

## Vitamin K Contents in Liver Tissue of Hepatocellular Carcinoma Patients

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Serum protein induced in vitamin K absence-II (PIVKA-II) is used as a tumor marker because it increases at a notably higher rate in patients with hepatocellular carcinoma. To clarify the mechanism causing the elevation of serum PIVKA-II, we measured the contents of vitamins K<sub>1</sub> (phyloquinone, PK) and K<sub>2</sub> (menaquinone, MK) (MK-4, MK-5, MK-6, MK-7, MK-8, MK-9, MK-10) in liver tissue resected from 21 hepatic cancer patients (12 patients with hepatocellular carcinoma and 9 patients with metastatic hepatic cancer), using HPLC combined with coulometric reduction and fluorometric detection. In the cancerous tissue of hepatocellular carcinoma patients, PK, MK-7, MK-8, and MK-10 were significantly lower than that found in the noncancerous tissue. Furthermore, MK-6, MK-7, MK-8, and MK-10 in the cancerous tissue of hepatocellular carcinoma patients were significantly lower than that in the cancerous tissue of metastatic hepatic cancer patients. These data suggested that one of the mechanisms of the elevation of serum PIVKA-II levels in hepatocellular carcinoma patients is a vitamin K deficiency in the local cancerous tissue.

Key words: Phylloquinone — Menaquinone — Protein induced in vitamin K absence-II — Hepatocellular carcinoma — Metastatic hepatic cancer

Vitamin K is a general term for nutrients or 2-methyl-1,4-naphthoquinone compounds that show antihemorrhagic action when given to animals with vitamin K deficiency. Vitamin K<sub>1</sub> has a 2-methyl-1,4-naphthoquinone ring with a phytyl side chain at the third position and is generically called phylloquinone (PK). Vitamin K<sub>2</sub> has a 2-methyl-1,4-naphthoquinone ring with an isoprenyl side chain at the third position and is generically called menaquinone (MK). Homologues of menaquinone have 1 to 15 isoprenyl side chains. These homologues are designated as menaquinone-*n* (MK-*n*), with *n* denoting the number of isoprenyl side chains.<sup>1,2)</sup>

There are many vitamin K-dependent proteins in the body such as prothrombin (factor II), coagulation factor VII, coagulation factor IX, coagulation factor X, protein C, protein S, osteocalcin, and matrix Gla protein. The relevant genes encode precursor proteins (proteins induced in vitamin K absence: PIVKA). Vitamin K is an essential cofactor required for the reaction ( $\gamma$ -carboxylation) that modifies a specific glutamic acid residue, near the amino acid terminal of the precursor proteins, into  $\gamma$ -carboxyglutamic acid. When vitamin K is deficient, the conversion of the glutamic acid residue to  $\gamma$ -carboxyglutamic acid is inhibited, then the precursor proteins without  $\gamma$ -carboxyglutamic acid accumulate in cells and are partly released into the blood.<sup>3–6)</sup> PIVKA corresponding to prothrombin

(factor II) is called PIVKA-II and is measured clinically as a means to diagnose vitamin K deficiency.

In patients with hepatocellular carcinoma,  $\alpha$ -fetoprotein has been used as a tumor marker. In 1984, Liebman *et al.*<sup>7)</sup> measured serum PIVKA-II levels in hepatocellular carcinoma patients by means of competition radioimmunoassays using a polyclonal antibody and reported that it was increased in 91% of those patients. Motohara *et al.*<sup>8)</sup> subsequently developed a highly sensitive method for measuring serum PIVKA-II using a monoclonal antibody. Several clinical studies<sup>9–13)</sup> with this method supported the view that serum PIVKA-II is a useful tumor marker with high specificity for hepatocellular carcinoma. However, the underlying mechanism causing the elevation of serum PIVKA-II levels in hepatocellular carcinoma patients remains unclear.

The aim of this study is to clarify this mechanism by measuring PK and MK homologue contents in the cancerous and noncancerous liver tissue of hepatocellular carcinoma patients and the cancerous liver tissue of metastatic hepatic cancer patients.

### MATERIALS AND METHODS

**Subjects** Portions of liver tissue resected from 21 hepatic cancer patients, who were admitted to our university hospital to undergo hepatic resection between April 1995 and December 1996, were used for this study. Informed consent was obtained from all the patients. Among them, 12

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Table I. Clinicopathological Characteristics of 12 Patients with Hepatocellular Carcinoma

| Patients | Age (yr) | Sex <sup>a)</sup> | Etiology <sup>b)</sup> | PIVKA-II <sup>c)</sup> (AU/ml) | PT <sup>d)</sup> (%) | aPTT <sup>e)</sup> (s) | Liver histology <sup>f)</sup> |           | Cancer size (cm×cm) |
|----------|----------|-------------------|------------------------|--------------------------------|----------------------|------------------------|-------------------------------|-----------|---------------------|
|          |          |                   |                        |                                |                      |                        | Cancer                        | Noncancer |                     |
| 1        | 59       | M                 | B                      | 12.60 (+)                      | 75                   | 30.3                   | Well                          | Cirrhosis | 7.0×8.0             |
| 2        | 69       | M                 | C                      | 10.90 (+)                      | 73                   | 32.4                   | Mod                           | Cirrhosis | 5.0×5.0             |
| 3        | 76       | M                 | C                      | 0.97 (+)                       | 75                   | 29.5                   | Mod                           | Normal    | 4.8×4.5             |
| 4        | 63       | M                 | C                      | 0.15 (+)                       | 64                   | 32.2                   | Well                          | Cirrhosis | 4.0×3.0             |
| 5        | 72       | M                 | C                      | 0.12 (+)                       | 85                   | 31.3                   | Mod                           | Cirrhosis | 3.0×2.8             |
| 6        | 67       | M                 | C                      | 0.07 (–)                       | 86                   | 30.9                   | Well                          | Cirrhosis | 1.2×1.2             |
| 7        | 80       | M                 | C                      | 0.06 (–)                       | 100                  | 30.4                   | Mod                           | Normal    | 2.2×1.5             |
| 8        | 63       | M                 | B                      | 0.02 (–)                       | 50                   | 33.3                   | Mod                           | Cirrhosis | 3.3×2.6             |
| 9        | 60       | M                 | C                      | <0.06 (–)                      | 83                   | 28.2                   | Well                          | Cirrhosis | 2.3×2.2             |
| 10       | 61       | M                 | C                      | <0.06 (–)                      | 63                   | 36.8                   | Well+Mod                      | Cirrhosis | 3.0×3.0             |
| 11       | 70       | F                 | C                      | <0.06 (–)                      | 55                   | 35.5                   | Mod                           | Cirrhosis | 2.0×1.8             |
| 12       | 66       | F                 | C                      | <0.06 (–)                      | 78                   | 28.4                   | Mod                           | Cirrhosis | 4.0×4.5             |

a) M, male; F, female.

b) B, hepatitis B; C, hepatitis C.

c) PIVKA-II, protein induced in vitamin K absence-II (positive value, (+)>0.1 AU/ml).

d) PT, prothrombin time (reference value≥70%).

e) aPTT, activated partial thromboplastin time (reference value, 24.9 to 33.2 s).

f) Cancer, cancerous liver tissue; Noncancer, noncancerous liver tissue; Well, well differentiated hepatocellular carcinoma; Mod, moderate differentiated hepatocellular carcinoma.

patients had hepatocellular carcinoma and 9 had metastatic hepatic cancer. Normal human liver tissues were not used as controls because of ethical problems.

The clinicopathological characteristics of the 12 patients with hepatocellular carcinoma are shown in Table I. Ten patients were males and 2 females. Their average ( $\pm$ SD) age was 67.1 ( $\pm$ 6.5) years, ranging from 59 to 80 years. None had received supplemental vitamin K for at least 1 month before the operation and oral intake of all patients was good before surgery. None had been administered fat emulsion or anticancer agents. Four had received intravenous administration of 1 g of cefmetazole immediately before surgery. The serum PIVKA-II level was measured by using an enzyme immunoassay kit (Eitest Mono P-II, Eisai, Tokyo),<sup>10,11)</sup> which employs an improved enzyme immunoassay using a monoclonal antibody developed by Motohara *et al.*<sup>8)</sup> Serum PIVKA-II levels above 0.1 arbitrary unit (AU)/ml were considered positive. Five patients were positive for serum PIVKA-II and 7 were negative. Prothrombin time and activated partial thromboplastin time revealed no apparent prolongation. Nine patients had hepatitis C and 2 had hepatitis B. Histopathological findings of the resected cancerous tissue of hepatocellular carcinoma patients were well differentiated hepatocellular carcinoma in 4 patients, moderately differentiated hepatocellular carcinoma in 7, and a combination of these in 1. The resected cancerous tissues were 7.0×8.0 cm maximum and 1.2×1.2 cm minimum in size. Histopathological findings of the resected noncancerous tissue of hepatocellular

carcinoma patients were liver cirrhosis in 10 patients and normal in 2.

Of the 9 patients with metastatic hepatic cancer, seven patients were males and 2 females. Their average ( $\pm$ SD) age was 63.2 ( $\pm$ 6.2) years, ranging from 57 to 78 years. None had received vitamin K for at least 1 month before surgery. Five patients had good ingestion, 3 had had no oral intake for 3 days, and 1 had had no oral intake for a week before surgery. Fat emulsion was administered to 1 patient before surgery. Six patients had received kanamycin and clindamycin orally the day before surgery. Prothrombin time and activated partial thromboplastin time showed no apparent prolongation. The underlying disease (primary tumor) was cancer of the sigmoidal colon in 6 patients, cancer of the ascending colon in 2, and rectal cancer in 1. Histopathological findings of the resected cancerous tissue of metastatic hepatic cancer patients were all adenocarcinoma, and their sizes were 10.0×8.0 cm maximum and 1.0×1.0 cm minimum.

Thirty-three samples of the resected cancerous and noncancerous liver tissue (only the cancerous tissue for metastatic cancer patients) were stored frozen at  $-30^{\circ}\text{C}$ . Their PK and MK homologue contents were measured later.

**Measurement of PK and MK homologue contents in liver tissue** PK and MK homologue (MK-4, MK-5, MK-6, MK-7, MK-8, MK-9, MK-10) contents in the resected liver tissue were measured by using HPLC combined with coulometric reduction and fluorometric detection,<sup>14)</sup> as previously described. A 1.0 g sample of freeze-dried liver tis-

sue was homogenized in 5 ml of 66% isopropyl alcohol. The homogenate was mixed with 6 ml of *n*-hexane. The mixture was centrifuged at 800*g* for 5 min. A 5 ml portion of the upper layer was evaporated to dryness under reduced pressure at room temperature. The residue was dissolved in 2 ml of *n*-hexane, and the solution was applied to a Sep-Pak silica cartridge. This was washed with 10 ml of *n*-hexane and vitamin Ks were eluted with 5 ml of *n*-hexane–diethyl ether (96:4, v/v). The eluate was evaporated to dryness under reduced pressure at room temperature. The residue was dissolved in 150  $\mu$ l of *n*-hexane and the solution was applied as a 13 cm band to a silica gel plate. The plate was dried for 5 min in air at room temperature. The 13 cm rectangular silica gel layer was scraped and the materials were extracted with 7 ml of chloroform and centrifuged at 800*g* for 5 min. A 5 ml portion of the chloroform layer was evaporated to dryness under reduced pressure at room temperature. The residue was dissolved in 200  $\mu$ l of ethanol and 50  $\mu$ l of the solution was injected into the HPLC system. At the same time, 50  $\mu$ l of the working standard solution for calibration was also injected into the system. The PK and MK homologue contents in the sample extract were measured by the peak-height method and calculated from their calibration graphs.

**Measurement of plasma PK and MK homologue concentrations** Plasma samples were collected from the 12 patients with hepatocellular carcinoma immediately before surgery and the plasma PK and MK homologue (MK-4, MK-5, MK-6, MK-7, MK-8) concentrations were measured by HPLC combined with coulometric reduction and fluorometric detection, as previously described.<sup>15,16)</sup>

**Statistical analysis** A statistical analysis was performed with the Wilcoxon signed-rank test to compare the vitamin K homologue content in the cancerous tissue of hepatocellular carcinoma patients with that of the noncancerous tissue. We used the Mann-Whitney *U* test to compare the vitamin K homologue content in the cancerous tissue of hepatocellular carcinoma patients with that in the cancerous tissue of metastatic hepatic cancer patients; to compare that in the cancerous tissue of serum PIVKA-II positive hepatocellular carcinoma patients with that in the cancerous tissue of serum PIVKA-II negative patients; and to compare the plasma vitamin K concentration in the serum PIVKA-II positive hepatocellular carcinoma patients with that in the serum PIVKA-II negative patients. A value of *P*<0.05 was considered statistically significant.

Regarding the detection limit of measurements of various liver tissues, if the level was less than 1.00 ng/g in the analysis of vitamin K content of the cancerous tissue of hepatocellular carcinoma patients, for example, we treated it as 1.00 ng/g, and if less than 1.00 ng/g in the analysis of the noncancerous tissue of hepatocellular carcinoma

patients or the cancerous tissue of metastatic hepatic cancer patients, for example, we treated it as 0 ng/g.

## RESULTS

**PK and MK homologue contents in the cancerous liver tissue of hepatocellular carcinoma patients** Only PK was detected in all patients and the average ( $\pm$ SD) was 3.12 ( $\pm$ 4.83) ng/g. MK-4 and MK-7 were detected in all but 1 patient and the average values ( $\pm$ SD) of 11 patients were 2.03 ( $\pm$ 3.22) ng/g and 1.35 ( $\pm$ 0.92) ng/g, respectively. MK-6 was at the detection limit or below in 6 patients, MK-8 was similar in 7, and MK-9 and MK-10 were similar in 11 each. MK-5 was at the detection limit or below in all patients.

No significant differences were seen in PK and MK homologue contents in the cancerous liver tissue between serum PIVKA-II positive patients and negative patients (Fig. 1).

**PK and MK homologue contents in the noncancerous liver tissue of hepatocellular carcinoma patients** PK, MK-4, and MK-7 were detected in all patients, and the average ( $\pm$ SD) was 10.50 ( $\pm$ 14.15) ng/g, 2.42 ( $\pm$ 2.33) ng/g, and 32.76 ( $\pm$ 43.15) ng/g, respectively. MK-6, MK-8, and MK-10 were detected in all but 1 patient, and the average values ( $\pm$ SD) of 11 patients were 4.81 ( $\pm$ 8.50) ng/g, 15.71 ( $\pm$ 35.34) ng/g, and 15.35 ( $\pm$ 29.19) ng/g, respectively. MK-9 was at the detection limit or below in 9 patients and MK-5 was the same in all patients.

**PK and MK homologue contents in the cancerous liver tissue of metastatic hepatic cancer patients** PK, MK-6,

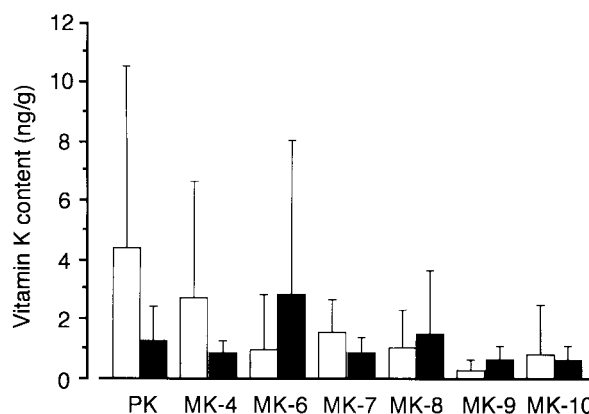


Fig. 1. Comparison of PK and MK homologue contents in the cancerous liver tissue between the serum PIVKA-II positive and negative patients with hepatocellular carcinoma. The results are expressed as average  $\pm$ SD. MK-5 is omitted. The apparent differences between the serum PIVKA-II positive and negative patients with hepatocellular carcinoma were not significant, Mann-Whitney *U* test.  $\square$  PIVKA-II negative (*n*=7),  $\blacksquare$  PIVKA-II positive (*n*=5).

MK-7, MK-8, and MK-10 were detected in all patients, and the average values ( $\pm$ SD) were 9.17 ( $\pm$ 9.87) ng/g, 7.23 ( $\pm$ 7.90) ng/g, 117.98 ( $\pm$ 248.95) ng/g, 6.14 ( $\pm$ 7.09) ng/g, and 23.08 ( $\pm$ 12.27) ng/g, respectively. MK-4 was at the detection limit or below in 4 patients, MK-9 was similar in 3, and MK-5 was similar in all patients.

**Comparison of PK and MK homologue contents between the cancerous and the noncancerous liver tissue of hepatocellular carcinoma patients** PK, MK-7, MK-8, and MK-10 contents in the cancerous tissue were significantly lower than those in the noncancerous tissue (Fig. 2).

**Comparison of PK and MK homologue contents in the cancerous liver tissue between hepatocellular carcinoma patients and metastatic hepatic cancer patients** MK-6, MK-7, MK-8, and MK-10 contents in the cancerous tissue of hepatocellular carcinoma patients were significantly lower than those in the metastatic hepatic cancer patients (Fig. 3).

No differences were seen in PK and MK homologue contents between the noncancerous tissue of hepatocellular carcinoma patients and the liver tissue of metastatic hepatic cancer patients.

**Plasma PK and MK homologue concentrations of hepatocellular carcinoma patients** Only PK was detected in all patients and the average ( $\pm$ SD) was 0.72 ( $\pm$ 0.62) ng/ml. MK-6 was detected in 8 patients, MK-7 in 10, and MK-8 in 7, and the average ( $\pm$ SD) levels of the detected patients were 0.15 ( $\pm$ 0.11) ng/ml, 0.98 ( $\pm$ 1.30) ng/ml, and 0.54 ( $\pm$ 0.83) ng/ml, respectively. MK-4 was at

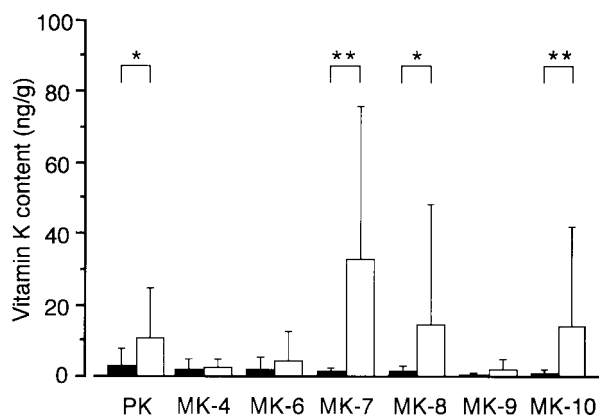


Fig. 2. Comparison of PK and MK homologue contents between the cancerous and the noncancerous liver tissue of hepatocellular carcinoma patients. The results are expressed as average $\pm$ SD. MK-5 is omitted. \* $P$ <0.05, \*\* $P$ <0.01, Wilcoxon signed-rank test. ■ cancerous liver tissue of hepatocellular carcinoma patients ( $n$ =12), □ noncancerous liver tissue of hepatocellular carcinoma patients ( $n$ =12).

the detection limit or below in 8 patients and MK-5 was the same in all patients.

No significant differences were seen in the plasma PK and MK homologue concentrations between serum PIVKA-II positive patients and negative patients (Fig. 4).

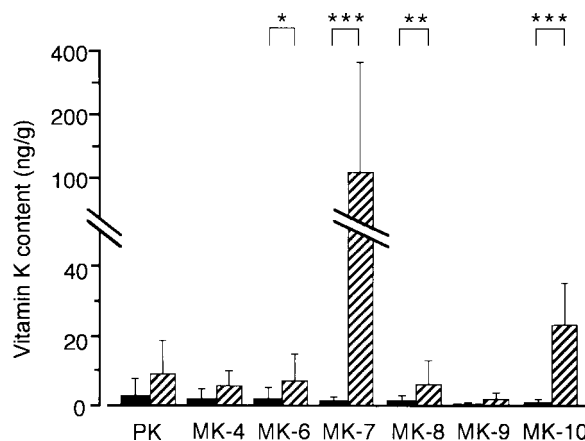


Fig. 3. Comparison of PK and MK homologue contents in the cancerous liver tissue between hepatocellular carcinoma and metastatic hepatic cancer patients. The results are expressed as average $\pm$ SD. MK-5 is omitted. \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001, Mann-Whitney  $U$  test. ■ cancerous liver tissue of hepatocellular carcinoma patients ( $n$ =12), ▨ cancerous liver tissue of metastatic hepatic cancer patients ( $n$ =9).

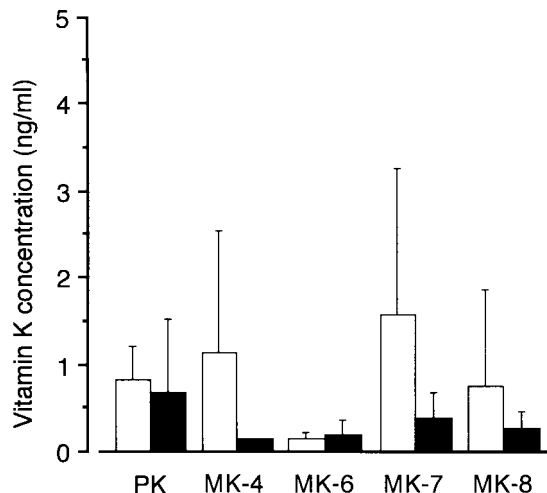


Fig. 4. Comparison of plasma PK and MK homologue concentrations between the serum PIVKA-II positive and the negative patients with hepatocellular carcinoma. The results are expressed as average $\pm$ SD. MK-5 is omitted. The apparent differences between the serum PIVKA-II positive and negative patients with hepatocellular carcinoma are not significant, Mann-Whitney  $U$  test. □ PIVKA-II negative ( $n$ =7), ■ PIVKA-II positive ( $n$ =5).

## DISCUSSION

The mechanism causing the elevation of serum PIVKA-II levels in hepatocellular carcinoma patients is proposed to be as follows; (1) vitamin K deficiency in local cancerous tissue due to an abnormal vitamin K uptake of hepatic cancer cells<sup>17-19</sup>; (2) excessive production of a prothrombin precursor (PIVKA) in hepatic cancer cells<sup>18-20</sup>; (3) decline of  $\gamma$ -glutamylcarboxylase activity in hepatic cancer cells<sup>20</sup>; (4) decline of the ability to use vitamin K due to oxidation-reduction cycle abnormality in hepatic cancer cells; or (5) prothrombin gene abnormality in hepatic cancer cells.<sup>20, 21</sup> But details of the mechanism are not known.

Huisse *et al.*,<sup>17</sup> Ono *et al.*,<sup>18</sup> and Yamagata *et al.*<sup>19</sup> reported vitamin K contents in hepatocellular carcinoma. Huisse *et al.* measured PK and MK contents (sum of MK-4–MK-10) in the cancerous and the noncancerous tissues of 10 patients with hepatocellular carcinoma and in the normal liver tissues of 10 patients with metastatic carcinoma of the liver, and reported that MK contents in the cancerous tissues of hepatocellular carcinoma patients are significantly more decreased than in the noncancerous tissues of hepatocellular carcinoma patients or the normal liver tissues of metastatic liver carcinoma patients. Furthermore, they demonstrated that MK contents are lower in the cancerous tissues from the patients with elevated serum levels of PIVKA-II than in the cancerous tissues from those with normal serum levels of PIVKA-II. They concluded that deficiency of vitamin K content in the cancerous tissues plays a critical role in the elevation of serum PIVKA-II associated with human hepatocellular carcinoma. However, they did not compare PK and MK contents in the cancerous tissues of hepatocellular carcinoma with those of metastatic liver carcinoma. For this reason, their results do not support the idea that MK contents in the cancerous tissues of hepatocellular carcinoma patients are particularly lower than those of metastatic liver carcinoma patients. Ono *et al.* measured only PK, MK-4, and MK-7 contents in the cancerous and noncancerous tissues of their hepatocellular carcinoma patients, and Yamagata *et al.* measured only PK and MK-4 contents in these types of tissues of their hepatocellular carcinoma patients. In these studies, there were no significant differences in vitamin K contents between the cancerous and the noncancerous tissues. They concluded that the main mechanism of serum PIVKA-II elevation in patients with hepatocellular carcinoma is excessive synthesis of PIVKA-II. Thus, there is conflict concerning the mechanism of serum PIVKA-II elevation in hepatocellular carcinoma patients.

Therefore, we measured the contents of PK and each menaquinone from MK-4 to MK-10 in the cancerous tissue and the noncancerous tissue of hepatocellular carcinoma patients, and furthermore in the cancerous tissue of metastatic hepatic cancer patients. The contents of PK and

MKs in the cancerous tissue of metastatic hepatic cancer patients had not been measured in previous studies. As shown above, we found that PK, MK-7, MK-8, and MK-10 contents in the cancerous tissue of hepatocellular carcinoma patients were significantly lower than that in the noncancerous tissue, and that the contents of MK-6, MK-7, MK-8, and MK-10, which have long isoprenyl side chains, in the cancerous tissue of hepatocellular carcinoma patients were significantly lower than those of metastatic hepatic cancer patients. Also, no differences were seen in plasma PK and MK homologue concentrations between the hepatocellular carcinoma patients and healthy adults, whose plasma PK and MK homologue concentrations were measured by the same method.<sup>15, 16</sup> This indicates that PK and MK homologue contents are especially lower in the cancerous tissue of hepatocellular carcinoma patients.

Although some reports<sup>22, 23</sup> suggest that MKs with long isoprenyl side chains, which showed lower levels in the cancerous liver tissue of our hepatocellular carcinoma patients, do not participate in  $\gamma$ -carboxylation, other reports<sup>24-27</sup> indicate that they do participate. Therefore, we believe that the low levels of MKs with long isoprenyl side chains in the cancerous tissue of hepatocellular carcinoma patients play a role in the serum PIVKA-II increase in these patients. The mechanism of vitamin K content decreases in the cancerous tissue of hepatocellular carcinoma patients is presumed to involve decreased vitamin K uptake into hepatic cancer cells, vitamin K nuclear receptor abnormality, vitamin K hypermetabolism, and a decrease in vitamin K supply to cancerous tissue due to blood distribution abnormality. Further studies are needed on this question.

We measured PK and MK contents of only 2 normal liver tissues and 10 cirrhotic tissues in the noncancerous portion from 12 hepatocellular carcinoma patients. Usui *et al.*<sup>28</sup> reported vitamin K contents in the noncancerous portion of 38 human liver specimens from patients with primary or metastatic liver cancer. They included 6 normal liver tissues and 22 cirrhotic liver tissues. In their study, PK, MK-7, MK-8 and MK-9 contents were not significantly different between normal liver tissues and cirrhotic liver tissues, but MK-10, MK-11 and MK-12 contents of cirrhotic liver tissues were significantly lower than those of normal liver tissues. In our study, PK, MK-7, MK-8 and MK-10 contents of the cancerous tissues from hepatocellular carcinoma patients were significantly lower than those of the noncancerous tissues, which were histologically close to liver cirrhosis. This indicates that the PK, MK-7, MK-8 and MK-10 contents of the cancerous tissues of hepatocellular carcinoma patients are significantly lower than those of cirrhotic liver tissues and normal liver tissues.

We found no differences in the vitamin K contents of the cancerous tissue between the serum PIVKA-II positive

and negative patients with hepatocellular carcinoma. One reason for this may be the size of the hepatocellular carcinomas in patients enrolled in this study, i.e., the larger the hepatocellular carcinoma, the higher the serum PIVKA-II.<sup>11)</sup> Our largest cancer was 7.0×8.0 cm. Serum PIVKA-II concentration reflects the total PIVKA-II produced by the whole cancer rather than the PIVKA-II production per unit of liver tissue weight, so it is natural that the vitamin K content per unit of liver tissue weight does not always correlate to the serum PIVKA-II. In our study, the serum PIVKA-II level was 12.6 AU/ml at the highest and there were no cases with a marked increase of serum PIVKA-II. These findings suggest that the cancer sizes in our hepatocellular carcinoma patients were relatively small and the

total PIVKA-II production was low. Another reason may be the small number of study patients.

In conclusion, a vitamin K deficiency in the cancerous tissue might account for at least a portion of the elevation of serum PIVKA-II levels in hepatocellular carcinoma patients.

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