# Cytokeratin 19 expression in hepatocellular carcinoma predicts early postoperative recurrence

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(Received February 27, 2003/Revised July 4, 2003/Accepted August 5, 2003)

**Clinicopathologic features and postoperative outcomes were investigated for patients who underwent curative surgery for biliary marker (CK7 and CK19)-positive hepatocellular carcinoma (HCC). Of 157 HCCs, 93 were CK7(−)CK19(−), 49 were CK7(+)-CK19(−), 1 was CK7(−)CK19(+), and 14 were CK7(+)-CK19(+). Semiquantitative analysis of expression levels demonstrated a significant correlation between CK7 and CK19 expression. Of various clinicopathologic parameters, tumor differentiation exhibited a significant correlation with CK7 and CK19 expression. All 15 patients with CK19-positive HCC also had anti-HBc. Log-rank test revealed that CK7 expression, CK19 expression, high aspartate aminotransferase (AST) activity, low albumin concentration, portal invasion, intrahepatic metastasis, and severe fibrosis (cirrhosis) reduced the tumor-free survival rate. Multivariate analysis demonstrated that CK19 expression, intrahepatic metastasis, and severe fibrosis were independent predictors of postoperative recurrence, while CK7 expression was not. Twelve of 15 patients with CK19-positive HCC had tumor recurrence within 2 years after surgery, a significantly higher incidence of early recurrence than for CK19-negative HCC. The incidence of extrahepatic disease, especially lymph node metastasis, was significantly higher for patients with CK19-positive HCC. These findings indicate that CK19 expression is a predictor of early postoperative recurrence due to increased invasiveness. (Cancer Sci 2003; 94: 851–857)**

Patients with intrahepatic cholangiocarcinoma (ICC), including combined hepatocellular and cholangiocarcinoma (cHCC-CC), exhibit poorer postoperative prognosis than those with hepatocellular carcinoma (HCC) due to more aggressive invasion and higher frequency of extrahepatic metastasis.[1–6] Thus, it is important for surgeons to take into account the presence of CC components within hepatic tumors in selecting a surgical strategy.[6] Cytokeratins (CK) are cytoskeletal intermediate filaments present in both normal and malignant epithelial cells.[7] Characteristic combinations of CK polypeptides are expressed in different epithelia depending on the organ and/or type of differentiation.[7, 8] In normal human liver, hepatocytes express CK8 and CK18, while bile duct cells also contain CK7 and CK19.[8–11] Since this CK pattern has been believed to be preserved during neoplastic transformation, HCC would be expected to express CK8 and CK18, but not CK7 or CK19.[8–11] Thus, expression of biliary-specific CK (CK7 and CK19) is widely used to distinguish ICC from HCC.[6, 8–12] Maeda *et al*.[6] recommended that HCC with "suggestive" CC components (incomplete gland formation without mucin production) should be categorized as cHCC-CC when "suggestive" CC components are positive for biliary markers. However, some HCCs have been reported to express biliary markers even with typical HCC growth pattern and morphologic appearance,[13–17] although the histogenesis of such biliary marker-positive HCC is not well-understood. A previous report indicated that without treatment, the prognosis is poorer for patients with biliary differentiation marker [AE1–AE3, cytokeratin (CK) 19]-positive HCC than for those without it.[16] There have, however, been no studies on the clinical usefulness of biliary marker expression by HCC in surgical patients. In this study, we compared the postoperative outcomes of patients with biliary-specific CK (CK7 and CK19)-positive HCC with those of CK-negative HCC patients.

## Materials and Methods

Liver specimens were obtained'' from 157 patients with HCC who underwent curative resection in the Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Hospital, from 1994 to 2000. There were 129 men and 28 women with an age range of 34 to 80 (mean 62) years. Ninety patients underwent anatomic resection (33, bisegmentectomy; 32, segmentectomy; and 25, subsegmentectomy), and 67 patients received a limited resection of less than Couinaud's segment.[18] Liver specimens were fixed in 10% formalin for 48 h. They were cut into 0.5-cm-thick slices, and slices obtained through the maximum diameter of the tumor were used for histologic analysis. Paraffin sections were stained with hematoxylin and eosin (H-E) and alcian blue. The diagnosis of HCC was made on the basis of typical clinical and laboratory findings and also the following histologic criteria. Tumor cells had prominent nucleoli, eosinophilic cytoplasm, and intercellular bile canaliculi, and exhibited the trabecular or pseudoglandular arrangement characteristic of HCC cells. It was also confirmed that there was neither glandular formation nor mucin production. Stages of dedifferentiation (well, moderate, and poor) and cell patterns (trabecular, pseudoglandular, solid) were determined according to the modification[2] of Edmondson and Steiner.[19] When multiple lesions were demonstrated, the largest nodule was identified as the principal tumor nodule and others were considered satellite lesions. Satellite lesions were classified as multicentric occurrence of HCC when well-differentiated HCC grew in a replacement growth pattern with moderately or poorly differentiated main tumor elsewhere.[20] Multiple HCCs that did not meet these criteria were assumed to be intrahepatic metastases originating from a main tumor. The grade and stage of chronic active hepatitis in non-cancerous liver were scored using the histologic activity index (HAI),[21, 22] which was calculated from four parameters: periportal necrosis with or without bridging necrosis, intralobular degeneration with focal necrosis, portal inflammation, and fibrosis. Patients were classified based on the presence or absence of expression of biliary markers. The clinicopathologic findings and postoperative outcomes were compared between these groups.

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.

**Immunohistochemistry.** Deparaffinized sections were incubated with 3% $H_2O_2$ in methanol for 30 min to block endogenous peroxidase activity. Sections were incubated with

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monoclonal antibody (mAb) against CK7 (1:100; Dako, Glostrup, Denmark) and mAb against CK19 (1:100; Dako) at room temperature for 60 min. After the primary antibody was washed off, the components of the Envision-plus (Dako) detection system were applied, including an antimouse polymer. Reaction products were visualized by incubation with 3,3'-diaminobenzidine. Two pathologists who did not know patient outcome determined the degree of staining, which was classified according to the percentage of positive cells as follows: +++, more than 75% of tumor cells were positive; ++, 25 to 74% were positive; +, 5 to 24% were positive; −, less than 5% were positive.

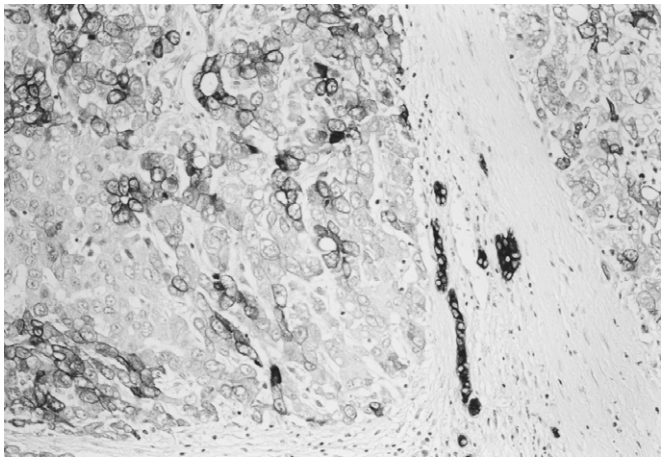

**Fig. 1.** Microscopic features of CK19-positive hepatocellular carcinoma. Cancer cells stained for CK19 antigen with variable intensity; bile duct epithelial cells also exhibited CK19, but hepatocytes did not. (original magnifications, ×200)

**Postoperative outcomes.** Tumor recurrence was investigated until the patient's death or the end of this study (December 31, 2002) by criteria including serum α-fetoprotein (AFP) assay, chest radiography, and ultrasound scanning or computed tomography conducted every 3 months after surgery. When recur-

**Table 1. Expression of cytokeratins 7 and 19 in hepatocellular carcinoma**

| Expression levels of CK19 | Expression levels of CK7 | | | | |
|---|---|---|---|---|---|
| | | + | ++ | +++ | Total |
| | 93 | 25 | 21 | 3 | 142 |
| + | 1 | 7 | 1 | 0 | 9 |
| ++ | 0 | 2 | 4 | 0 | 6 |
| +++ | 0 | 0 | 0 | 0 | 0 |
| Total | 94 | 34 | 26 | 3 | 157 |

CK7, cytokeratin 7; CK 19, cytokeratin 19.

**Table 2. Clinicopathologic findings in patients with hepatocellular carcinoma with and without cytokeratin 7 expression**

| | CK 7 expression | | *P* value |
|---|---|---|---|
| | Positive (*n*=63) | Negative (*n*=94) | |
| Age (years, mean±SD) | 61.0±7.2 | 63.0±7.5 | 0.11 |
| Gender (male:female) | 46:17 | 83:11 | 0.19 |
| Laboratory data | | | |
| Anti-HCV positive | 46 | 73 | 0.57 |
| HBs Ag positive | 12 | 13 | 0.38 |
| Anti-HBc positive | 51 | 63 | 0.05 |
| AST (IU/liter) | 58 (34, 100)[1)] | 57 (33, 100) | 0.74 |
| ALT (IU/liter) | 63 (32, 125) | 64 (30, 112) | 0.61 |
| Total bilirubin (mg/dl) | 0.9 (0.5, 1.3) | 0.8 (0.5, 1.3) | 0.19 |
| Albumin (g/dl) | 3.7 (3.3, 4.1) | 3.8 (3.4, 4.3) | 0.19 |
| Platelet count ($10^4$/μl) | 15.0 (8.4, 24.0) | 13.7 (7.5, 24.1) | 0.72 |
| AFP (>20 ng/ml) | 38 | 52 | 0.62 |
| Pathology | | | |
| 1) Non-cancerous portions | | | |
| HAI score | | | |
| Grade 0–2 | 52 | 74 | 0.68 |
| 3–4 | 11 | 20 | |
| Stage 0–3 | 43 | 67 | 0.72 |
| 4 (cirrhosis) | 20 | 27 | |
| 2) Cancerous portions | | | |
| Tumor size (cm, mean±SD) | 4.0±3.0 | 4.2±3.5 | 0.71 |
| Tumor differentiation | | | |
| Well | 1 | 8 | <0.01 |
| Moderate | 32 | 62 | |
| Poor | 30 | 24 | |
| Intrahepatic metastasis | 20 | 16 | 0.03 |
| Portal invasion | 23 | 29 | 0.49 |
| Type of operation | | | |
| Anatomic resection | 31 | 58 | 0.14 |
| Limited resection | 32 | 36 | |

*1*) Data are presented as the median with the 10th and 90th percentiles indicated in parentheses. CK7, cytokeratin 7; Anti-HCV, hepatitis C virus antibody; HBs Ag, hepatitis B surface antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-fetoprotein; HAI, histologic activity index; SD, standard deviation.

**Table 3. Clinicopathologic findings in patients with hepatocellular carcinoma with and without cytokeratin 19 expression**

| | CK 19 expression | | *P* value |
|---|---|---|---|
| | Positive (*n*=15) | Negative (*n*=143) | |
| Age (years, mean±SD) | 59.5±7.3 | 62.5±7.4 | 0.13 |
| Gender (male:female) | 10:5 | 120:22 | 0.15 |
| Laboratory data | | | |
| Anti-HCV positive | 12 | 107 | >0.99 |
| HBs Ag positive | 2 | 23 | >0.99 |
| Anti-HBc positive | 15 | 99 | 0.01 |
| AST (IU/liter) | 54 (40, 99)[1)] | 58 (33, 101) | 0.59 |
| ALT (IU/liter) | 55 (33, 110) | 65 (31, 120) | 0.94 |
| Total bilirubin (mg/dl) | 0.8 (0.5, 1.0) | 0.9 (0.5, 1.3) | 0.21 |
| Albumin (g/dl) | 3.8 (3.5, 4.2) | 3.7 (3.3, 4.1) | 0.61 |
| Platelet count ($10^4$/μl) | 12.5 (7.5, 22.5) | 14.8 (8.1, 24.5) | 0.36 |
| AFP (>20 ng/ml) | 11 | 79 | 0.27 |
| Pathology | | | |
| 1) Non-cancerous portions | | | |
| HAI score | | | |
| Grade 0–2 | 10 | 116 | 0.16 |
| 3–4 | 5 | 26 | |
| Stage 0–3 | 10 | 100 | 0.77 |
| 4 (cirrhosis) | 5 | 42 | |
| 2) Cancerous portions | | | |
| Tumor size (cm, mean±SD) | 3.7±2.5 | 4.2±3.4 | 0.63 |
| Tumor differentiation | | | |
| Well | 0 | 9 | 0.02 |
| Moderate | 5 | 89 | |
| Poor | 10 | 44 | |
| Intrahepatic metastasis | 6 | 30 | 0.11 |
| Portal invasion | 6 | 46 | 0.57 |
| Type of operation | | | |
| Anatomic resection | 10 | 79 | 0.41 |
| Limited resection | 5 | 63 | |

*1*) Data are presented as the median with the 10th and 90th percentiles indicated in parentheses. CK 19, cytokeratin 19; Anti-HCV, hepatitis C virus antibody; HBs Ag, hepatitis B surface antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-fetoprotein; HAI, histologic activity index; SD, standard deviation.

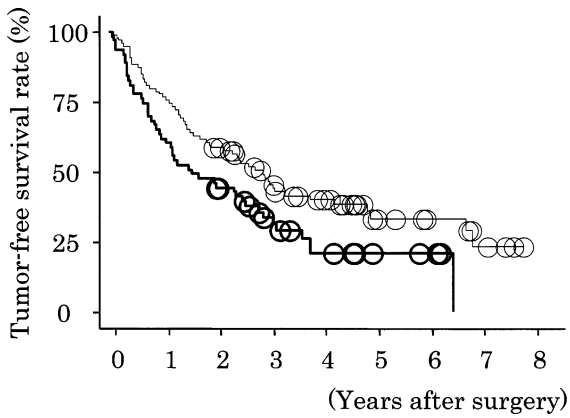


**Fig. 2.** Tumor-free survival rates after resection of hepatocellular carcinoma for patients with or without tumor expression of CK7. The thin line represents patients with CK7-negative cancers (*n*=94); the thick line represents patients with CK7-positive cancers (*n*=63).

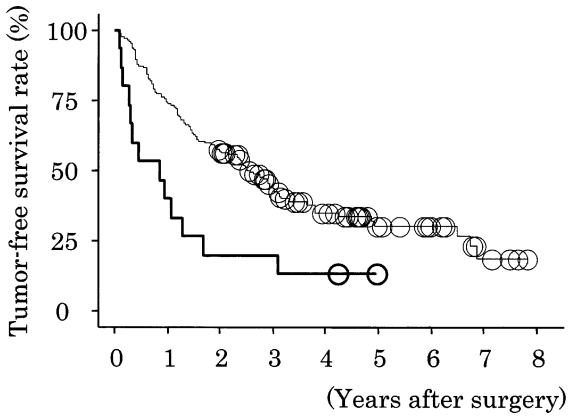


**Fig. 3.** Tumor-free survival rates after resection of hepatocellular carcinoma for patients with or without tumor expression of CK19. The thin line represents patients with CK19-negative cancers (*n*=142); the thick line represents patients with CK19-positive cancers (*n*=15).

rence was strongly suspected, selective hepatic angiography and ultrasound-guided biopsy were conducted for definitive diagnosis. Bone metastasis was examined by scintigraphy with $^{99m}$Tc diphosphonate.

**Statistics.** The correlation between level of CK7 expression and that of CK19 expression was analyzed by Spearman rank correlation coefficient determination. Student's *t* test was used to analyze differences in age and tumor size. The Mann-Whit-

**Table 4. Univariate analysis with respect to outcome**

| Factors | Number of patients | Tumor-free survival rate (95% CI) | | *P* value |
|---|---|---|---|---|
| | | 2 years (%) | 5 years (%) | |
| Cytokeratin 19 expression | | | | |
| Positive | 15 | 20 (0–40) | 13 (0–31) | 0.0018 |
| Negative | 142 | 40 (49–65) | 30 (21–39) | |
| Cytokeratin 7 expression | | | | |
| Positive | 63 | 46 (34–58) | 21 (10–33) | 0.0199 |
| Negative | 94 | 59 (49–68) | 33 (22–44) | |
| Age (years) | | | | |
| <60 | 49 | 55 (45–64) | 31 (21–40) | 0.6389 |
| ≥60 | 108 | 51 (37–65) | 24 (8–39) | |
| Gender | | | | |
| Male | 129 | 54 (35–72) | 41 (22–60) | 0.3687 |
| Female | 28 | 54 (45–62) | 25 (16–34) | |
| AST activity (IU/liter) | | | | |
| ≤40 | 30 | 67 (50–84) | 52 (28–76) | 0.0047 |
| >40 | 127 | 50 (42–59) | 23 (15–32) | |
| ALT activity (IU/liter) | | | | |
| ≤45 | 49 | 59 (45–73) | 34 (18–50) | 0.3428 |
| >45 | 108 | 51 (41–60) | 26 (12–31) | |
| Total bilirubin (mg/dl) | | | | |
| ≤1.0 | 122 | 53 (38–71) | 27 (18–37) | 0.7644 |
| >1.0 | 35 | 54 (44–62) | 34 (18–51) | |
| Albumin (g/dl) | | | | |
| <3.7 | 70 | 49 (37–60) | 21 (10–32) | 0.0330 |
| ≥3.7 | 87 | 58 (47–68) | 34 (22–46) | |
| Platelet count ($10^4$/$mm^3$) | | | | |
| <10 | 31 | 42 (24–59) | 11 (0–29) | 0.1212 |
| ≥10 | 126 | 56 (48–65) | 32 (23–41) | |
| Serum concentration of AFP (ng/ml) | | | | |
| <20 | 67 | 55 (43–67) | 27 (14–40) | 0.9622 |
| ≥20 | 90 | 52 (42–63) | 30 (19–40) | |
| Tumor size (cm) | | | | |
| <3.0 | 70 | 56 (44–67) | 24 (11–37) | 0.9911 |
| ≥3.0 | 87 | 52 (44–62) | 31 (21–42) | |
| Tumor differentiation | | | | |
| Poor | 55 | 40 (27–53) | 27 (11–42) | 0.0954 |
| Others | 102 | 61 (51–70) | 29 (19–39) | |
| Intrahepatic metastasis | | | | |
| Present | 36 | 25 (11–39) | 14 (1–26) | <0.0001 |
| Absent | 121 | 62 (53–71) | 33 (24–43) | |
| Portal invasion | | | | |
| Present | 52 | 39 (25–52) | 24 (9–38) | 0.0496 |
| Absent | 113 | 41 (32–50) | 31 (21–41) | |
| Histologic activity index score | | | | |
| Grade | | | | |
| 0–2 | 125 | 53 (44–62) | 30 (20–39) | 0.5286 |
| 3–4 | 31 | 58 (16–76) | 25 (6–44) | |
| Stage | | | | |
| 0–3 | 110 | 59 (50–68) | 34 (24–45) | 0.0216 |
| 4 (cirrhosis) | 47 | 40 (16–55) | 16 (3–28) | |
| Type of resection | | | | |
| Anatomic | 89 | 55 (45–65) | 33 (22–44) | 0.4843 |
| Limited | 68 | 52 (37–59) | 20 (6–35) | |

CI, confidence interval; IFN, interferon; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-fetoprotein; TAE, transcatheter arterial embolization.

ney *U* test was used to examine differences in laboratory test results. Fisher's exact test or the $\chi^2$ test was used to compare categorical data between groups.

The tumor-free survival rate was calculated by the Kaplan-Meier method, and rates are reported with 95% confidence intervals; differences were tested for significance using the log-rank test. The tumor-free survival time was measured from the date of resection until the detection of recurrent tumor or the end point of this study in patients without recurrence. Multivariate analysis was performed using a Cox regression model with forward stepwise selection.

## Results

On immunohistochemical examination, the hepatocytes and bile duct cells in the non-cancerous portion were CK7(−)-CK19(−) and CK7(+)CK19(+), respectively. On the other hand, HCC cells were variously stained for these markers. Of 157 tumors, 93 (59%) were CK7(−)CK19(−), 49 (31%) were CK7(+)CK19(−), 1 (1%) was CK7(−)CK19(+), and 14 (9%) were CK7(+)CK19(+). One example stained for CK19 is shown in Fig. 1. Expression of CK7 and CK19 was semiquantitatively measured, and the distribution of staining is shown in Table 1. Spearman rank correlation analysis demonstrated a significant correlation between the level of expression of CK7 and that of CK19 ($P<0.0001$; correlation coefficient=0.32).

The relationships of CK7 (Table 2) and CK19 (Table 3) expression with various clinicopathologic parameters were then analyzed. All 15 patients with CK19-positive HCC were found to have anti-hepatitis B core antibody (anti-HBc). The proportion of patients with anti-HBc was significantly higher among patients with CK19-positive HCC than among those CK19-negative HCC, while no significant differences were noted between CK19-positive and CK19-negative HCC in the proportion of patients with anti-hepatitis C virus or HBV surface antigen. Tumor differentiation was significantly correlated with CK7 and with CK19 expression, i.e., poorly differentiated HCC more frequently expressed CK7 and CK19 than well/moderately differentiated HCC. The prevalence of intrahepatic metastasis was significantly higher in patients with CK7-positive HCC than in those with CK7-negative HCC. Other parameters such as operative method, tumor size, portal invasion, and degree of inflammation and fibrosis in the non-cancerous portion were significantly correlated with neither CK7 nor CK19 expression.

No patient was lost to follow-up. The follow-up period from surgery until death or the endpoint of this study was 60 to 2881 days (mean 1308 days). During follow-up, 58 patients died, including 6 who died of causes unrelated to HCC or cirrhosis. The tumor-free survival rates for patients with CK7-positive and CK7-negative HCC were 62% and 78% at 1 year; 37% and 48% at 3 years; and 21% and 33% at 5 years, respectively (Fig. 2). Tumor-free survival rate was significantly higher for patients with CK7-negative HCC ($P=0.0199$). The tumor-free survival rates for patients with CK19-positive and CK19-negative HCC were 40% and 75% at 1 year; 20% and 44% at 3 years; and 13% and 30% at 5 years, respectively (Fig. 3). Patients with CK19-positive HCC had significantly lower tumor-free survival rates ($P=0.0018$). High aspartate aminotransferase (AST) activity, low albumin concentration, portal invasion, intrahepatic metastasis, and stage 4 disease (cirrhosis) also significantly decreased tumor-free survival rates, while other clinicopathologic parameters did not (Table 4). Multivariate analysis demonstrated that CK19 expression, intrahepatic metastasis, and stage 4 disease (cirrhosis) were independent predictors of postoperative recurrence, while CK7 expression was not (Table 5).

Of 157 patients, 106 (13 CK19-positive HCC and 93 CK19-negative) exhibited tumor recurrence during the follow-up period. Twenty-two patients had extrahepatic tumor recurrence. Early recurrence (i.e., within 2 years after surgery) occurred in 12 of 15 patients (73%) with CK19-positive HCC and in 61 (43%) of 142 with CK19-negative HCC. The incidence of early recurrence in patients with CK19-positive HCC was significantly ($P=0.01$) higher than in those with CK19-negative HCC (Table 6). Of the 12 patients with CK19-positive HCC and HCC recurrence within 2 years after surgery, 5 had tumor recurrence only in the liver, while 7 had extrahepatic metastasis. Five of the 7 patients with extrahepatic metastasis also had tumor recurrence in the liver, while 2 had lymph node metastasis without intrahepatic recurrence. Of the 61 patients with CK19-negative HCC and HCC recurrence, 15 had extrahepatic metastasis, and 13 of the 15 extrahepatic metastases occurred within 2 years after surgery. Thus, the frequency of extrahepatic metastasis within 2 years after surgery in patients with CK19-positive HCC was also significantly ($P<0.01$) higher than in those with CK19-negative HCC. Lymph nodes were the preferred site of metastasis with a significantly ($P<0.01$) higher frequency of involvement in CK19-positive HCC.

**Table 5. Multivariate analysis of factors predicting postoperative recurrence**

| Variable | Multivariate risk ratio | *P* value |
|---|---|---|
| Cytokeratin 19 expression | 2.2 (1.2–4.0)[1)] | 0.0082 |
| Intrahepatic metastasis | 2.8 (1.8–4.3) | <0.0001 |
| Stage 4 (cirrhosis) | 1.9 (1.2–2.8) | 0.0025 |

*1*) Percentage, with 95% confidence interval in parentheses.

**Table 6. Early tumor recurrence in patients with hepatocellular carcinoma with and without cytokeratin 19 expression**

| | CK 19-positive ($n$=15) | CK 19-negative ($n$=142) | *P* value |
|---|---|---|---|
| Early recurrence[2)] | 12 (73%) | 61 (43%) | 0.01[1)] |
| Extrahepatic recurrence | 7 | 13 | 0.01[1)] |
| Brain | 0 | 3 | >0.99[1)] |
| Lung | 2 | 4 | 0.25[1)] |
| Bone | 1 | 8 | >0.99[1)] |
| Lymph nodes | 5 | 2 | <0.01[1)] |
| Adrenal gland | 1 | 1 | 0.30[1)] |

*1*) Fisher's exact test.
*2*) Early recurrence within 2 years after surgery.

## Discussion

If hepatocytes were the cells of origin for HCC, these cells would be expected to express CK8 and CK18, but not CK7 or CK19. However, some investigators have reported expression of CK7 and/or CK19 in some human HCCs.[13–17] In this study, 64 of 157 HCCs demonstrated expression of biliary-specific CK (CK7 and/or CK19). Some investigators have postulated that biliary-specific CK-positive HCC might be generated through carcinogenesis of biliary marker-positive hepatic progenitor cells,[16, 17, 23–25] which are capable of differentiating into both hepatocytes and biliary epithelial cells.[26–28] However, the histogenesis of such biliary marker-positive HCC has not been established. CK pattern might not always be preserved during neoplastic transformation.[14] Our previous study indicated that prior HBV infection may enhance aberrant dedifferentiation of HCV-related HCC, since HCCs positive for biliary-specific CK were found only in patients with prior HBV infection (anti-HBc positive).[17] In this study, all patients with CK19-positive HCC also had anti-HBc.

Wu *et al.*[16] reported that survival in patients with biliary marker-positive HCC was significantly shorter than in those with negative HCC when patients received no medical or surgical treatment. Our study also demonstrated that patients with biliary marker-positive HCC have a lower tumor-free rate even when curative resection is performed. In this study, CK19 expression, intrahepatic metastasis, and stage 4 disease (cirrhosis) were independent predictors of postoperative recurrence, while CK7 expression was not. It has been reported that biliary marker-positive HCC exhibited features of more aggressive disease, i.e., poorer differentiation and higher proliferation, than biliary marker-negative HCC.[16] In this study, poor differentia-

tion was unrelated to postoperative recurrence, although poorly differentiated HCC more frequently expressed CK7 or CK19 than well/moderately differentiated HCC.

Postoperative HCC recurrence is believed to take place in two ways, intrahepatic metastasis in the residual liver and metachronous, multicentric hepatocarcinogenesis based on chronic hepatitis.[23–27] Some authors have postulated that early recurrence arises mainly from intrahepatic metastases, whereas late recurrence is more likely to be multicentric in origin.[24–27] It has been reported that postoperative multicentric hepatocarcinogenesis proceeds steadily in the non-cancerous liver portion, at a rate of 3.4% per year, even in the late follow-up period. On the other hand, residual intrahepatic metastasis is an early postoperative event, and the annual incidence of intrahepatic metastasis was 19.7% within 2 years after surgery.[24] Poon *et al*. reported that tumor factors, but not host factors, were linked to early recurrence, while the risk of late recurrence was dependent on underlying liver status.[25] In our study, tumor recurrence was noted in 12 of 15 patients with CK19-positive HCC within 2 years after surgery, and the incidence of early recurrence was significantly higher in patients with CK19-positive HCC than in those with CK19-negative HCC. The frequency of extrahepatic metastasis within 2 years after surgery in patients with CK19-positive HCC was also significantly higher than in those with CK19-negative HCC. These results indicate that recurrence in patients with CK19-positive HCC must be largely due to residual carcinoma cells that have not been removed at operation. To elucidate the origin of tumor recurrence, further studies based on clonal genetic analysis are needed.[28–30] However, it is too difficult to perform such analysis of all recurrent tumors.

It has been reported that patients with ICC have poorer postoperative prognosis due to their higher rate of metastasis, especially lymph node metastasis during the early postoperative period.[1–6] In the present study, the frequency of extrahepatic metastasis within 2 years after surgery in patients with CK19-positive HCC was also significantly higher than in those with CK19-negative HCC. Lymph nodes were the preferred site of metastasis, with a significantly ($P<0.01$) higher frequency in cases of CK19-positive HCC. These results indicated that CK19-positive HCCs might exhibit biological behaviors similar to those of ICCs. This tendency toward extrahepatic metastasis of CK19-positive HCC suggests a high degree of invasiveness; and we are now investigating adhesion molecule expression in this type of HCC.

In conclusion, CK19-positive HCCs exhibit a high incidence of early postoperative recurrence due to intra- and extrahepatic metastasis even after curative resection, which may be due to their malignant properties of progression and dissemination.

The authors thank Prof. Dr. S. Fukushima (Department of Pathology, Osaka City University Medical School) for his helpful comments on histology.

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