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Potential role of vitamin K_2 as a chemopreventive agent against hepatocellular carcinoma

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Vitamin K, a cofactor necessary for the production of several antihemorrhagic factors, can inhibit the growth of various types of cells derived from neoplasms. In hepatoma cells, vitamin K_2 causes cell-cycle arrest and apoptosis. Vitamin K_2 is widely used in Japan to treat osteoporosis. The safety, relatively low cost and ease of use of vitamin K_2 have led to good compliance with treatment. The result of preliminary clinical trials in patients with chronic liver diseases are intriguing and suggest that vitamin K_2 might reduce the risk of hepatocellular carcinoma (HCC) in patients with liver cirrhosis as well as prevent disease recurrence after curative therapy in patients with HCC. This article reviews the potential role of vitamin K_2 as a chemopreventive agent against HCC and discusses future directions for clinical trials.

Key words: hepatocellular carcinoma, vitamin K_2, viral hepatitis, liver cirrhosis, chemoprevention

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) arises almost exclusively in patients with chronic liver disease, especially hepatic cirrhosis. The annual incidence of HCC in patients with cirrhosis ranges 5–7%.[1–3] The rate of recurrence after curative treatment of primary HCC is high.[4–6] Epidemiological studies estimate that the number of deaths from HCC will increase by 2010–2015.[7] Decreased mortality from HCC requires preventive therapy. Prospective studies have been performed to evaluate the chemopreventive properties of interferon (IFN), "Sho-saiko-to", and an acyclic retinoid.[8–11]

The vitamin K family is known to inhibit the growth of human cancer cell lines. However, the mechanisms of this effect have yet to be fully explored. Recently, vitamin K_2 has attracted attention as a new chemopreventive agent against HCC.

BACKGROUND OF VITAMIN K

VITAMIN K IS a cofactor for the enzyme γ-glutamyl-carboxylase, which converts glutamate residues into γ-carboxy-glutamate. Vitamin K-dependent proteins include coagulation factors II (prothrombin), VII, IX, and X, protein C and S, osteocalcin, surfactant-associated proteins, and bone matrix protein. The vitamin K family of molecules comprises the natural forms vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinones) as well as the synthetic form vitamin K_3 (menadione). These naphthoquinone-containing molecules inhibit tumor cell growth in culture, with vitamin K_3 being more potent than either vitamin K_1 or K_2. Vitamin K_2 inhibits growth of human cancer cell lines and suppresses induction of differentiation in various human myeloid leukemia cell lines.[12,13] Clinically, myelodysplastic syndrome has been successfully treated with vitamin K_2.[14]

A number of findings indicate that vitamin K may have a role in controlling cell growth. Underlying mechanisms may involve redox cycling (as known for vitamin K_3), proteins with growth-inhibitory properties induced by vitamin K, such as prothrombin,[15] previously unidentified pathways involving arylation,[16] or growth arrest genes such as *gas 6*.[17] Geranylgeraniol (GGO), a side chain of vitamin K_2, strongly induces apoptosis of tumor cells, suggesting that GGO might inhibit cell growth.[18]

Recently, microarray analysis has shown that several genes are induced by treatment with vitamin K_2.[19] Protein kinase A (PKA) is a common activator of related

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Table 1 Baseline characteristics[21]

	Treatment ($n = 21$)	Control ($n = 19$)	*P*-value
Average age (years)	59.8 ± 8.7	61.4 ± 7.1	0.54
HBV/HCV	1/20	1/18	0.94
Albumin (g/dL)	3.9 ± 0.3	3.9 ± 0.3	0.87
Platelets (10^4/mm^3)	14.7 ± 5.4	12.1 ± 5.2	0.13
Total bilirubin (mg/dL)	0.8 ± 0.2	0.9 ± 0.4	0.47
ALT (IU/mL)	81.7 ± 42.7	70.4 ± 33.4	0.36
AFP (ng/mL)	13.4 ± 17.7	13.3 ± 8.7	0.99
IFN (+/−)	4/17	3/16	0.79

Mann–Whitney *U*-test for age, serum albumin, platelets, total bilirubin, alanine transferase (ALT) and α-fetoprotein (AF); χ^2 test for hepatitis B and C virus (HBV/HCV). IFN (+/−): Patents who received interferon(IFN) prior to enrollment; +, yes; −, no.

signaling pathways, identified by microarray analysis. Vitamin K_2 is thought to activate PKA, which inhibits RhoA activation. Alterations caused by high-dose treatment with vitamin K_2 result in cell-cycle arrest at the G1 and G2/M phases, accompanied by inhibition of tumor invasion. The effects of vitamin K_2 in doses used to treat osteoporosis are poorly understood, especially in the liver. However, the results of *in vitro* studies suggest that vitamin K_2 is one of the most promising agents for the chemoprevention of HCC.

PRIMARY CHEMOPREVENTION

WE PREVIOUSLY REPORTED a 2-year study showing that vitamin K_2 helps to prevent bone loss in women with viral cirrhosis of the liver.[20] Most of the subjects agreed to participate in an extended study designed to clarify the long-term effects of vitamin K_2 on bone loss associated with cirrhosis. The incidence of HCC was found to differ between women who received vitamin K_2 and those who did not.[21] In detail, the subjects of the initial 2-year study were 50 women with viral liver cirrhosis who were admitted to our department between 1996 and 1998. If the results of abdominal dynamic computed tomography and abdominal ultrasonography suggested the presence of HCC, abdominal angiography or needle biopsy was performed to confirm the diagnosis. Three patients in the treated group and four in the control group were confirmed to have HCC and were excluded from further study. The remaining 43 patients were randomly assigned by means of sealed envelopes to receive 45 mg/day of vitamin K_2 (Glakay; Eisai, Tokyo, Japan) p.o. (treated group) or no vitamin K_2 (control group). At the end of the first study (after 2 years of treatment), 21 patients in the treated group and 19 in the control group consented to participate in a longer trial. In a longer trial, all but one patient in each group had hepatitis C virus (HCV) infection; two other patients had hepatitis B infection. Seven patients, four in the control group and three in the treated group, had previously received IFN-α for their HCV infections, but HCV was not eradicated. No patient was given IFN therapy after study entry. Surveillance for HCC was done according to detailed guidelines for the follow up of patients with liver cirrhosis in Japan.[8] Compliance with vitamin K_2 in the treated group was good; no patient had adverse reactions or dropped out of the study. The two groups were similar with respect to age, virus type, platelets, alanine aminotransferase (ALT), α-fetoprotein (AFP) and other clinical findings (Table 1). After the first study commenced, HCC was detected in two of the 21 patients given vitamin K_2 and nine of the 19 controls; the cumulative proportion of patients with HCC was smaller in the treated group (log-rank test, $P = 0.024$; Fig. 1). On univariate analysis, the risk ratio for the development of HCC in the treated group versus the control group was 0.195 (0.042–0.913; $P = 0.038$). On multivariate analysis with adjustment for age, ALT activity, serum albumin, total bilirubin, platelet count, AFP, and history of treatment with IFN-α, the risk ratio for the development of HCC in patients given vitamin K_2 was 0.126 (0.016–0.992; $P = 0.049$) (Table 2).

The original goal of our trial was to assess the long-term effects of vitamin K_2 on bone loss in women with viral liver cirrhosis. Our trial thus had several important limitations when the data were used to assess the value of vitamin K_2 for the primary prevention of HCC in patients with liver cirrhosis. Factors limiting the value of our findings included the small study group, the inclusion of only women and the participation of only one

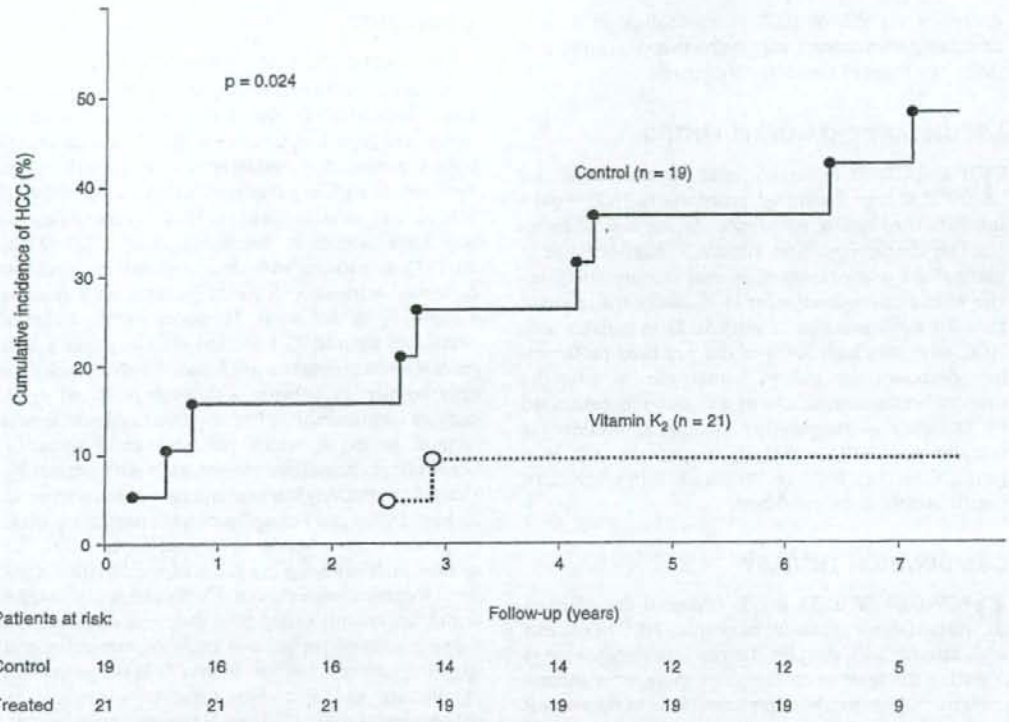

Figure 1 Cumulative incidence of hepatocellular carcinoma (HCC) diagnosed in patients treated with vitamin K_2 and in a control group.[21] All patients were followed up for at least 6 years. Vertical marks on curves show the latest follow-up to date for the 15 patients monitored for less than 7 years.

center. However, similar to previously reported randomized controlled studies of cirrhosis in which the primary end-point was the development of HCC, patients with evidence of HCC on highly sensitive imaging studies were excluded, and the two study groups were similar with respect to risk factors for HCC, including age, severity of cirrhosis, history of IFN therapy and type of hepatitis virus infection. Our results indicate that vitamin K_2

Table 2 Adjusted odds ratios for the development of hepatocellular carcinoma (HCC)[21]

	Odds ratio	95% CI	*P*-value
VK_2/control	0.126	0.016–0.992	0.0491
Total bilirubin (mg/dL) (1.0+/<1.0)	0.294	0.042–2.044	0.2161
Albumin (g/dL) (<3.5/3.5+)	33.434	2.362–473.352	0.0094
Platelets (10^4/mm^3) (<100/100+)	2.235	0.458–10.900	0.3200
ALT (IU/mL) (<80/80+)	0.393	0.071–2.164	0.2831
AFP (ng/mL) (20+/<20)	1.689	0.306–9.335	0.5477
IFN (+/–)	1.260	0.201–7.903	0.8053

Adjusted for age and all other variables in this table. IFN (+/–): Patents who received IFN prior to enrollment; +, yes; –, no. CI, confidence interval; VK_2, vitamin K_2.

decreases the risk of HCC to approximately 20% as compared with control, suggesting that vitamin K_2 may delay the onset of hepatocarcinogenesis.

SECONDARY CHEMOPREVENTION

THE RATE OF recurrence after curative therapy for HCC is high. Improved outcomes in HCC require inhibition of tumor recurrence. Before our study on primary chemoprevention, vitamin K_2 has been used to prevent the development of second primary malignancies after curative therapy for HCC. Koike *et al.* showed that the administration of vitamin K_2 to patients with HCC who have high levels of des-γ-carboxy prothrombin decreased the risk of portal vein invasion by tumor.[22] Preliminary results of a study being conducted by Mizuta *et al.* suggest that vitamin K_2 inhibits the recurrence of HCC, especially in patients with HCV (unpubl. results). However, this study is in progress; its results remain to be published.

COMBINATION THERAPY

PREVIOUS STUDIES HAVE evaluated the effectiveness of single agents for preventing HCC in patients with chronic liver diseases. To our knowledge, studies assessing the value of combination therapy for chemoprevention have not been reported. One of the reasons for the lack of studies evaluating combined treatment is concern about adverse effects associated with different agents. For example, adverse effects of IFN therapy include fever, leukopenia and thrombocytopenia. In contrast, vitamin K_2 has not been associated with serious side-effects in patients with osteoporosis. Vitamin K_2 may therefore be able to be used concomitantly with other chemopreventive agents, without increasing the risk of adverse reactions. Yoshiji *et al.* reported that a combination of vitamin K_2 and perindopril, an angiotensin-converting enzyme (ACE) inhibitor, was more effective for chemoprevention than either agent alone in a diethylnitrosamine-induced rat hepatocarcinogenesis model.[23] The number and size of enzyme-altered preneoplastic lesions were both significantly reduced, and the expression of CD31, a marker of neovascularization, was decreased in rats given combination treatment. Their findings suggested that a low dose of vitamin K_2 (1 μM) inhibits the proliferation of endothelial cells. Clinical trials examining whether vitamin K_2 plus an ACE inhibitor prevents HCC in patients with chronic liver diseases thus appear to be warranted.

CONCLUSION

AVAILABLE EVIDENCE SUGGESTS that vitamin K plays a role in controlling cell growth. The mechanisms responsible for the vitamin K_2-mediated inhibition of cell growth remain unexplained. Clinical studies have suggested that treatment vitamin K_2 reduces the incidence of HCC in patients with chronic liver diseases. Indeed, the annual incidence of HCC in control patients was 8.8%, similar to the incidence of HCC (7.9%; 32/107) in patients with liver cirrhosis in Japan,[3] as compared with only 1.6% in patients who received vitamin K_2 in our study. However, previous clinical studies of vitamin K_2 have focused on patients with specific characteristics or risk factors for HCC, including only women or patients with high levels of des-γ-carboxy prothrombin. Future investigations should attempt to define which patients would optimally benefit from chemopreventive therapy with vitamin K_2. The safety, relatively low cost and ease of use of vitamin K_2 have led to good compliance with treatment. These properties make vitamin K_2 a suitable candidate for clinical trials assessing the value of combination treatment for chemoprevention or chemotherapy in patients at risk for, or with a confirmed diagnosis of, HCC.

The results of preliminary trials are intriguing and suggest a potential role for vitamin K_2 in the prevention of primary and secondary hepatocarcinogenesis in patients with hepatic cirrhosis. However, currently available results must be verified by multicenter randomized controlled studies in which the primary end-point is the prevention of HCC by vitamin K_2.

CONFLICT OF INTEREST

NO CONFLICT OF interest has been received from the authors.

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症例報告

肝切除後に総肝動脈リンパ節転移を来した肝細胞癌の1例

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松山 光春[2)] 堀井 勝彦[2)] 清水 貞利[2)] 玉森 豊[2)]
東野 正幸[2)] 久保 正二[1)]

肝癌切除後の孤立性リンパ節転移を摘除することで，術後2年6か月の現在，無再発生存中の症例を経験したので報告する．症例は58歳の男性で，C型慢性肝炎に伴う肝癌に対して肝切除術を2回施行されていた．経過観察中のCT像上，肝尾状葉に約4cm大の腫瘤性病変を認め，AFP，PIVKA-II値の著明な上昇がみられた．腹部血管造影像では腫瘤は中肝動脈および左胃動脈より栄養される腫瘍濃染像として描出され，肝癌の尾状葉再発と診断し開腹した．腫瘍は肝尾状葉に接するように総肝動脈の腹側に存在していたが，肝臓からは独立しており肝癌の総肝動脈幹リンパ節転移と考え摘除した．病理組織学的検査では中分化型肝癌のリンパ節転移と診断された．AFP，PIVKA-IIは術後2か月目に標準値範囲内へ低下し，以来，再発徴候を認めていない．原発巣がコントロールされた肝癌の孤立性リンパ節転移は摘除により良好な予後が得られる可能性が示唆された．

はじめに

肝細胞癌（以下，肝癌）のリンパ節転移は剖検例で約30％と比較的高率に認められるが[1)2)]，肝癌の臨床経過においてリンパ節転移が問題となることは比較的まれである．このため，肝癌リンパ節転移に対する治療選択および成績についての報告は少数である．今回，我々は肝癌切除後に総肝動脈幹リンパ節転移を孤立性に認め，摘除により長期間無再発生存しえた症例を経験したので報告する．

症　例

患者：58歳，男性

主訴：症状なし．

家族歴，既往歴：特記事項なし．

現病歴：十数年前よりC型肝炎のため近医にて経過観察されていた．2001年4月の超音波検査にて肝外側区域に約1cmおよび前区域に約2cmの肝腫瘤を認め，AFPは65.6ng/mlであった．腹部血管造影像上，同部位に腫瘍濃染像がみられたため，肝癌と診断し開腹した．術中超音波検査にて，前区域に新たに約1cm大の腫瘤を認めたため，それぞれに対して肝部分切除術を施行した．術後の病理組織学的検査において中分化型肝癌で，門脈腫瘍栓所見はなかった．非癌部肝組織は肝硬変像を呈していた．

術後リザーバー動注（5-FU 1,500mg＋CDDP 5mg＋leucovorin 12mg　計6回）を行っていたが，術後約1年目にAFPが71ng/mlと上昇し，CTにて肝内側区域に肝癌再発を認めたため，再度肝部分切除術を施行した．病理組織学的検査では広範な壊死を伴う低分化型肝癌と診断された．門脈腫瘍栓および肝内転移を認めなかった．再手術後2か月目にはAFPは正常範囲内となったが，再手術後1年目には，AFPは3,955ng/mlと再上昇したため，精査加療目的に入院となった．

入院時現症：身長161cm，体重61kg．皮膚，眼球結膜に黄染は認められず，腹部は平坦，軟で，肝脾とも触知しなかった．また，体表リンパ節は触知しなかった．

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