Yamanashi Med. J. 6(4), 193~206, 1991

Histopathological Analysis of Intrahepatic Multiple Hepatocellular Carcinomas—Possibility of Differential Diagnosis of Their Origins by Clonal Study

Masanori MATSUDA, Masayuki YAMAMOTO, Kaoru NAGAHORI, Kazuo MIURA, and Yoshiro MATSUMOTO

First Department of Surgery, Yamanashi Medical College

Abstract: We selected 7 (23.3%) synchronously occurring multicentric hepatocellular carcinomas (HCC) from the resected liver specimens of 30 cases of HCC with multiple lesions. We used the following pathological criteria for selection. 1) Remote and smaller nodules showing microscopically well-differentiated HCC, besides the major nodule showing poorer differentiation; 2) multiple well-differentiated HCC; and 3) multiple HCC indicating "nodule-in-nodule" form. In 7 patients with synchronous multicentric HCC lesions (MC), less microscopic infiltration of tumor capsule (MC=42.9%, IM=73.9%) and invasion of portal vein (MC=28.6%, IM=60.9%) were observed than in the main lesions in 25 cases of intrahepatic metastatic group (IM). In MC group, non-cancerous liver tissue indicated chronic hepatitis in 4 cases and liver cirrhosis in 3 cases, while the non-cancerous liver tissue in IM group indicated chronic hepatitis in 3 cases, liver cirrhosis in 14 cases, and liver fibrosis in 6 cases. In 3 HBV carriers whose clinical history or histological analysis had suggested multicentric occurrence, clonal analysis, using Southern blot hybridization technique, was performed to ascertain multicentricity. In 2 patients, analysis of the intergration patterns of HBV DNA to multiple HCC was helpful in determining whether tumor origin was multicentric or metastatic, but in one patient whose lesions were very well-differentiated HCC and contained interior portal structures, clonal analysis did not help in this determination, as no discrete bands appeared.

From a clinical viewpoint, we believe that the determination of clonarity of intrahepatic multiple HCC become more important for treatment of HCC patients.

Key words: Hepatocellular carcinoma, Multicentric occurrence, Intrahepatic metastasis, Southern blot hybridization, Hepatitis B virus DNA integration

INTRODUCTION

Whether hepatocellular carcinoma (HCC) occurs unicentrically or multicentrically has long been the subject of discussion[1),2)]. The outcome of such discussions was not very important for clinical treatments in earlier times, since, by the time the tumors were diagnosed, they were too large and too advanced for any determination of their clonarity to lead to alternative treatments or improved prognosis. However, recent developments of imaging techniques have enabled us to detect multiple small intrahepatic tumors and we have been able to then choose a suitable treatment to increase patient survival. In view of its clinical importance, we have paid attention to the modes of occurrence and progression of HCC, to its multicentric development and intrahepatic metastases.

In this study we analyzed HCC multicentricity in 31 patients with intrahepatic multiple

Tamaho, Nakakoma, Yamanashi 409-38, Japan
Received June 5, 1991
Accepted September, 4, 1991

lesions, and we compared the histopathological features of the multicentric HCC with those of intrahepatic metastatic HCC. Further, we analyzed multicentricity by Southern blot hybridization method[3] in 3 hepatitis B virus (HBV) carriers. In one of these patients, histological criteria did not confirm multicentric occurrence but clinical history did not deny this possibility. The significance and the present limits of this method for detection of multicentricity will be discussed.

Materials and Methods

Cases

The subjects were 30 cases of HCC (Nos. 1–30) with intrahepatic multiple lesions which had been removed surgically. These operations were performed between January, 1985 and December, 1990 (Table 1). Case 31 showed a single tumor at liver resection, but 4 years after the resection, rapidly growing tumors appeared in the remnant liver. The patient died one year after the tumors appeared and 5 years after the primary liver resection for HCC. Autopsie specimens were then obtained from this patient.

Histopathological study

The main- and sublesions of liver specimens were examined histologically. The pathological status of the non-cancerous portion of the liver was also examined. Pathological classification of HCC was performed according to the criteria of the Liver Cancer Study Group of Japan[4]. Histological grading of HCC was performed according to the criteria of Edmondson and Steiner[5]. With no reference to the clinical informations of each case, pathological studies were performed.

Multicentricity was then determined according to the following microscopic findings:

1) Remote and smaller nodules showing microscopically well-differentiated HCC, besides the major nodule showing poorer differentiation; 2) multiple well-differentiated HCC; and 3) multiple HCC indicating "nodule-in-nodule" form[6].

Thirty cases (Nos. 1–30), were then divided into two groups; A) MC group which had multicentric HCC lesions and B) IM group which had only intrahepatic metastatic tumors. The histopathological features of the group A) and B) were compared.

Clonal analysis

In 3 cases of HBV carriers (Nos. 1, 2 and 31), whose detailed clinical histories or histological analyses had suggested that their lesions were of multicentric occurrence, clonal analysis, using Southern blot hybridization technique[3], was carried out to ascertain multicentricity. When the integration patterns of HBV DNA in host nuclear DNA in each intrahepatic lesion are different, the lesions are regarded as being of multicentric occurrence; when these patterns are the same the lesions are regarded as metastatic tumors[7)–15)]. Results obtained by this method in these 3 cases were analyzed and compared with the results of histopathological analyses.

Specimens about 2.0 g of non-cancerous portions of the liver, as well as specimens of each intrahepatic lesion were obtained during liver resection or by autopsy, and were then stored at −80°C until used for DNA extraction. The samples (300–1,000 mg), in 10 volumes of TNE solution (10 mM Tris-Hcl pH 7.8, 10 mM NaCl, 2 mM EDTA-3Na), were treated with proteinase K (100 mg/m*l*, Merck, Darmstadt, Germany) and 2% sodium dodecyl sulfate (SDS) overnight at 37°C. The DNA was extracted first with phenol and then with chloroform/isoamyl alcohol (24/1), following which, it was precipitated with cold ethanol and then treated with RNase (Boehringer, Germany). Ten μg of purified DNA was digested with *Bam*HI, *Hind*III or *Eco*RI (Takara, Japan) overnight at 37°C, using 50 units of each enzyme. The sample was applied to 0.8% agarose gel, electrophoresed, and then transferred to a nylon membrane (Hybond-N+, Amersham, UK). A full length HBV-DNA (type adr) probe (Clonit, Milano, Italy)

was labeled with [^{32}P] deoxycytidine 5-triphosphate using a Multiprimer system (Amersham, UK). Hybridization was performed for 4 hours at 65°C in rapid hybridization buffer (Amersham, UK) containing 2×10^6 cpm/m*l* of denatured probe. The filter was then washed and autoradiographed.

Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) were measured by radioimmunoassay and hepatitis C antibody (HCVAb) was measured by enzymoimmunoassay using an anti-C100–3.

Profiles of 3 HBV Carrier cases of putative multicentric HCC (Nos. 1, 2 and 31)

Case 1. In December 1985, a 52-year-old male was admitted to our hospital complaining of abdominal pain. Two hypoechoic nodules were detected in the lateral and medial segments of the liver by ultrasonography (US). These two tumors were suspected to be HCC following angiography. At admission, serum laboratory data were: total bilirubin (T-Bil) 2.9 mg/*l*, glutamic oxalacetic transaminase (GOT) 109 IU/*l*, glutamic pyruvic transaminase (GPT) 65 IU/*l*, lactic dehydrogenase (LDH) 1828 IU/*l*, alpha-fetoprotein (AFP) 12.3 ng/m*l*, and positive HBsAg and HBeAg. The patient did not have a history of drinking or of blood transfusion. An extended left lobectomy was performed on February 20, 1986. He survived for 4 years after liver resection, without showing any distant organ metastasis.

Case 2. A 49-year-old male. Two hyperechoic lesions with diameters of 2.7 cm and 2.4 cm, respectively, were detected in the posterior segment of the liver by US in May 1990. Angiography showed no tumor stains. Fine needle aspiration biopsy revealed that the tumors were so-called early HCC (eHCC)[16),17)] keeping portal structures inside. Serum laboratory data were: T-BIl 0.8 mg/*l*, GOT 57 IU/*l*, GPT 55 IU/*l*, LDH 54 IU/*l*, gammaglutamyl transpeptidase (γ-GPT) 71 U/*l*, AFP 39 ng/m*l*, positive HBsAg and HBeAb, and negative HBeAg. There was a history of gastrectomy for gastric ulcer at the age of 22. On June 19, 1990 right lobectomy was performed.

Case 31. A 43-year-old male. He had been treated for liver dysfunction, and a space-occupying lesion was detected in the liver by computed tomography (CT) in January 1985. Angiography revealed the tumor suggestive of HCC in the anterior segment of the liver. No other tumors were detected by CT or angiography. Serum laboratory data were: T-BIl 0.4 ng/*l*, GOT 54 IU/*l*, GPT 81 IU/*l*, LDH 579 IU/*l*, γ-GTP 81 U/*l*, AFP 25 ng/m*l*, and positive HBsAg and HBeAg. There was no history of blood transfusion. On February 5, 1985, anterior-inferior subsegmentectomy of the liver was performed. The resected specimen contained a single encapsulated nodular tumor. No intrahepatic metastases were found. The followup study of the patient did not reveal any recurrence in the remnant liver until about 4 years later. In September 1989, after hemodialysis was initiated, rapidly growing tumors were detected in the posterior segment of the liver. Transarterial infusion chemotherapy was performed. In March 1990, multiple lung metastases were detected. The patient died of liver failure on December 2, 1990, and autopsy was performed.

Results

Histopathological study of the cases with intrahepatic multiple HCC lesions (Table 1)

Of 30 cases examined, 7 (Nos. 1–7, 23.3%) were classified as being in the synchronous multicentric HCC (MC group). Twenty three cases (Nos. 8–30) were classified as having intrahepatic metastases (IM group). Patient 31 was suspected to have metachronous multicentric occurrence tumor, but from the result of the histopathological study, we were not able to determine its multicentricity.

In MC group (Nos. 1–7), microscopic infiltration of tumor capsule (MC=42.9%, IM=73.9%) and invasion of portal vein (MC=28.6%, IM=60.9%) were observed less than in the main lesions of IM group. Capsular

Table 1. Review of 31 cases with intrahepatic multiple lesions

	Case No.	age sex	main lesion						sublesion					non-cancerous liver	hepatitis	
			location*	max. diameter (cm)	differentiation**	fc	fc-inf	vp	location*	no. of lesions	max. diameter (cm)	histology	differentiation**		HBsAg	HCV Ab***
A)	1	52M	S_3	6.0	III	+	−	vp_0	S_4	1	3.5	HCC	II	LC	+	?
	2	49M	S_7	2.7	I	−		vp_0	S_7	1	2.4	HCC	I	CH	+	−
	3	64M	S_8	2.0	II	+	−	vp_0	S_7	1	1.2	HCC	I	CH	−	−
	4	63F	S_6	2.9	I~II	+	−	vp_0	S_8	1	1.6	HCC	I	LC	−	+
									S_7	1	0.2	HCC	I			
	5	70M	S_5	3.5	II	+	+	vp_0	S_4	1	2.5	HCC	I~II	LC	+	?
	6	63M	S_{5678}	9.4	III~IV	+	+	vp_2	S_{5678}^{1234}	>4	3.0	HCC	III~IV	CH	−	−
									S_5	1	0.5	HCC	I			
	7	71M	S_8	4.5	III	+	+	vp_1	S^2	1	2.5	HCC	II	CH	−	+
									S_5	1	3.8	HCC	II~III			
B)	8	48F	S_{67}	7.0	II	+	+	vp_2	S_{67}	>4	0.8	HCC	II	LC	+	?
	9	67M	S_6	12.0	I~II	+	+	vp_1	S_{678}	>4	3.0	HCC	II	LC	−	?
	10	52M	S_5	3.0	II	+	+	vp_0	S_6	1	1.0	HCC	II	fibrosis	+	?
	11	57M	S_6	6.5	II	+	+	vp_0	S_7	1	0.8	HCC	II	fibrosis	−	?
	12	56M	S_{78}	13.0	II	+	+	vp_1	S_{5678}	>4	1.0	HCC	II	LC	+	?
	13	58M	S_{67}	12.0	II~III	+	+	vp_1	S_{567}	3	1.0	HCC	III	LC	+	?
	14	71F	S_5	10.0	II~III	−		vp_1	S_{5678}	>4	1.8	HCC	III	fibrosis	−	?
	15	54M	S_{67}	12.0	II	−		vp_2	S_{4567}	>4	0.5	HCC	II	LC	+	?
	16	53M	S_3	4.6	II	+	+	vp_1	S_{63}	2	3.0	HCC	II	LC	−	?
	17	63M	S_7	2.0	II	+	+	vp_0	S_7	1	1.5	HCC	II	fibrosis	−	?
	18	72M	S_{45678}	22.0	I~II	+	+	vp_1	S_{45678}	>4	3.0	HCC	II	fibrosis	−	−
	19	60M	S_8	3.3	II	+	−	vp_0	S_4	1	3.0	HCC	II	fibrosis	−	+
	20	64M	S_4	1.0	III	+	−	vp_1	S_{86}	>4	2.2	HCC	III	LC	−	+
	21	42M	S_{67}	3.8	III	+	+	vp_1	S_{5678}^{1234}	>4	2.0	HCC	III	LC	+	?
	22	33M	S_{5678}	6.0	III	+	+	vp_0	S_4	>4	0.1	HCC	III	LC	+	+
	23	62M	S_3	1.4	II	−		vp_0	S_3	2	0.2	HCC	II	CH	−	+
	24	50M	S_5	6.5	II	+	+	vp_0	S_{58}	>4	1.5	HCC	II	LC	−	+
	25	66F	S_3	2.5	III	+	+	vp_1	S_{23}	>4	0.8	HCC	III	LC	−	+
	26	58M	S_4	10.0	II	+	+	vp_2	S_4	>4	1.0	HCC	II	CH	+	+
	27	65M	S_6	2.2	II	+	+	vp_0	S_5	3	2.0	HCC	II	LC	−	+
	28	76F	S_{67}	7.3	III	−		vp_2	S_{67}	>4	2.0	HCC	III	CH	−	−
	29	65M	S_4	7.0	II	+	+	vp_2	S_{4567}	>4	1.5	HCC	II	LC	−	−
	30	65M	S_3	2.5	III	+	+	vp_0	S_3	1	0.4	HCC	III	LC	+	+
	31	43M	S_5	2.5	II	+	−	vp_0	S_{234678}****	>4	12.0	HCC	III	LC	+	?

A)multicentric HCC group, B) intrahepatic metastatic group,
*Couinaud's classification[18], **Edmondson's grade, ***anti-C100-3, ****autopsy, fc: capsule formation,
fc-inf: capsule infiltration, vp: portal vein invasion, (fc, fc-inf, vp are histological findings) LC: liver cirrhosis,
CH: chronic hepatitis,

infiltration or portal vein invasion was observed in 21 of 23 cases in IM group (91.3%). In MC group, the non-cancerous liver tissues showed the pattern of chronic hepatitis in 4 cases and liver cirrhosis in 3 cases, while in IM group, chronic hepatitis was indicated in 3 cases, liver cirrhosis in 14 cases, and liver fibrosis in 6 cases. No apparent positive relationship was observed between hepatitis B and/or C virus infection and multicentric occurrence.

Pathological study of 3 cases of putative multicentric HCC

Case 1. The cut surface of the lateral segment of the liver contained a well encapsulated yellowish nodular tumor, measuring 6.0×6.0×6.0 cm, and showing extrahepatic growth (Fig. 1a). The tumor in the medial segment measuring 3.5×3.5×3.5 cm and had no capsule formation (Fib. 1b). Histological findings for the tumor in the lateral segment showed moderately differentiated HCC of trabecular type, with nuclear atypism (Edmondson Grade III). There was no capsule infiltration or portal vein invasion (Fig. 1c). The tumor in the medial segment was well to moderately differentiated HCC (Edmondson Grade II) (Fig. 1d). The non-cancerous liver tissue was cirrhotic. There was a histological difference between the two tumors and they could be regarded as of multicentric occurrence according to criterion 1), described in MATERIALS AND METHODS.

Case 2. Two yellowish lesions were found. They had not destroyed the preexisting architecture of the liver lobule (eHCC) (Fig. 2a). Histological findings for the larger lesions (2.7×2.0×1.8 cm, T1) showed well-differentiated HCC with thin trabecular arrangements. Portal tract structures were observed inside the lesion. The cancer cells showed a replacing growth (Fig. 2b). The histological findings for the smaller lesion (2.4×2.0×2.0 cm, T2) were almost the same as those for the larger one. The non-cancerous liver tissue was the pattern of chronic hepatitis. The occurrence of HCC in this case was suggestive of synchronous multicentric origin, according to criterion 2), described in MATERIALS AND METHODS.

Case 31. The resected specimen at operation contained a single nodular encapsulated tumor measuring 2.5×2.5×2.0 cm (Fig. 3a). Histological findings for the tumor showed moderately differentiated HCC of clear cell type (Edmondson Grade II). There was no capsule infiltration or portal vein invasion (Fig. 3b). The non-cancerous liver tissue was cirrhotic.

Autopsy findings: A massive tumor, measuring 12.0×12.0×8.0 cm, and its metastatic lesions were found in the liver. A tumor embolus was found in the right portal vein. Intrahepatic metastases were found in the lateral segment (Fig. 3c) and multiple metastatic tumors were found in both lungs. Microscopic findings for the main tumor of the right lobe of the liver showed poorly differentiated thick-trabecular type HCC (Edmondson Grade III) (Fig. 3d). The histological characteristics of the portal vein embolus, the intrahepatic metastases and the metastatic lesion in the lung were almost same as those of the recurrent main tumor. In this case, the clinical course had suggested metachronous multicentric occurrence, but we were unable to determine, by histological examination of the reappearing tumors, whether they were metachronous multicentric tumors or intrahepatic metastases of the primary tumor that had been resected 5 years earlier.

Clonal study of 3 cases of putative multicentric HCC

Case 1. Southern blot analysis of DNA prepared from HCC in the lateral and HCC in the medial segments showed discrete bands that were hybridized by HBV DNA following digestion with *Hind*III or *Bam*HI. The integration patterns of HBV DNA were different in the two tumors. Non-cancerous liver tissue showed a smear pattern (Fig. 4).

These results indicated that the two HCC were different clonal origin[11),14),15)] (synchronous multicentric tumor-occurrence).

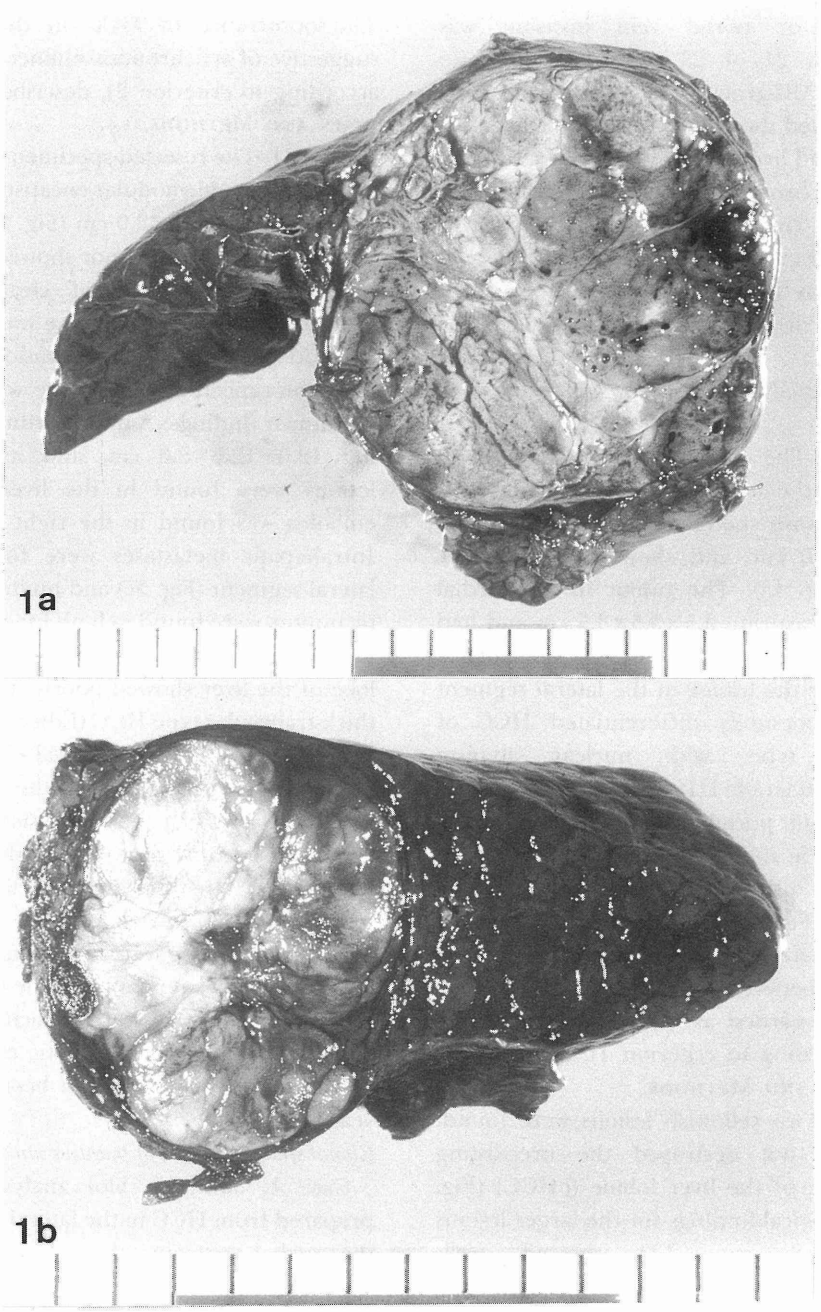

Fig. 1. a) Cut surface of tumor in the lateral segment of the liver in case No. 1.
b) Cut surface of tumor in the medial segment in the same case.

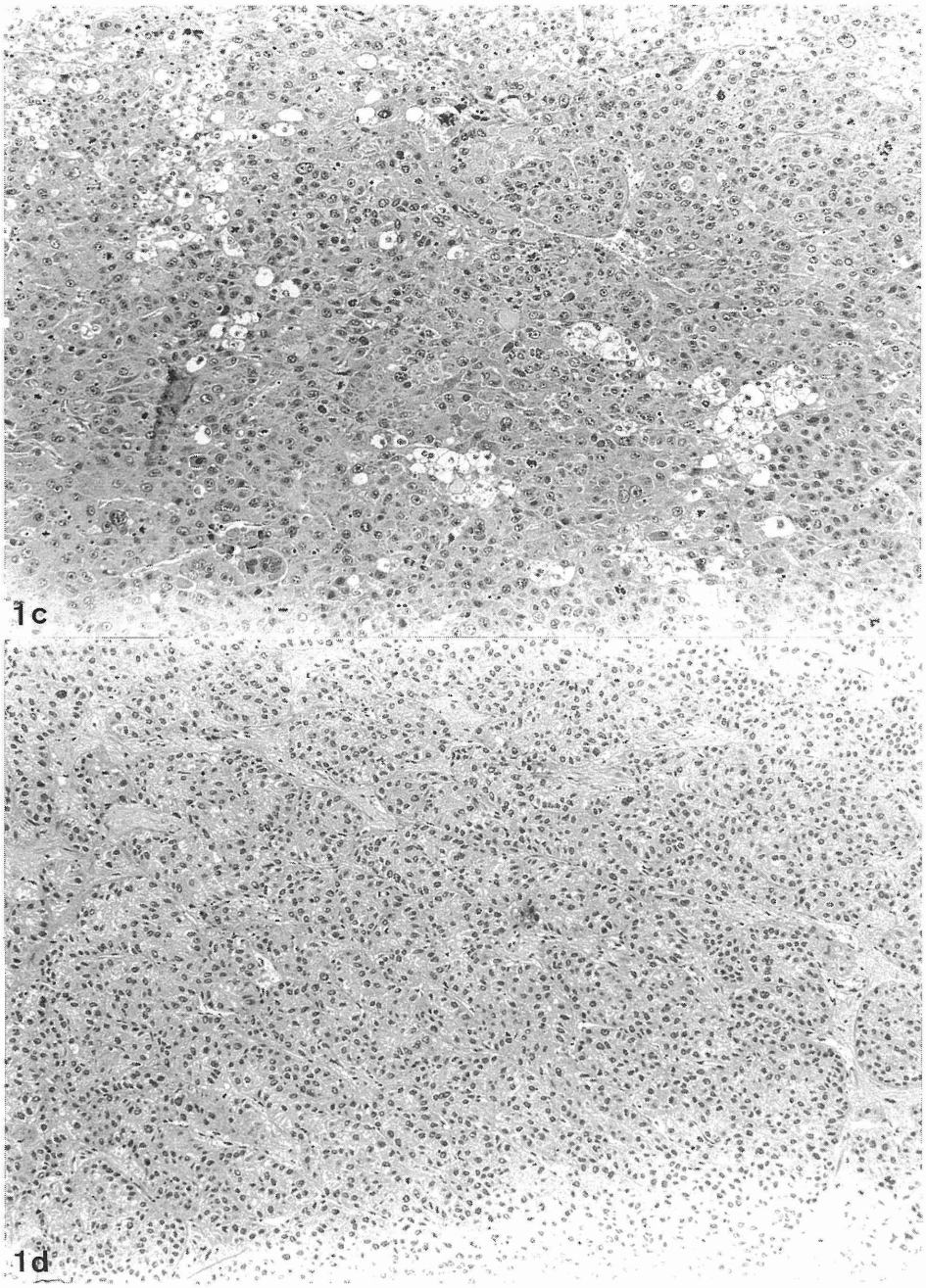

Fig. 1. c) Histological findings for the tumor in the lateral segment. HE ×100.
d) Histological findings for the tumor in the medial segment. HE ×100.

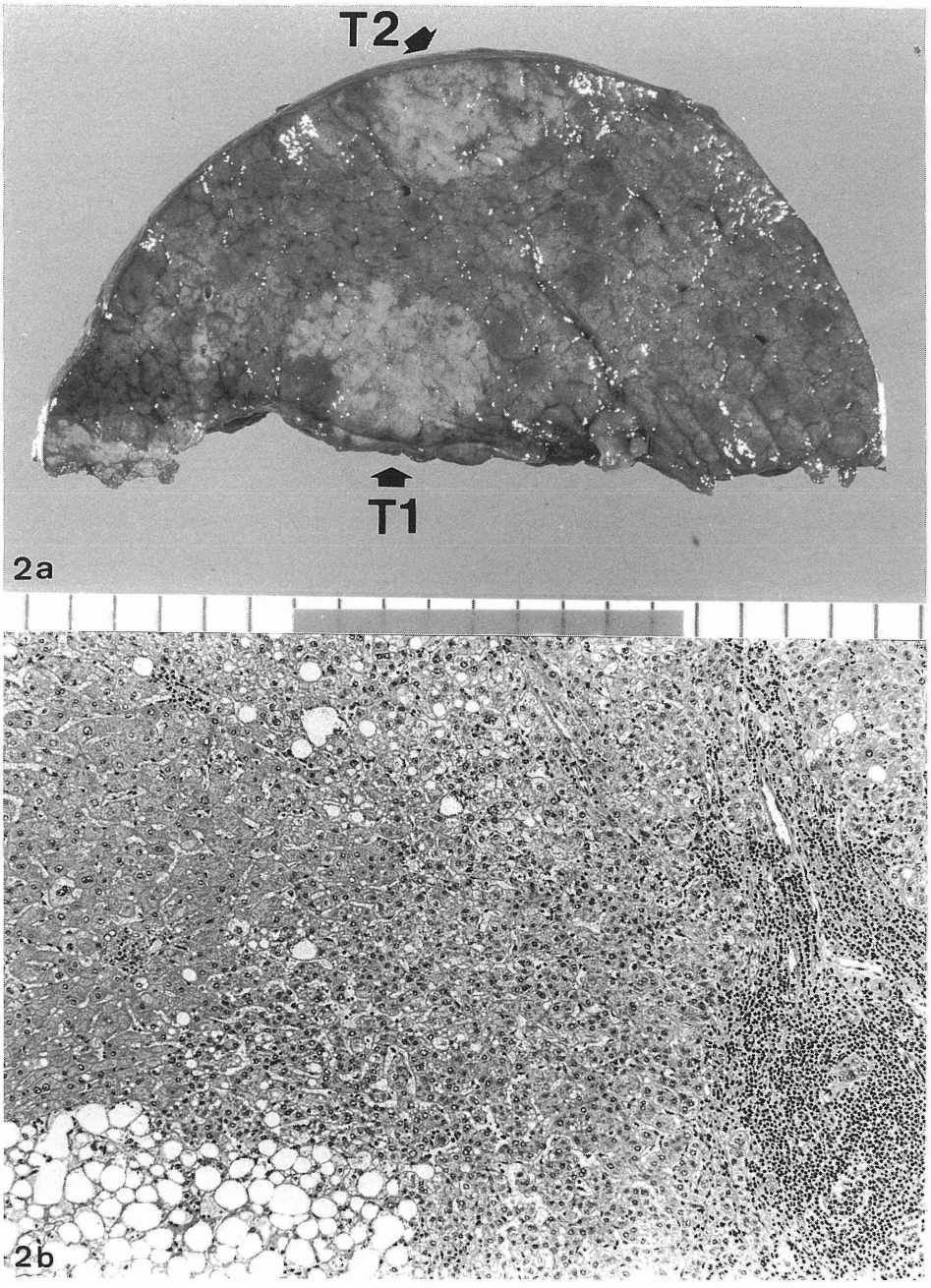

Fig. 2. a) Cut surface of resected liver in case No. 2. T1: the larger lesion, T2: the smaller lesion.
b) Histological findigns for the larger lesions. HE ×100.

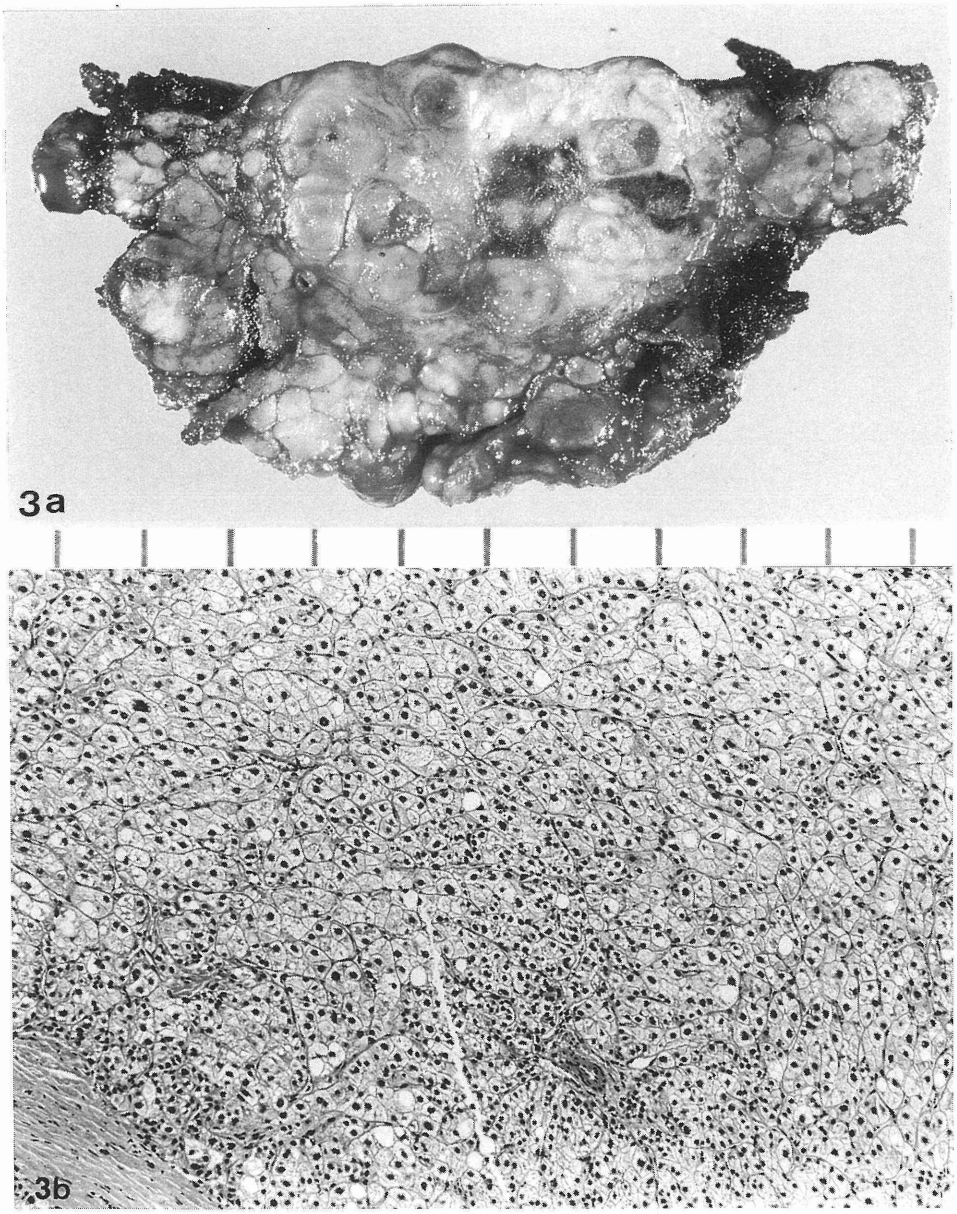

Fig. 3. a) Cut surface of resected specimen in case No. 31, at operation.
b) Histological findings for the resected tumor. HE ×100.

Case 2. Southern blot analysis of two HCC in the posterior segment and of non-cancerous liver tissue showed smear patterns; no discrete band was detected following digestion with *Eco*RI, *Hind*III or *Bam*HI (Fig. 5). These results indicate that the HBV DNA was integrated with DNA of both tumors as well as with that of the non-cancerous liver tissue. It is possible that non-cancerous tissue inside the lesions may interfere with appearance of discrete bands (multicentricity was not verified by this clonal study).

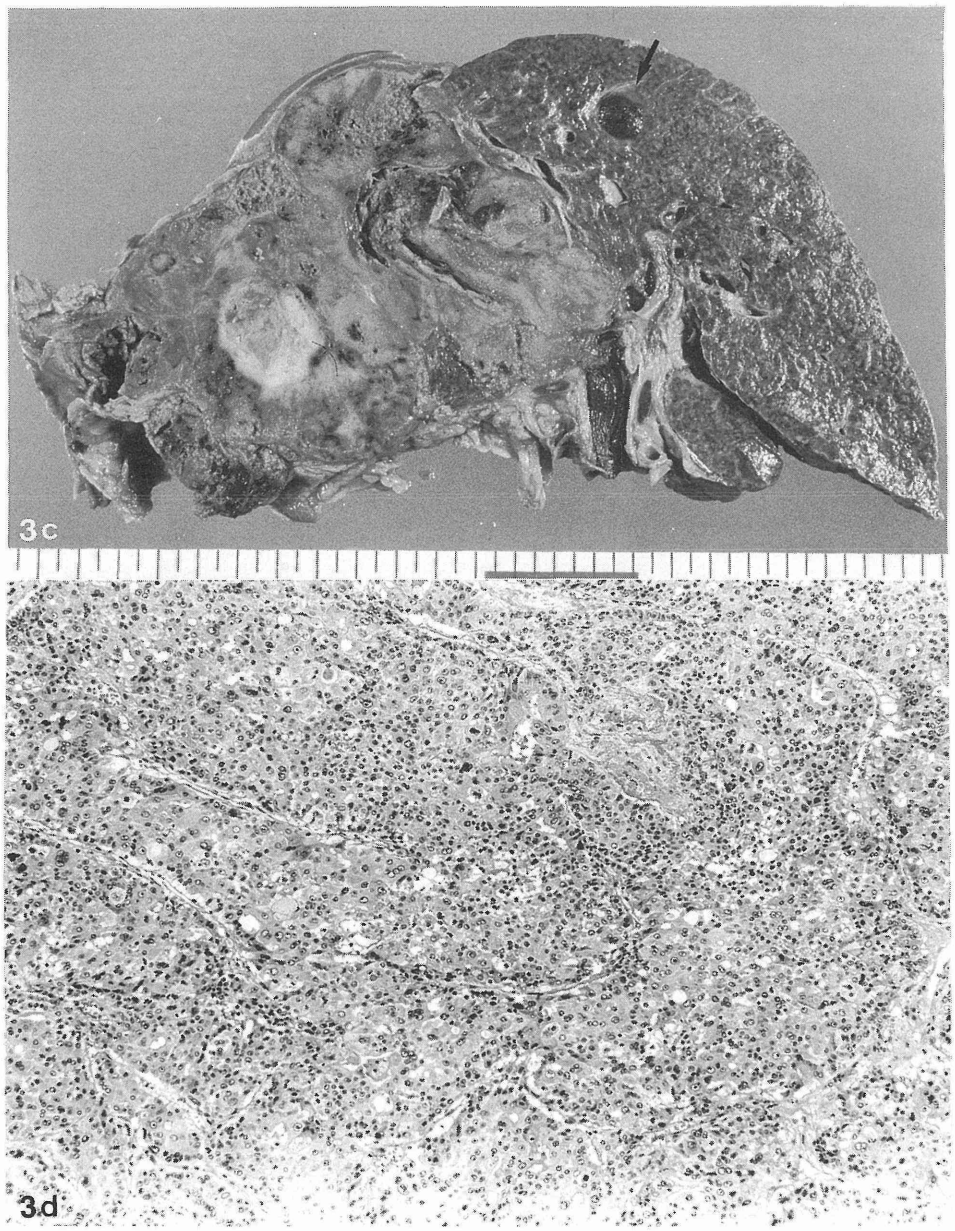

Fig. 3. c) Cut section of the liver in the same case at autopsy. Arrow: tumor embolus in the right portal vein.
d) Histological findings for the main tumor at autopsy. HE ×100.

Case 31. Southern blot analysis of the tumor resected at primary operation, the main liver tumor that reappeared, the portal vain embolus, the intrahepatic metastasis in the lateral segment, and the metastatic lesions in the lung showed the same discrete band hybridized by HBV DNA following digestion with *Hind*III or *Bam*HI. No discrete band was detected in the non-cancerous liver tissue (Fig. 6). The same integration pattern of HBV DNA in all tumors

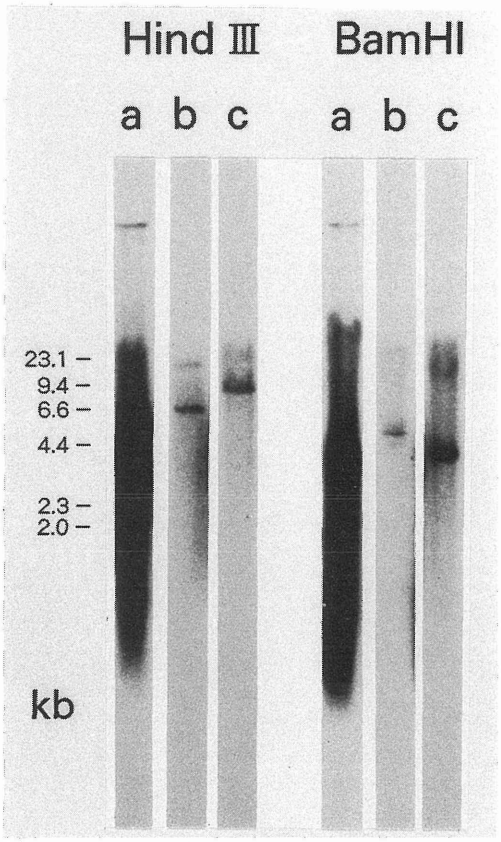

Fig. 4. Southern blot analysis in case No. 1. a: Non-cancerous liver. b: HCC in the lateral segment. c: HCC in the medial segment.

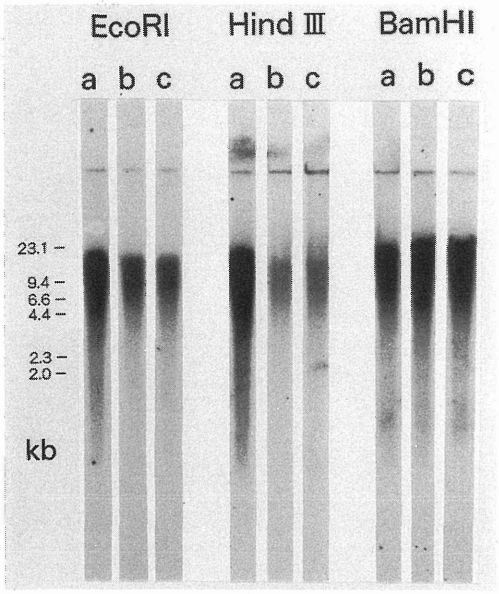

Fig. 5. Southern blot analysis in case No. 2. a: Non-cancerous liver. b: The larger lesion. c: The smaller lesion.

indicated that all tumors obtained by autopsy were of the same clone as the primary HCC resected 5 years previously[14] (metachronously developing intrahepatic metastasis).

Discussion

According to the UICC classification of primary malignant tumors, HCC patients with intrahepatic multiple tumors in both lobes are categorized as Stage IV-A, but our clincial experiences revealed that not all of these patients had a poor prognosis[19]. One reason for this fact is the existence of two types of Stage IV-A; one in which there is a cluster of slow growing HCC caused by the multicentric occurrence of HCC, and the other, in which main tumors and their metastatic lesions are found. In the latter type, there is a greater possibility of accompanying vascular invasions and early occurrence of distant organ metastasis. The recognition of this difference is very important clinically. Judged only on the basis of multiple tumors, Stage IV-A patients have been regarded as "unresectable" and have been treated conservatively. HCC determined by our criteria of multicentric occurrence appear to be less malignant, that is, they are slow growing tumors. If the large main tumor were removed in these patients, the remaining smaller tumors could be controlled by subsequent multidisciplinary treatment. From a clincal viewpoint, we believe that the determination of clonarity of intrahepatic multiple HCC become more important for treatment of HCC patients.

By histopathological study, 23.3% of the cases with multiple intrahepatic lesions were determined to have multicentric HCC. Our criteria of multicentlic occurrence are based on the fact that HCC originate as relatively well-

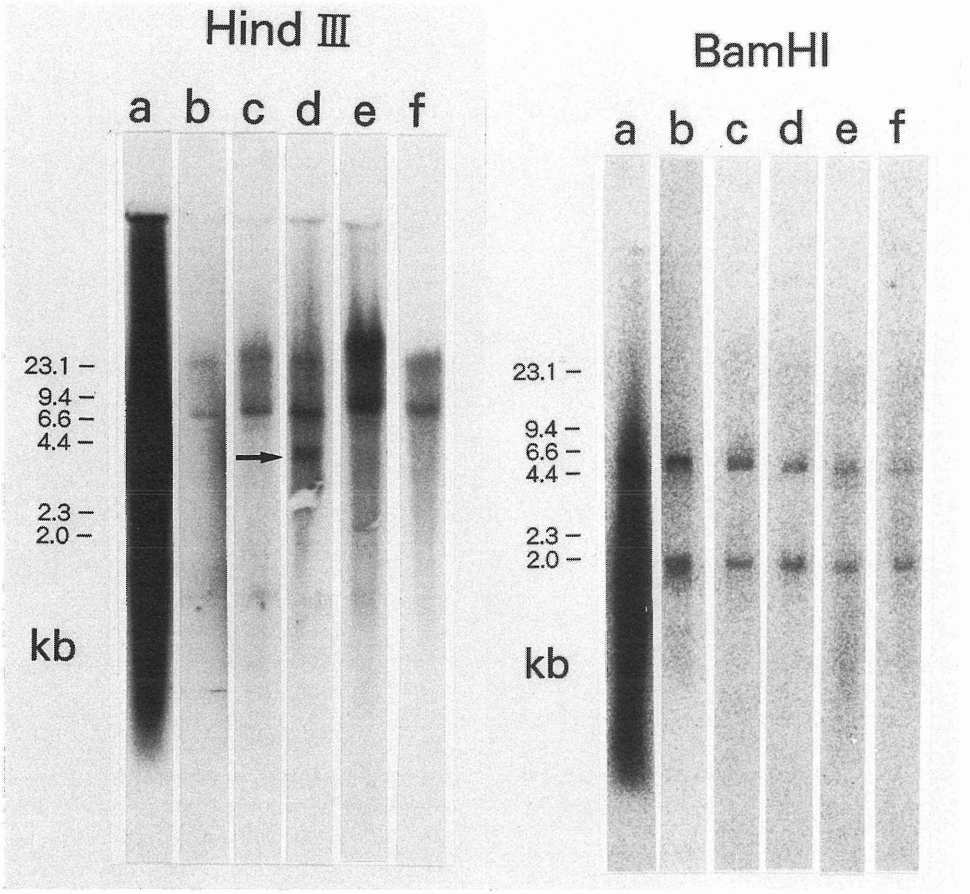

Fig. 6. Southern blot analysis in case No. 31. a: Non-cancerous liver. b: Main tumor at autopsy. c: Intrahepatic metastasis in the lateral segment. d: Portal vein embolus. e: Lung metastasis. f: Primary tumor resected at operation 5 years prior to autopsy. Arrow: Free HBV DNA.

differentiated tumor and become progressively less differentiated at a later stage of their development[20], and well-differentiated HCC hardly has an ability of matastasis. Moreover, "nodule-in-nodule" form indicates that the tumor originated and progressed at the place and not metastatic HCC cells have grown. Tumor capsule infiltration or portal vein invasion was present in 42.9% of this MC group, while these features occurred in 91.3% of the intrahepatic metastatic group. Therefore, in the cases with multiple HCC and no tumor-capsule infiltration or portal vein invasion, we must keep in mind the possiblity of multicentric occurrence. Chronic hepatitis was observed more in the non-cancerous liver tissue of MC group than in such tissue in IM group. It may be possible that multicentric HCC occur more frequently in patients whose liver cells are exposed to continuous inflammatory stimuli.

Hsu *et al.* reported that clonal analysis using Southern blot hybridization was not only a valuable tool for the study of clonal origin of tumor and evolution of HBV-related HCC, but it also provided valuable information to better understanding of biological behavior[15]. In this study, clonal analysis in cases Nos. 1 and 31, clearly demonstrated tumor origin, but in case No. 2, the limit of this method was

reached. Even in HCC with HBV DNA integration, very well-differentiated HCC which retain the portal tract structures inside[16),17)] do not show discrete bands, apparently due to interference from the non-cancerous tissue inside. On the other hand, as a verification of the uniclonal development of HCC, if Southern blot DNA analysis of multiple tumors shows the same restriction pattern in all specimens, even in those metachronously sampled in reoperation or autopsy, and in those of extrahepatic metastasis, it is possible to say that all these tumors are of monoclonal origin[11),15)]. Chen *et al.* examined 5 pairs of HCC resected from individual HBsAg carrior and concluded that recurrent HCC originated from the first tumor in some cases but represent de novo neoplasms in others[14)]. Our present study failed to analyze the clonarity of the HCC lesions of 9 cases of positive HBsAg in IM group, because of no enough sampling of intrahepatic metastatic lesions for clonal study. Hsu *et al.* verified by Southern blot analysis, the unicentric origin (intrahepatic metastasis) of 15 (93.5%) of 16 patients with multiple HCC histologically regarded as unicentric in origin[15)]. Further investigations are necessary on clonarity of HCC lesions histologically regarded as intrahepatic metastases, especially in lesions located relatively far from the primary tumor. Integrated HBV DNA has been demonstrated in some HBsAg-seronegative HCC by Southern blot analysis, ranging 1.7[21)] to 12%[22)]. Clonal analysis can be applied not only to HBsAg carriers but also to some HBsAg-seronegative patients.

Clonal study is a valuable method for determination of the mode of occurrence of HCC and it provides important information for better treaments of multinodular HCC patients.

A part of this work was reported at the XXVI. Congress of the European Society for Surgical Research (ESSR) in Salzburg on May 11th, 1991.

References

1) Berman C. Primary carcinoma of the liver. Adv Cancer Res 158; **5**: 55–96.
2) Higgins GK. The pathologic anatomy of primary hepatic tumors. *In*: Pack GT, Islami AH, eds. Tumor of the liver. Heidelbrg, Berlin: Springer, 1970: 15–37.
3) Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975; **98**: 503–517.
4) Liver cancer study group of Japan. The general rule for the clinical and pathological study of primary liver cancer. Jpn J Surg 1989; **19**: 98–129.
5) Edmondson HA, Steiner PE. Primary carcinoma of the liver: A study of 100 cases among 48,900 necropsies. Cancer 1954; **7**: 462–503.
6) Arakawa M, Kage M, Sugihara S, *et al.* Emergence of malignant lesions within an adenomatous hyperplastic nodule in cirrhotic liver. Gastroenterology 1986; **91**: 198–208.
7) Brechot C, Pourcel C, Louise A, *et al.* Presence of integrated hepatitis B virus DNA sequence in cellular DNA of human hepatocellular carcinoma. Nature 1980; **286**: 533–535.
8) Shafritz DA, Shouval D, Sherman HI, *et al.* Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. N Engl J Med 1981; **305**: 1067–1073.
9) Hino O, Kitagawa T, Koike K, *et al.* Detection of hepatitis B virus DNA in hepatocellular carcinoma in Japan. Hepatology 1984; **4**: 90–95.
10) Esumi M, Aritaka T, Arii M, *et al.* Clonal origin of human hepatoma determined by integration of hepatitis B virus DNA. Cancer Res 1986; **46**: 5767–5771.
11) Sakamoto M, Hirohashi S, Tsuda H, *et al.* Multicentric indipendent development of hepatocellular carcinoma revealed by analysis of hepatitis B virus integration pattern. Am J Surg Pathol 1989; **13** (12): 1064–1067.
12) Esumi M, Tanaka Y, Tozuka S, *et al.* Clonal state of human hepatocellular carcinoma and non-tumor hapatocytes. Cancer Chemother Pharmacol 1989; **23**: (Suppl): S1–S3.
13) Tsuda H, Hirohashi S, Shimosato Y, *et al.* Clonal origin of atypical adenomatous hyperplasia of the liver and clonal identity with hepatocelullar carcinoma. Gastroenterology 1988; **95**: 1664–1666.

14) Chen PJ, Chen DS, Lai MY, *et al.* Clonal origin of recurrent hepatocellular carcinomas. Gastroenterology 1989; **96**: 527–529.
15) Hsu HC, Chiou TJ, Chen JY, *et al.* Clonality and evolution of hepatocellular carcinoma with multiple nodules. Hepatology 1991; **13**: 923–928.
16) Kanai T, Hirohashi S, Upton MP, *et al.* Pathology of small hepatocellular carcinoma. Cancer 1987; **60**: 810–819.
17) Sakamoto M, Hirohashi S, Shimosato Y. Early stage of multistep hepatocarcinogenesis. Human Pathol 1991; **22**: 172–178.
18) Couinaud C. Lobes et segments hépatiques Notes sur lārchitecture anatomique et chirurgical du fole. Presse Medicale 1954; **62**: 709–712.
19) Iizuka H, Yamamoto M, Kohno T, *et al.* Continuous infusion of IL-2 and adriamycin injections selectively to advanced hepatocellular carcinoma through the irrigating hepatic artery. Frontiers of mucosal immunology 1991; **2**: 513–516.
20) Kenmochi K, Sugihara S, Kojiro M. Relationship of histologic grade of hepatocellular carcinoma (HCC) to tumor size, and demonstration of tumor cells of multiple different grades in single small HCC. Liver 1987; **7**: 18–26.
21) Imazeki F, Omata M, Yokosuka O, *et al.* Integration of hepatitis B virus DNA in hepatocellular carcinoma. Cancer 1986; **58**: 1055–1060.
22) Hino O, Kitagawa T, Sugano H. Relationship between serum and histochemical markers for hepatitis B virus and rate of viral integration in hepatocellular carcinomas in Japan. Int J Cancer 1985; **35**: 5–10.