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A Case of Hepatocellular Carcinoma Producing Prothrombins Induced by Vitamin K Absence or Antagonist (PIVKA-II)

—Immunohistological Detection of PIVKA-II—

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SUMMARY

A 78-year old man presented with PIVKA-II (prothrombins induced by vitamin K absence or antagonist) producing hepatocellular carcinoma. The level of plasma PIVKA-II showed positive value in the diagnosis of hepatocellular carcinoma. The tumor was detected in the liver as a high-echoic mass with portal venous tumor thrombus by ultrasonographic examination. A percutaneous liver biopsy was performed under an ultrasonographic guidance. Histological examination revealed hepatocellular carcinoma and immunoperoxidase staining for PIVKA-II was positive in the cytoplasma of the malignant cells. Increased plasma PIVKA-II levels were probably produced by hepatocellular carcinoma cells.

Key words : PIVKA-II, Immunohistochemistry, Hepatocellular carcinoma

INTRODUCTION

PIVKA-II (prothrombins induced by vitamin K absence or antagonist) was recently found as a useful marker of hepatocellular carcinoma (HCC) in laboratory diagnosis(3). Monitoring of PIVKA-II concentration in the plasma is useful for the diagnosis and treatments of patients with HCC. However, there is very little information on the PIVKA-II producing-cells. In this report, we describe the relation between the level of plasma PIVKA-II and immunolocalization in the tumor with an antibody to PIVKA-II in a case of HCC.

Abbreviation: PIVKA-II, prothrombins induced by vitamin K absence or antagonist

CASE REPORT

A 78-year-old man was admitted to our hospital with HCC on November 1, 1989. He had a 14-year history of mitral valve regurgitation (MR), aortic valve stenosis and regurgitation (ASR), and a 12-year history of liver cirrhosis. He has been imbibing 900 ml daily of japanese sake for 55 years. There was no significant family history. The patient was medicated for MR, ASR and liver cirrhosis at our hospital. The patient was first noted by a small liver mass under ultrasonographic examination (US) at the right anterior superior segment of the liver on May 8, 1989. However, a computed tomography (CT) scan failed to show any liver mass. A liver biopsy was performed under a US guidance and histological examination revealed a nodule of liver cirrhosis. The level of plasma PIVKA-II, but not of AFP, was slightly elevated. During the course of examination of a US, the liver tumor was gradually enlarged and the level of plasma PIVKA-II was rapidly increased. He was not treated with vitamin K antagonists or gamma-glutamyl-carboxylase inhibitors.

Table 1 Laboratory data on admission

Blood			
WBC	5,300	IgG	923 mg/d*l*
RBC	402X10⁴	IgM	51.3 mg/d*l*
Hb	12.8 g/d*l*	IgA	81.4 mg/d*l*
Ht	37.5 %	ZTT	4.5 U
Plt	10.6X10⁴	TTT	1.4 U
Hepaplastin test	68 %	BUN	21.3 mg/d*l*
Na	139 mEq/L	Ccr	43.4 m*l*/min
K	4.0 mEq/L	ChE	1093 IU/L
Cl	106 mEq/L	HBsAg	(−)
Ca	8.8 mg/d*l*	HBsAb	(−)
GOT	133 IU/L	HBcAb	(−)
GPT	150 IU/L	AFP	148 ng/m*l*
LDH	358 IU/L	PIVKA-II	28 AU/m*l*
Al-p	180 IU/L	CEA	2.8 ng/m*l*
GGT	212 IU/L	Urinalysis	
T-chol	178 mg/d*l*	glucose	(−)
T. P.	5.9 g/d*l*	protein	(++)
alb	3.75 g/d*l*	bilirubin	(−)
α_1-glb	0.28 g/d*l*	urobilinogen	(−)
α_2-glb	0.77 g/d*l*		
β-glb	0.41 g/d*l*		
γ-glb	0.69 g/d*l*		

His height was 168 cm, his weight was 64 kg. The temperature was 36.3℃, the pulse was 90/min (irregular). The blood pressure was 180/90 mmHg. A Grade 2 diastolic and holosystolic heart murmurs were audible. The liver was felt 5 cm at the right hypochondral region, the spleen was also palpitated. Laboratory examination revealed that the concentration of AST and ALT were abnormal (Table 1). The concentration of plasma PIVKA-II was 28 AU/ml and that of serum AFP was 140 ng/ml. Creatinin clearance showed a low value. A chest X-ray showed that the cardiac silhouette was enlarged.

A CT scan of the abdomen showed an abnormal low-density mass in the right lobe of the liver, swelling of the liver and the spleen (Fig. 1).

A US showed a high-echoic mass in the right anterior segment and an abnormal iso-echoic mass in the right portal vein (Fig. 2).

A magnetic resonance image (MRI) showed a low-intensity mass with a T1 sequence and a high-intensity mass with a T2 sequence in the right lobe of the liver (Fig. 3).

Hepatic angiography was not performed because of renal impairment. These image modalities demonstrated that the patient was suffered from HCC and portal venous tumor thrombus. A percutaneous biopsy of the liver tumor

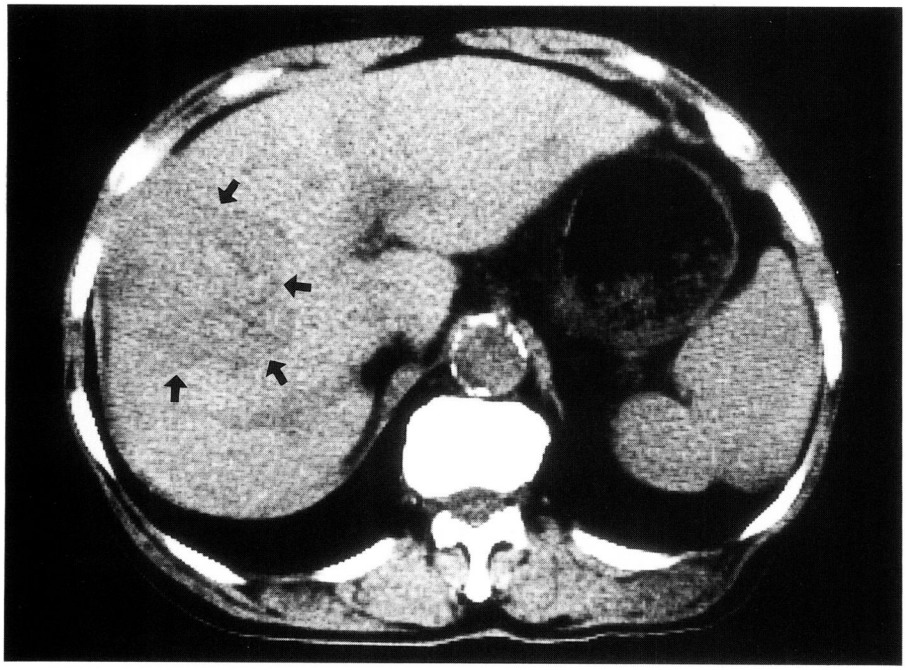

Fig. 1 A computed tomography scan without contrast medium showing a low density tumor in the right lobe in the liver (arrows).

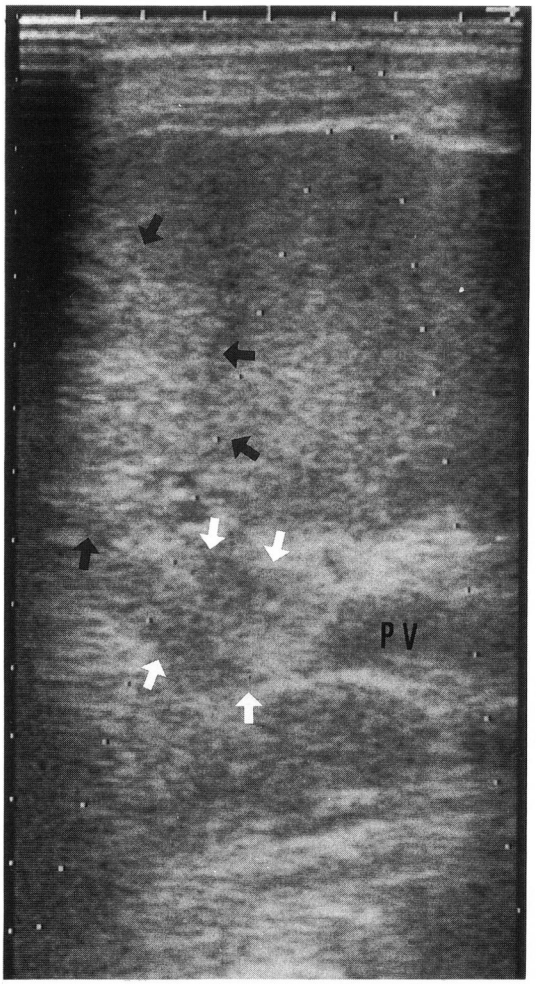

Fig. 2 An ultrasonography showing a high-echoic tumor in the right anterior segment in the liver (black arrows) and an iso-echoic tumor in the right portal vein (white arrows).

was performed under US guidance.

Serial levels of PIVKA-II and AFP is shown in Fig. 4. The level of PIVKA-II was positive on initial screening and rapidly increased after an elapse of 6 months, but the level of AFP showed a negative value for diagnosis of HCC.

IMMUNOHISTOCHEMISTRY

Immunohistochemical staining for PIVKA-II was performed by the ABC method, briefly described in the following procedure. The liver specimen was fixed with

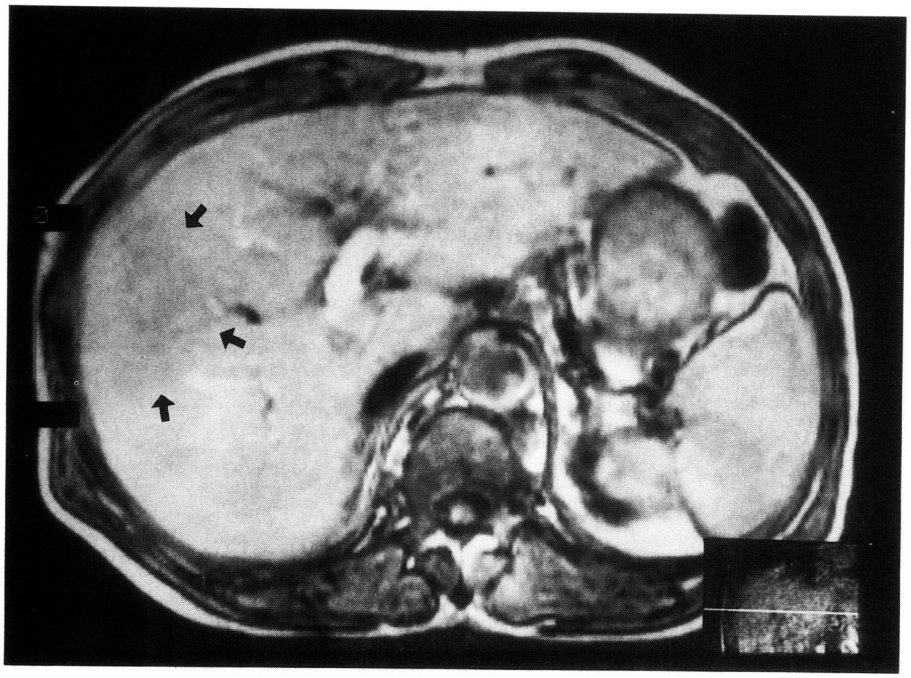

Fig. 3 A magnetic resonance Tl weight image showing a low intensity tumor in the right lobe in the liver (arrows).

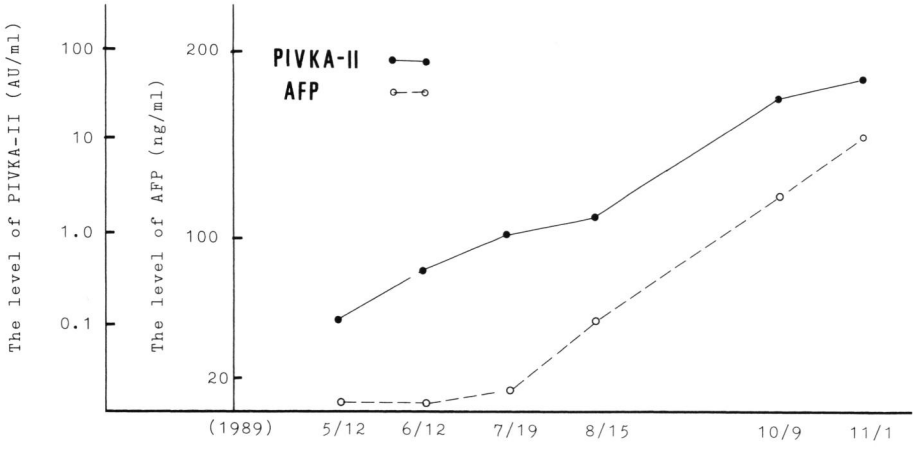

Fig. 4 Serial levels of the plasma PIVKA-II and the serum AFP.

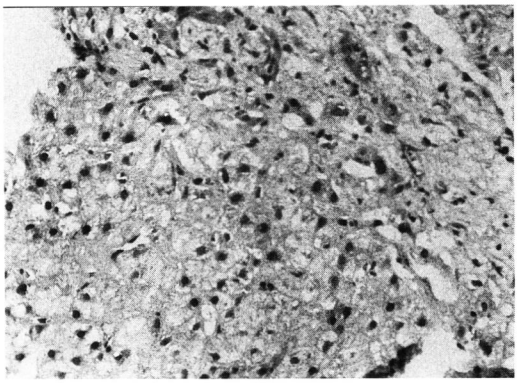

Fig. 5a The lesion consists of nodular proliferation of normal hepatocytes (hematoxylin-eosin staining, original magnitude ×20).

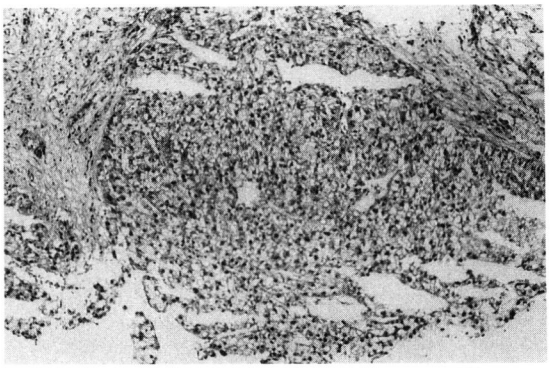

Fig. 5b The lesion consists of thick trabecular arrangement of the malignant cells (hematoxylin-eosin staining, original magnitude ×10).

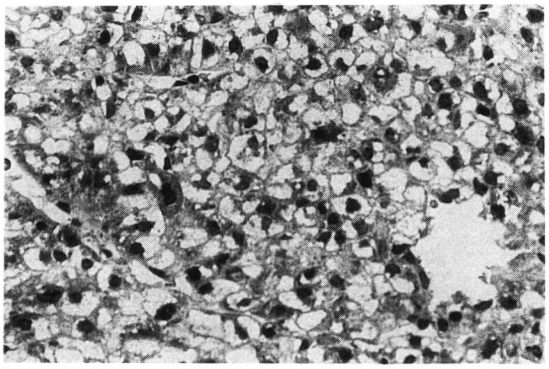

Fig. 5c The lesion consists of clear cell type of the malignant cells (hematoxylin-eosin staining, original magnitude ×20).

10% formalin and embedded in OCT compound at −20℃. The sections were treated with methanol containing 0.3% H_2O_2 for the purpose of blocking endogenous peroxidase activity and incubated in anti-human PIVKA-II monoclonal antibody(4) at 4°C overnight. This was incubated in biotin labeled anti-mouse IgG antibody at 37°C for 30 min, then treated with avidin-biotin peroxidase complex (Vector Lab. Burlingame, Calif.) at 37°C for 30 min. The sections were washed by phosphate buffered saline at each step. Then, the liver sections were treated with 3-3'-diaminobenzidine and H_2O_2 in Tris-HCℓ buffer. A control study was performed with a normal mouse serum instead of murine monoclonal antibody against PIVKA-II.

RESULTS

Histology and Immunohistochemistry

Histology revealed a cirrhotic nodule (Fig. 5a). In addition, tumor cells grew in thick cords and were separated by prominent sinusoid. Fibrous connective tissue was surrounding the tumor nodule (Fig. 5b). The tumor was predominantly composed of cells with clear cytoplasma. Their nuclei were large and hyperchromatic (Fig. 5c). Edmondson and Steiner's classification was Grade II.

Immunostaining with antibody to PIVKA-II was positive in numerous cells of HCC (Fig. 6a). PIVKA-II was localized in the cytoplasma of the tumor cells (Fig. 6b). The cells of cirrhotic nodule showed negative staining (Fig. 7).

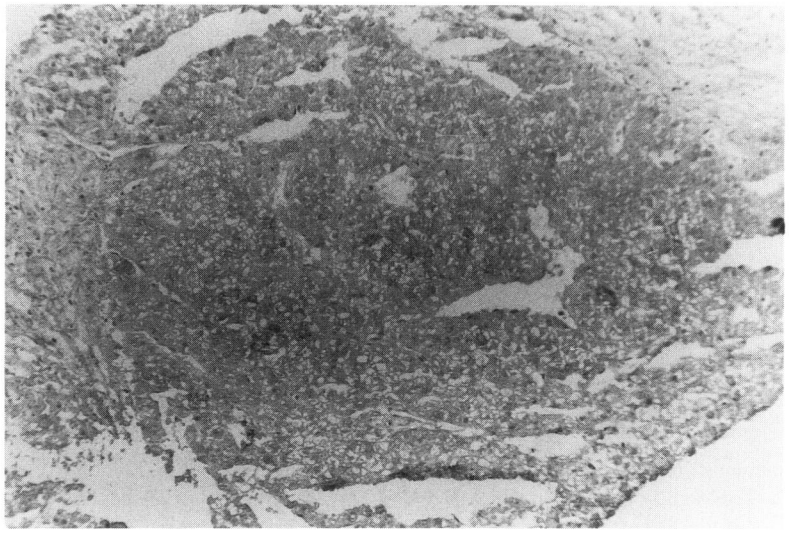

Fig. 6a Immunostaining of PIVKA-II using the ABC method. The site of HCC nodule showed positive staining for PIVKA-II (counter stained with hematoxylin, original magnitude ×10).

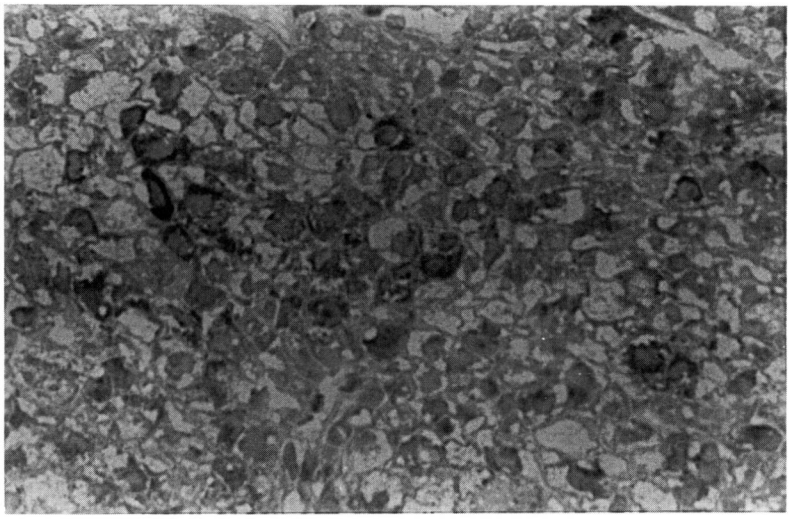

Fig. 6b The cytoplasma of the malignant cells showed positive staining (counter stained with hematoxylin, original magnitude ×20).

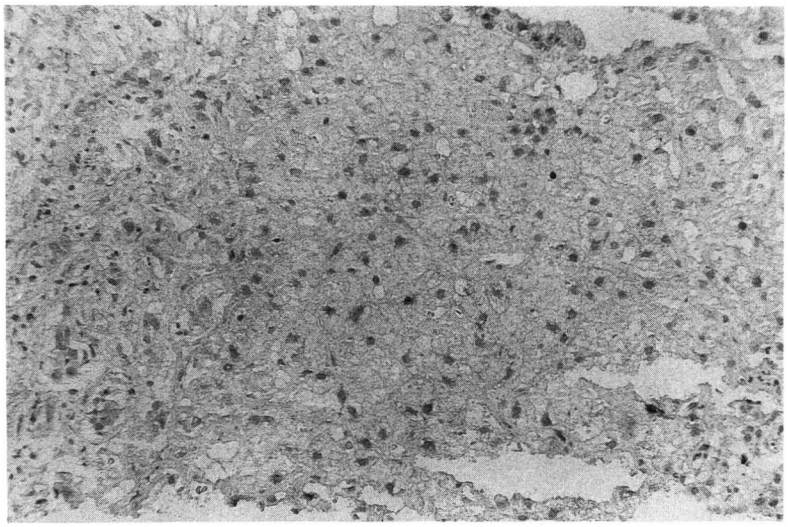

Fig. 7 Immunostaining of PIVKA-II using the ABC method. The site of cirrhotic nodule showed negative staining for PIVKA-II (counter stained with, hematoxylin, original magnitude ×20).

DISCUSSION

PIVKA-II was utilized as a tumor marker of HCC. We reported that PIVKA-II was positive in 52% of 82 patients with HCC(2). The producing cell of PIVKA-II was still not quite clearly demonstrated. We previously reported that the levels of PIVKA-II were decreased after effective treatments and increased in parallel with tumor growth(1). Okuda et al. reported that the levels of PIVKA-II were increased in the culture medium of HCCs, but not in the others(6). It was reported that the concentration of PIVKA-II was increased in HCC tissue compared with non-cancerous liver tissues(7). These reports suggested that PIVKA-II might be produced by HCC cells.

The examination of the relationship between the level of plasma PIVKA-II and the presence of immunoreactive PIVKA-II in the tumor was required. Therefore, we attempted to examine the immunostaining of PIVKA-II in the patients with HCC. However, we could not find any evidence of immunoreactive PIVKA-II in HCC tissues which were fixed with 10% formalin and embedded in paraffin. There were two possibilities that we could have succeeded in detection of the immunolocalization of PIVKA-II in this case. First, the sections were embedded in OCT compound at −20°C instead of paraffin in consideration that PIVKA-II might be degraded at high temperature(5). Second, the tissue localization of PIVKA-II could be detected in this patient who showed very high concentrations of the plasma PIVKA-II.

The cytoplasma localization of PIVKA-II were observed in HCC cells. This result suggested that these sites were capable of producing PIVKA-II. In contrast, the nonmalignant cells in cirrhotic nodule showed negative staining.

This case demonstrated that PIVKA-II in the plasma might be derived mainly from HCC cells with a relative vitamin K deficiency(6) or an acquired vitamin K-dependent gamma-carboxylation deficiency(8), but not from the non-malignant cells.

In conclusion, there would be a close correlation between the plasma level and the tissue localization of PIVKA-II in HCC. However, further studies are needed to clarify the immunohistochemistry for PIVKA-II in HCCs and/or other benign liver-diseases.

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REFERENCES

1. ARIMA, K., KODAMA, T., SUGA, M., SAKAMOTO, H., OHE, Y. and YACHI, A.: **Jpn. J. Cancer Chemother. 16**, 3049-3052 (1989).
2. KODAMA, T., YOKOI, T., ARIMA, K., SUGA. M., IMAI, K., YACHI, A., TAKAHASHI, A. and SHIMAMURA, Y.: **Tumor Res. 22**, 63-70 (1987).
3. LIEBMAN, H. A., FURIE, B. C., TONG, M. L., BLANCHARD, R. A., LO, K., LEE, S., COLEMAN, M. S. and FURIE, B.: **N. Engl. J. Med. 310**, 1427-1431 (1984).
4. MOTOHARA, K., KUROKI, Y., KAN, H., ENDO, F. and MATSUDA, I.: **Pediatr. Res. 19**, 354-357 (1985).
5. NARAKI, T., WATANABE, K., SHIMOZURU, B., NAMBA, Y., MORIMOTO, T., MOTOHARA, K. and MATSUDA, I.: **Clin. Immunol. 18**, 479-492 (1986).
6. OKUDA, H., NAKANISHI, T., FURUKAWA, R., FURUKAWA, M. and OBATA, H.: **Kanzo 29**, 47-51 (1988).
7. ONO, M., OHOTA, I., SEKIYA, T. and NAMIKI, M.: **Kanzo 30**, 1042 (1989).
8. YOSHIKAWA, Y., SAKATA, Y., TODA, G. and OKA, H.: **Hepatology 8**, 524-530 (1988).