Des-gamma-carboxy prothrombin as an important prognostic indicator in patients with small hepatocellular carcinoma

Kenichi Hakamada, Norihisa Kimura, Takuya Miura, Hajime Morohashi, Keinosuke Ishido, Masaki Nara, Yoshikazu Toyoki, Shunji Narumi, Mutsuo Sasaki

AIM: To clarify the effect of a high des-gamma-carboxy prothrombin (DCP) level on the invasiveness and prognosis of small hepatocellular carcinoma.

METHODS: Among 142 consecutive patients with known DCP levels, who underwent hepatectomy because of hepatocellular carcinoma, 85 patients met the criteria for small hepatocellular carcinoma, i.e. one ≤ 5 cm sized single tumor or no more than three ≤ 3 cm sized tumors.

RESULTS: The overall survival rate of the 142 patients was 92.1% for 1 year, 69.6% for 3 years, and 56.9% for 5 years. Multivariate analysis showed that microscopic vascular invasion (P = 0.03) and serum DCP ≥ 400 mAU/mL (P = 0.02) were independent prognostic factors. In the group of patients who met the criteria for small hepatocellular carcinoma, DCP ≥ 400 mAU/mL was found to be an independent prognostic factor for recurrence-free (P = 0.02) and overall survival (P = 0.0005). In patients who did not meet the criteria, the presence of vascular invasion was an independent factor for recurrence-free (P = 0.02) and overall survivals (P = 0.01). In 75% of patients with small hepatocellular carcinoma and high DCP levels, recurrence occurred extrahepatically.

CONCLUSION: For small hepatocellular carcinoma, a high preoperative DCP level appears indicative for tumor recurrence. Because many patients with a high preoperative DCP level develop extrahepatic recurrence, it is necessary to screen the whole body.

© 2008 WJG. All rights reserved.

Key words: Small hepatocellular carcinoma; Hepatic resection; Des-gamma-carboxy prothrombin; Vascular invasion; Prognostic factor

Peer reviewer: Dusan M Jovanovic, Professor, Institute of Oncology, Institutski Put 4, Sremska Kamenica 21204, Serbia

Hakamada K, Kimura N, Miura T, Morohashi H, Ishido K, Nara M, Toyoki Y, Narumi S, Sasaki M. Des-gamma-carboxy prothrombin as an important prognostic indicator in patients with small hepatocellular carcinoma. World J Gastroenterol 2008; 14(9): 1370-1377. Available from: URL: http://www.wjgnet.com/1007-9327/14/1370.asp DOI: http://dx.doi.org/10.3748/wjg.14.1370

INTRODUCTION

Des-gamma-carboxy prothrombin (DCP) is a tumor marker specific for hepatocellular carcinoma[1]. It is believed that the elevation of the serum DCP level correlates with the presence of vascular invasion or intrahepatic metastases[2-8]. Furthermore, DCP has been reported to be an independent prognostic factor for recurrence and survival after hepatic resection[9-13], liver transplantation[14,15], ablation treatment[16,17], and transarterial chemoembolization (TAE) treatment[18]. However, the rate of detectable serum DCP levels in patients with small hepatocellular carcinoma is low[15-17]. Although methods have been improved[18,19], sensitivity is still at about 50% for most small cell carcinoma[20,21]. Thus, almost all reports on the biological nature of DCP and its prognostic value are based on analyses of patients with larger or more advanced tumors with various degrees of hepatic functional reserve. Reports on the relevance of preoperative DCP level as a prognostic marker in small hepatocellular carcinoma patients are rare.

This study thus aimed analysing the predictive value of preoperative serum DCP level on tumor recurrence and prognosis, particularly in hepatocellular carcinoma patients who had undergone liver resection and who met the criteria for small hepatocellular carcinoma[21], i.e. a single
tumor with all dimensions being 5 cm or less, or no more than three tumors with dimensions of 3 cm or less.

**MATERIALS AND METHODS**

From a total of 172 consecutive patients who had undergone a first curative hepatic resection for hepatocellular carcinoma at the Hirosaki University Hospital from 1990 to 2004, 142 patients whose preoperative DCP level was measured by a highly-sensitive assay were enrolled. Patients who met the criteria of small hepatocellular carcinoma, i.e. a single tumor with the largest dimension being 5 cm or less, or no more than three tumors with the largest dimension being 3 cm or less, were compared to the others. Sixteen clinical parameters were recorded [age, gender, Child-Pugh score, serum total bilirubin level, serum albumin level, prothrombin activity, 15-min retention rate of indocyanine green (ICG R15), status of hepatitis virus infection, the number of tumors, the largest dimension of the tumor, the degree of tumor differentiation, the presence or absence of macroscopic and microscopic vascular invasion, the extent of tumor (stage), DCP, and alpha-fetoprotein (AFP)] and the predictivity for probability of recurrence and prognosis of survival were evaluated.

DCP was measured by the chemiluminescent immunoassay using a sensitive anti-DCP antibody (Eisai Co., Ltd., Tokyo, Japan), and threshold values were set to 40, 100, 200, and 400 mAU/mL for determining the presence or absence of a positive reaction. Moreover, the extent of tumor was classified according to the stage classification by the Liver Cancer Study Group of Japan.

**Statistical analysis**

Comparisons between the two groups were carried out by the Chi-square test for categorical data and Student's t-test for continuous data. The continuous variables were reported as the mean ± SD. A Cox proportional hazards model was used to test the significance of 16 parameters as predictors of recurrence-free and overall survivals. Kaplan-Meier method and long rank test were also adapted to compare the effect of these factors on survival. These statistical analyses were performed using the SPSS 11.0 statistical software program. P values < 0.05 were considered to be statistically significant.

**RESULTS**

In this series, 85 of 142 patients met the criteria of small hepatocellular carcinoma, accounting for 60% of the total. Patients’ characteristics are given in Table 1. The mean age was 63.0 ± 10.6 years; that of the patients with small hepatocellular carcinoma was significantly higher than that of the patients with greater tumors. No difference was observed between males and females. Concerning Child-Pugh score, Class B was observed more commonly in patients who did not meet the criteria.

Other liver function tests as serum total bilirubin level, albumin level, platelet counts, or ICG R15 were also found to be lower in patients with small hepatocellular carcinoma. A positive status for hepatitis virus, either hepatitis B or C virus, was more frequent in small hepatocellular carcinoma patients. The largest dimension of the tumor was 2.9 ± 1.2 cm in patients who met the criteria for small hepatocellular carcinoma; it was 7.6 ± 4.6 cm in patients who did not meet the criteria, and lesions > 5 cm in size accounted for more than 70% of the cases in the latter group. Ninety percent of the patients who met the criteria for small hepatocellular carcinoma had a solitary lesion, whereas a significantly larger number of patients outside the criteria had multiple lesions. Pathological evaluation of tumor specimens revealed a significantly higher rate of poorly differentiated tumor cells and a more frequent presence of microscopic vascular invasion in patients who did not meet the criteria for small hepatocellular carcinoma compared to those who did. According to the TNM-Staging by the Liver Cancer Study Group of Japan, approximately 90% of the patients who met the criteria were classified as being in Stage I or II, whereas two-thirds of the patients outside the criteria were classified as being in Stage III or higher. Serum AFP level were not found to be different between the two groups, when threshold was set to 40 ng/mL. However, when a threshold of ≥ 200 ng/mL was chosen, the positive rate among patients outside the criteria was high. On the other hand, the serum DCP showed a lower positive rate among patients who met the criteria for small hepatocellular carcinoma compared to those who did not independently on the threshold value.

The recurrence-free survival and the overall survival of the whole group of patients was 60.3% for 1 year, 29.5% for 3 years, and 13.9% for 3 years, and 92.1% for 1 year, 69.6% for 3 years, and 56.9% for 5 years, respectively. The recurrence-free survival of the patients who met the criteria was 66.2% for 1 year, 34.1% for 3 years, and 16.6% for 5 years, with the overall survival of 97.5% for 1 year, 82.5% for 3 years, and 67.9% for 5 years, which compared particularly well to the recurrence-free survival of patients who did not meet the criteria as 49.9% for 1 year, 20.1% for 3 years, and 7.5% for 5 years (P = 0.0195), and the overall survival being 82.8% for 1 year, 46.8% for 3 years, and 36.5% for 5 years (P < 0.0001).

An univariate analysis, including all patients revealed the number of tumors, the degree of tissue differentiation, vascular invasion, tumor stage, any of the DCP thresholds, and AFP ≥ 20 ng/mL, as significant prognostic factors for recurrence. Concerning overall survival, the tumor diameter was also a significant prognostic factor (Table 2). Consequently, a multivariate analysis indicated that microscopic vascular invasions (P = 0.03) and DCP ≥ 400 mAU/mL (P = 0.02) were independent prognostic factors for survival prognosis (Table 3). There was no independent factor reflecting the recurrence-free survival.

The univariate analysis of the 85 patients with small hepatocellular carcinoma showed that DCP of various cut-off values was the most significant prognostic factor (Table 4), and a multivariate analysis showed that DCP ≥ 400 mAU/mL was the only independent prognostic factor for recurrence-free survival (Hazard ratio (HR): 3.32; 95% confidence interval (CI): 1.20-9.17, P = 0.02) and overall survival (HR: 1.20; 95% CI: 2.98-50.00, P = 0.0005) (Table 5).

On the other hand, a multivariate analysis of the 57 patients outside the criteria showed that microscopic
vascular invasion was the only independent prognostic factor for recurrence-free survival (HR: 2.97; 95% CI: 1.17-7.58, \( P = 0.02 \)) and overall survival (HR: 3.92; 95% CI: 1.38-11.24, \( P = 0.01 \)) (Table 6).

By performing a Kaplan-Meier analysis of small hepatocellular carcinoma patients, the period of recurrence-free survival was found to be significantly shorter in the group of patients with DCP levels \( \geq 400 \) mAU/mL (\( P = 0.02 \)), and more than 70% of the patients experienced some recurrence within 1 year (Figure 1A). Tumor recurrence after surgery occurred within the liver in 95% of patients with DCP levels < 400 mAU/mL, but extrahepatically in 75% of patients with DCP levels \( \geq 400 \) mAU/mL (\( P < 0.0001 \)). At the time of tumor recurrence, an elevation of DCP levels was observed among all patients. Most of the patients with tumor recurrence received TAE, local ablation therapy, or a second hepatectomy, but the overall survival also significantly decreased for the group of DCP \( \geq 400 \) mAU/mL (\( P < 0.0001 \)) (Figure 1B).

**DISCUSSION**

In this series, a high preoperative level of DCP was the only prognostic indicator for recurrence and poor prognosis in patients who underwent hepatectomy for a small hepatocellular carcinoma. Presence of microscopic vascular invasion, on the other hand, was the independent predictor of poor prognosis of both recurrence-free and overall survivals in more advanced hepatic carcinomas. Thus, different results were obtained for the prognostic factors, depending on disease progression.

Table 1  Comparison of demographic and clinical data

| Factor                          | Conforming to the criteria (\( n = 85 \)) | Outside the criteria (\( n = 57 \)) | \( P \) value |
|---------------------------------|------------------------------------------|-------------------------------------|---------------|
| Age (yr)                        | 65.0 ± 8.4                               | 60.1 ± 12.7                         | 0.007         |
| Gender                          | Male                                      | 62 (73%)                            | 44 (77%)      | 0.57         |
|                                | Female                                    | 23 (27%)                            | 13 (23%)      |              |
| Child-Pugh score                |                                          |                                     |               |
| Class A                         | 71 (84%)                                  | 54 (95%)                            | 0.04          |
| Class B                         | 14 (16%)                                  | 3 (5%)                              |               |
| Class C                         | 0                                        | 0                                   |               |
| Total bilirubin (mg/dL)         | 0.90 ± 0.55                              | 0.74 ± 0.32                         | 0.04          |
|                                | < 1                                       | 42 (49%)                            | 41 (72%)      | 0.008        |
|                                | \( \geq 1 \)                              | 43 (51%)                            | 16 (28%)      |              |
| Albumin (g/dL)                  | 3.7 ± 0.5                                 | 3.8 ± 0.50                          | 0.04          |
|                                | \( \leq 3.5 \)                            | 39 (46%)                            | 14 (25%)      | 0.01         |
|                                | \( > 3.5 \)                               | 46 (54%)                            | 43 (75%)      |              |
| Prothrombin time (%)            | 83.0 ± 15.2                               | 85.9 ± 17.6                         | 0.30          |
|                                | \( \leq 80 \)                             | 35 (41%)                            | 25 (44%)      | 0.75         |
|                                | \( > 80 \)                                | 50 (59%)                            | 32 (56%)      |              |
| Platelet count \( (\times 10^4 / \text{mm}^3) \) | 11.8 ± 6.3                               | 17.5 ± 8.6                          | <0.0001       |
|                                | \( < 10 \)                                | 35 (41%)                            | 13 (23%)      | 0.02         |
|                                | \( \geq 10 \)                             | 50 (59%)                            | 44 (77%)      |              |
| ICG R15 (%)                    | 19.8 ± 9.9                                | 14.1 ± 1.2                          | 0.0005        |
|                                | \( \leq 15 \)                             | 26 (31%)                            | 35 (61%)      | 0.0003       |
|                                | \( > 15 \)                                | 59 (69%)                            | 22 (39%)      |              |
| Hepatitis virus                |                                          |                                     |               |
| C positive                     | 68 (80%)                                  | 26 (46%)                            | <0.0001       |
| B positive                     | 8 (10%)                                   | 15 (26%)                            | 0.008         |
| Tumor number                   |                                          |                                     |               |
| Single                         | 77 (91%)                                  | 29 (51%)                            | <0.0001       |
| Multiple                       | 8 (9%)                                    | 28 (49%)                            |              |
| Tumor size (cm)                | 2.9 ± 1.2                                 | 7.6 ± 4.6                           | <0.0001       |
|                                | \( \leq 3 \)                              | 52 (61%)                            | 5 (9%)        | <0.0001      |
|                                | \( 3-5 \)                                 | 33 (39%)                            | 11 (19%)      |              |
|                                | \( > 5 \)                                 | 0                                   | 41 (72%)      |              |
| Histology                      |                                          |                                     |               |
| Well or moderately differentiated | 68 (87%)                                  | 39 (71%)                            | 0.02         |
| Poorly differentiated           | 10 (13%)                                  | 16 (29%)                            |              |
| Vascular invasion              |                                          |                                     |               |
| Macroscopically positive       | 0                                        | 5 (8.8%)                            | 0.005         |
| Microscopically positive       | 8 (10%)                                   | 18 (32%)                            | 0.0008        |
| TNM Staging by the LCSGJ \(^1\) |                                          |                                     |               |
| I/I + II/III/IV-A              | 25/5/18/1                                 | 2/17/25/13                          | <0.0001       |
| I + II                         | 76 (99%)                                  | 19 (33%)                            | <0.0001       |
| III + IV-A                     | 3 (41%)                                   | 38 (67%)                            |              |
| AFP (ng/mL)                    | 933 ± 660                                 | 31473 ± 192949                      | 0.15          |
|                                | \( \geq 20 \)                             | 52 (61%)                            | 35 (61%)      | 0.98         |
|                                | \( \geq 100 \)                            | 28 (33%)                            | 26 (46%)      | 0.13         |
|                                | \( \geq 200 \)                            | 16 (19%)                            | 24 (42%)      | 0.003        |
|                                | \( \geq 400 \)                            | 11 (13%)                            | 23 (40 %)     | 0.0002       |
| DCP (mAU/mL)                   | 780 ± 4129                                | 8168 ± 28247                        | 0.02          |
|                                | \( \geq 40 \)                             | 45 (53%)                            | 43 (75%)      | 0.007        |
|                                | \( \geq 100 \)                            | 26 (31%)                            | 36 (63%)      | 0.0001       |
|                                | \( \geq 200 \)                            | 16 (19%)                            | 30 (53%)      | <0.0001      |
|                                | \( \geq 400 \)                            | 11 (13%)                            | 27 (47%)      | <0.0001      |

\(^1\) Liver Cancer Study Group of Japan.

www.wjgnet.com

Table 1  Comparison of demographic and clinical data
with invasiveness and the metastasizing property of carcinoma[2-8]. Shirabe et al[8] reported that the preoperative DCP level, tumor diameter, and histologic differentiation correlated with the presence or absence of microscopic vascular invasion in a study on 218 patients who had undergone hepatic resection. Sakon et al[2], Sugimoto et al[3], and Nanashima et al[4] also reported that a high preoperative DCP level correlated with the presence of microscopic vascular invasion in patients undergoing hepatectomy. Shimada et al[5] reported in a study on 40 patients who had undergone a living donor liver transplantation DCP levels $\geq$ 300 mAU/mL to be correlated with the presence of microscopic vascular invasion and DCP thus to be a poor prognostic factor.

However, the above reports regarding the invasive character of carcinoma among patients with a high level of DCP were based on the analyses of those with various cancer stages, including more advanced tumors and those with various degrees of liver cirrhosis. There are only a few reports on patients who had undergone a resection for small hepatocellular carcinoma, because vascular invasion or intrahepatic metastases are seldom seen in this group with earlier stage.

It has been reported that the rate of detectable levels of DCP is higher in patients with larger tumors[9]. On the other hand, the positive rate of DCP detectability is low for small hepatocellular carcinoma[9,24]. Okuda et al[9] reported that it was 81.3% with a tumor diameter of $\geq$ 3 cm, while it was 30.4% for $\leq$ 2 cm. Sassa et al[10] reported that it was 44.3% for $\leq$ 2 cm. An assay that employs higher DCP diagnostic sensitivity has been introduced[15,20], but the diagnostic sensitivity for small hepatocellular carcinoma still remains at about 50%[27-30]. Thus, most reports on biological properties of DCP present hepatic
Table 4  Univariate analysis for recurrence-free and overall survivals in 85 patients who met the criteria

| Factor                  | Covariate (n) | Reference (n) | Recurrence-free survival | Overall survival |
|-------------------------|---------------|---------------|--------------------------|-----------------|
|                         |               |               | HR  | 95% CI | P    | HR  | 95% CI | P    |
| Gender                  | Female (23)   | Male (62)     | 0.81 | 0.44-1.48 | 0.48 | 0.91 | 0.43-1.94 | 0.81 |
| Child-Pugh score        | B (14)        | A (71)        | 0.83 | 0.43-1.61 | 0.58 | 1.06 | 0.46-2.43 | 0.89 |
| Total bilirubin (mg/dL) | ≥ 1 (43)      | < 1 (42)      | 1.37 | 0.82-2.30 | 0.23 | 1.77 | 0.83-3.79 | 0.14 |
| Albumin (mg/dL)         | < 3.5 (39)    | > 3.5 (46)    | 0.92 | 0.55-1.52 | 0.74 | 0.56 | 0.28-1.09 | 0.09 |
| Prothrombin time (%)    | < 80 (35)     | ≥ 80 (50)     | 1.25 | 0.75-2.08 | 0.40 | 0.82 | 0.42-1.61 | 0.56 |
| Platelet (× 10^9/mm³)  | < 10 (35)     | ≥ 10 (50)     | 1.05 | 0.63-1.76 | 0.84 | 0.98 | 0.50-1.92 | 0.95 |
| RCG R1S                 | > 15% (59)    | < 15% (26)    | 1.37 | 0.79-2.38 | 0.27 | 1.51 | 0.70-3.26 | 0.29 |
| Hepatitis C virus       | Positive (68) | Negative (76) | 1.42 | 0.75-2.70 | 0.28 | 1.46 | 0.60-3.52 | 0.40 |
| Hepatitis B virus       | Positive (8)  | Negative (76) | 0.63 | 0.25-1.57 | 0.32 | 0.56 | 0.13-2.34 | 0.43 |
| Number of tumor         | Multiple (8)  | Single (77)   | 1.12 | 0.48-2.61 | 0.79 | 1.29 | 0.50-3.36 | 0.60 |
| Size of tumor (cm)      | > 3 (33)      | ≤ 3 (52)      | 1.08 | 0.64-1.81 | 0.78 | 1.27 | 0.63-2.56 | 0.50 |
| Histology               | Poor¹ (10)    | Well-mod² (68) | 1.32 | 0.56-3.11 | 0.53 | 3.03 | 0.89-10.29 | 0.08 |
| Vascular invasion       | Present (8)   | Absent (75)   | 0.96 | 0.38-2.06 | 0.77 | 0.88 | 0.31-2.54 | 0.82 |
| Stage by LCSGJ³         | III + IV (9)  | 1 + II (76)   | 1.41 | 0.64-3.12 | 0.39 | 1.80 | 0.74-4.35 | 0.20 |
| AFP (ng/mL)             | ≥ 20 (52)     | < 20 (33)     | 1.47 | 0.87-2.49 | 0.15 | 1.50 | 0.75-3.02 | 0.25 |
|                         | > 200 (16)    | < 200 (69)    | 0.53 | 0.25-1.13 | 0.10 | 0.50 | 0.18-1.42 | 0.20 |
|                         | > 400 (11)    | < 400 (74)    | 0.57 | 0.23-1.43 | 0.23 | 0.53 | 0.13-2.21 | 0.38 |
| DCP (mAU/mL)            | ≥ 40 (45)     | < 40 (40)     | 1.73 | 1.03-2.92 | 0.04 | 1.46 | 0.75-2.82 | 0.27 |
|                         | ≥ 100 (26)    | < 100 (59)    | 1.67 | 0.96-2.91 | 0.07 | 1.71 | 0.83-3.50 | 0.14 |
|                         | ≥ 200 (16)    | < 200 (69)    | 1.69 | 0.84-3.40 | 0.14 | 5.61 | 2.56-13.16 | <0.0001 |
|                         | ≥ 400 (11)    | < 400 (74)    | 2.41 | 1.12-5.18 | 0.02 | 5.71 | 2.38-13.70 | <0.0001 |

n: Number of patients; HR: Hazard ratio; CI: Confidence interval. ¹Poorly differentiated; ²Well or moderately differentiated hepatocellular carcinoma; ³LCSGJ: Liver Cancer Study Group of Japan.

Table 5  Multivariate analysis for recurrence-free and overall survivals in 85 patients who met the criteria

| Factor                  | Covariate (n) | Reference (n) | Recurrence-free survival | Overall survival |
|-------------------------|---------------|---------------|--------------------------|-----------------|
|                         |               |               | HR  | 95% CI | P    | HR  | 95% CI | P    |
| Number of tumor         | Multiple (8)  | Single (77)   | 0.66 | 0.06-7.45 | 0.74 | 0.46 | 0.006-35.08 | 0.72 |
| Histology               | Poor¹ (10)    | Well-mod² (68) | 0.70 | 0.24-2.01 | 0.51 | 0.37 | 0.07-2.12 | 0.27 |
| Vascular invasion       | Present (8)   | Absent (75)   | 1.04 | 0.44-2.46 | 0.95 | 0.96 | 0.31-2.99 | 0.95 |
| Stage by LCSGJ³         | III + IV (9)  | 1 + II (76)   | 1.90 | 0.17-21.28 | 0.60 | 3.97 | 0.05-333.33 | 0.53 |
| AFP (ng/mL)             | ≥ 20 (52)     | < 20 (33)     | 1.28 | 0.71-2.29 | 0.41 | 1.20 | 0.54-2.66 | 0.66 |
|                         | ≥ 100 (26)    | < 100 (59)    | 1.67 | 0.96-2.91 | 0.07 | 1.71 | 0.83-3.50 | 0.14 |
|                         | ≥ 200 (16)    | < 200 (69)    | 1.69 | 0.84-3.40 | 0.14 | 5.61 | 2.56-13.16 | <0.0001 |
| DCP (mAU/mL)            | ≥ 400 (11)    | < 400 (74)    | 3.32 | 1.20-9.17 | 0.02 | 1.20 | 2.98-50.00 | 0.0005 |

n: Number of patients; HR: Hazard ratio; CI: Confidence interval. ¹Poorly differentiated; ²Well or moderately differentiated hepatocellular carcinoma; ³LCSGJ: Liver Cancer Study Group of Japan.

carcinoma are based on more advanced diseases[7].

Many reports state high DCP levels to be of poor prognostic value. It has been shown that patients with high DCP and high AFP levels show poor prognostic factors[4,6,10-12,14-16]. Thus, a prognostic staging system in which DCP has been incorporated is being suggested. From an analysis of 141 patients Kawakita et al[15] concluded DCP ≥ 100 mAU/mL to be a prognostic factor, and the authors suggested a stage classification in which DCP ≥ 100 mAU/mL should be incorporated into
DCP is an abnormal prothrombin that is produced by under-carboxylation of normal prothrombin.\cite{57} Suzuki et al.\cite{40} reported that DCP exerts a mitogenic effect on hepatocellular carcinoma cells via a Met-Janus kinase 1-STAT3 signaling pathway. On the other hand, it has been demonstrated that the antiproliferative effect of vitamin K on hepatic carcinoma is not due to a depressed production of DCP, but rather caused by protein kinase A\cite{39}. Regarding the above, the mechanism of the antiproliferative effect of DCP on hepatic carcinoma is still unknown. However, in this study, it was clarified that a high DCP level is an important prognostic factor for recurrence, even in a condition in which small hepatocellular carcinoma before the histological invasion of carcinoma such as vascular invasion becomes obvious. This result corresponds to reports by Koike et al.\cite{40} and Hagiwara et al.\cite{40} showing that patients who have a high DCP level can expect the expression of future vascular invasion. Moreover, in many of our patients tumor recurred extrahepatically early after resection. Regarding the precise mechanism of DCP on hepatic carcinoma development, it would be necessary to study its effect on the initiation of vascular invasion and on proliferative activity.

Figure 1 Postoperative recurrence-free (A) and overall (B) survival of patients with small hepatocellular carcinoma. In 85 patients who met the criteria for small hepatocellular carcinoma, the period of recurrence-free survival was significantly shorter in patients with DCP $\geq 400$ mAU/mL than in those with DCP $< 400$ mAU/mL ($P = 0.02$). More than 70% of recurrences occurred within a year (A). Overall survival was also significantly shorter in patients with DCP $\geq 400$ mAU/mL (B).

The univariate analyses of 16 clinical parameters in all patients indicated that the number of tumors, the tumor diameter, the degree of histologic differentiation, vascular invasion, the tumor staging, serum AFP level, and DCP level to be significant prognostic factors. These results are in accordance with previous reports.\cite{8,10,12,15-19,34-36} However, limiting this to small hepatocellular carcinoma, DCP alone is the independent prognostic factor for both tumor recurrence and patients’ survival, while presence of microscopic vascular invasion is a prognostic factor in patients outside the criteria. Regarding the mechanism of these different results, we assume the following. Specifically, a high DCP level constitutes a risk factor of microscopic vascular invasion and in small hepatocellular carcinoma, DCP shows positive before the development of microscopic vascular invasion and becomes an independent prognostic factor. As a tumor becomes larger, the frequency of the detection of microscopic vascular invasion increases and the independence of DCP disappears, thus showing a stronger correlation with the prognosis than does DCP.\”

The CLIP score. Nanashima et al.\cite{10} concluded that DCP $\geq 400$ mAU/mL was a poor prognostic factor and suggested modified CLIP scoring. Moreover, Omagari et al.\cite{12} suggested the SLiDe score combined with stages and liver damage, and Toyoda et al.\cite{13} suggested the BALAD score that can predict the prognosis only by measuring bilirubin, albumin, AFP-L3, AFP, and DCP using preoperative serum samples, according to an analysis of 2600 patients.

In this study, we used the criteria of small hepatocellular carcinoma “a 5-cm single tumor or no more than three 3-cm tumors” as suggested by Mazzaferro et al.\cite{14}. These criteria are internationally accepted as inclusion criteria for liver transplantation for unresectable small hepatocellular carcinoma, because they reflect a restriction of the tumor to the liver\cite{14}.

The univariate analyses of 16 clinical parameters in all patients indicated that the number of tumors, the tumor diameter, the degree of histologic differentiation, vascular invasion, the tumor staging, serum AFP level, and DCP level to be significant prognostic factors. These results are in accordance with previous reports.\cite{8,10,12,15-19,34-36} However, limiting this to small hepatocellular carcinoma, DCP alone is the independent prognostic factor for both tumor recurrence and patients’ survival, while presence of microscopic vascular invasion is a prognostic factor in patients outside the criteria. Regarding the mechanism of these different results, we assume the following. Specifically, a high DCP level constitutes a risk factor of microscopic vascular invasion and in small hepatocellular carcinoma, DCP shows positive before the development of microscopic vascular invasion and becomes an independent prognostic factor. As a tumor becomes larger, the frequency of the detection of microscopic vascular invasion increases and the independence of DCP disappears, thus showing a stronger correlation with the prognosis than does DCP.\”

### REFERENCES

1. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee
Clinical utility of plasma des-gamma-carboxy prothrombin and alpha-fetoprotein in hepatocellular carcinoma. J Hepatol 2007; 47: 235-240.

Ando E, Tanaka M, Yamashita F, Kuromoto R, Takada A, Fukushima K, Yonehara Y, Sumie S, Okuda K, Kumashiro R, Saito M. Diagnostic clues for recurrent hepatocellular carcinoma: comparison of tumour markers and imaging studies. Eur J Gastroenterol Hepatol 2003; 15: 641-648.

Sassa T, Kumada T, Nakano S, Uematsu T. Clinical utility of simultaneous measurement of serum high-sensitivity des-gama-carboxy prothrombin and Lens culinaris agglutinin A-reactive alpha-fetoprotein in patients with small hepatocellular carcinoma. Eur J Gastroenterol Hepatol 1999; 11: 1387-1392.

Shimauchi Y, Tanaka M, Kuromatsu R, Ogata R, Tateishi Y, Itano S, Ono N, Yutani S, Nagamatsu H, Matsugaki S, Yanagida H, Saito Y, Yamashita F, Saito M, Tanaka K, Sata M. A simultaneous monitoring of Lens culinaris agglutinin A-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin as an early diagnosis of hepatocellular carcinoma in the follow-up of cirrhotic patients. Oncol Rep 2000; 7: 249-256.

Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Yamaguchi A, Isogai M, Kaneoka Y, Washizu J. Prognostic significance of simultaneous measurement of three tumour markers in patients with hepatocellular carcinoma. Clin Gastroenterol Hepatol 2006; 4: 111-117.

Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of preoperative des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. Cancer 1998; 82: 5682-691.

Shirabe K, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, Aishima S, Maehara Y. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma: with special reference to the serum levels of des-gamma-carboxyprothrombin. J Hepatol 2002; 37: 917-924.

Kikuchi H, Aisawa S, Mihara M, Sugimachi K. Des-gamma-carboxyprothrombin in candidates for liver transplantation with hepatocellular carcinoma. Hepatol Res 2002; 23: 165-171.

Okuda H, Nakanishi T, Kadomatsu T, Umezawa H, Okuda K, Saito R, Nikaido T, Yamanaka Y, Yamaguchi A, Sugimachi K. Des-gamma-carboxy prothrombin: an early marker of recurrent hepatocellular carcinoma. Hepatology 1997; 25: 1527-1531.

Nakamura S, Nakashima T, Ogasawara M, Shoji S, Takagi K, Hayashi S, Sato S. Clinical evaluation of plasma des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma in patients with tumors of various sizes. J Gastroenterol 1998; 33: 682-691.

Nakamura S, Kouko S, Sakamoto K, Kamei A, Fujita K, Kumada T, Osaki Y, Oka H, Urano F, Kudo M, Nakanishi T, Takatsu K, Saito A, Hayashi N, Kaito M, Ishihara T, Nakagawa N, Kamei A, Fujita K, Wise M, Satomura S. Clinical evaluation of plasma des-gamma-carboxy prothrombin and proliferative activity of hepatocellular carcinoma. Surgery 1995; 117: 682-691.

Sugimoto H, Takeda S, Inoue S, Kaneko T, Watanabe K, Nakao A. Des-gamma-carboxy prothrombin (DCP) ratio, a novel parameter measured by monoclonal antibodies MU-3 and 3C11 for a new prognostic indicator for hepatocellular carcinoma. Liver Int 2003; 23: 38-44.

Shimada M, Yonemura Y, Iijichi H, Harada N, Shiotsani S, Ninomiya M, Terashi T, Yoshizumi T, Soejima Y, Maehara Y. Living donor liver transplantation for hepatocellular carcinoma: a special reference to a preoperative des-gamma-carboxy prothrombin value. Transplant Proc 2003; 37: 1177-1179.

Carr BI, Kanne W, Wise M, Satomura S. Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. Dig Dis Sci 2007; 52: 776-782.

Shirabe K, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, Aishima S, Maehara Y. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma: with special reference to the serum levels of des-gamma-carboxyprothrombin. J Hepatol 2002; 37: 917-924.

Kikuchi H, Aisawa S, Mihara M, Sugimachi K. Des-gamma-carboxy prothrombin in candidates for liver transplantation with hepatocellular carcinoma. Hepatol Res 2002; 23: 165-171.

Nakamura S, Nakashima T, Ogasawara M, Shoji S, Takagi K, Hayashi S, Sato S. Clinical evaluation of plasma des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma in patients with tumors of various sizes. J Gastroenterol 1998; 33: 682-691.

Nakamura S, Kouko S, Sakamoto K, Kamei A, Fujita K, Kumada T, Osaki Y, Oka H, Urano F, Kudo M, Nakanishi T, Takatsu K, Saito A, Hayashi N, Kaito M, Ishihara T, Nakagawa N, Kamei A, Fujita K, Wise M, Satomura S. Clinical evaluation of plasma des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma in patients with tumors of various sizes. J Gastroenterol 1998; 33: 682-691.
Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; 334: 693-699

Liver Cancer Study Group of Japan. Classification of Primary Liver Cancer. 1st ed. Tokyo: Kanehara, 1997: 2-22

Furukawa H, Shimamura T, Suzuki T, Taniguchi M, Yamashita K, Kamiyama T, Matsushita M, Todo S. Living-donor liver transplantation for hepatocellular carcinoma. *J Hepatobiliary Pancreat Surg* 2006; 13: 393-397

Hagiwara S, Kudo M, Kawasaki T, Nagashima M, Minami Y, Chung H, Fukunaga T, Kitano M, Nakatani T. Prognostic factors for portal venous invasion in patients with hepatocellular carcinoma. *J Gastroenterol* 2006; 41: 1214-1219

Hamamura K, Shiratori Y, Shiina S, Imamura M, Obi S, Sato S, Yoshida H, Omata M. Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. *Cancer* 2000; 88: 1557-1564

Tateishi R, Shiina S, Yoshida H, Teratani T, Obi S, Yamashiki N, Yoshida H, Akamatsu M, Kawabe T, Omata M. Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. *Hepatology* 2006; 44: 1518-1527

Naraki T, Kohno N, Saito H, Fujimoto Y, Ohhira M, Morita T, Kohgo Y. gamma-Carboxyglutamic acid content of hepatocellular carcinoma-associated des-gamma-carboxy prothrombin. *Biochim Biophys Acta* 2002; 1586: 287-298

Suzuki M, Shiraha H, Fujikawa T, Takaoka N, Ueda N, Nakanishi Y, Koike K, Takaki A, Shiratori Y. Des-gamma-carboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. *J Biol Chem* 2005; 280: 6409-6415

Otsuka M, Kato N, Shao RX, Hoshida Y, Iijichi H, Koike Y, Taniguchi H, Moriyyama M, Shiratori Y, Kawabe T, Omata M. Vitamin K2 inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A activation. *Hepatology* 2004; 40: 243-251

Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, Yoshida H, Shiina S, Omata M. Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. *Cancer* 2001; 91: 561-569