Age-Related, Different Clinicopathologic Features of Hepatocellular Carcinoma Patients

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Objective
The authors attempted to clarify the clinicopathologic differences of hepatocellular carcinoma (HCC) patients, according to age distribution, and to investigate whether these differences contribute a certain hepatocarcinogenesis.

Summary Background Data
Hepatitis-associated viruses causing HCC have been investigated, and the infection of the viruses and etiologically, the peak age of the disease vary according to geographic barriers. However, a correlation between clinicopathologic differences and the age distribution of the patients is not well understood.

Methods
The authors reviewed their institutional experience from 1978 to 1990 in treating 428 patients with HCC. The carrier rate for hepatitis B surface antigen (HBsAg), the frequency of occurrence of high serum alpha-fetoprotein (AFP) of 2000 ng/mL, the degree of liver damage represented by the retention rate of indocyanine green dye at 15 minutes ($ICGR_{15}$), and the incidence of accompanying liver cirrhosis were investigated and compared in each decade of age.

Results
The HBsAg carrier rate and the frequency of high serum AFP values were significantly prominent in the younger patients (20–49 yrs). The degree of liver damage and the incidence of liver cirrhosis were prominent in the elderly patients (older than 70 yrs) or the middle-aged patients (50–69 yrs); however, these four values in the middle-aged patients were intermediate with respect to those observed in the other two age groups. In addition, there was a positive correlation between the HBsAg carrier rate and the frequency of high serum AFP values or between the degree of liver damage represented by $ICGR_{15}$ and the incidence of liver cirrhosis, showing that the former correlation was inversely related to the latter.

Conclusions
The authors' study indicates that there are age-related differences of clinicopathologic features in HCC patients, suggesting that there are different steps or mechanisms of hepatocarcinogenesis according to the patient's age-distribution.

South Africa, Southeast Asia, China, Taiwan, Japan, and the Pacific Islands have a high incidence of hepatocellular carcinoma (HCC), presenting a correlation between age of the onset and frequency of the disease.[1,2] The peak age of onset of HCC varies considerably with geographic differences—i.e, the 30s in South Africa,[3,4] the 40s in Southeast Asia,[5] the late 50s in Japan,[5] and older than 60 years in the United States and western Europe.[6] These differences indicate that geographic barriers have a certain relation to hepatocarcinogenetic patterns and suggest that the patients in areas with high incidences of HCC might have been carriers of the hepatitis B surface antigen (HBsAg) from an early age, or may have suffered different degrees of liver damage according to their age distribution.

However, a correlation between the clinicopathologic characteristics and the age distribution of the patients with HCC is not well understood. We reviewed the records of 428 patients with HCC to investigate age-related clinicopathologic differences of the disease, and tried to clarify the characteristics leading to the cause of hepatocarcinogenesis.

In this study, we found clinicopathologic differences in the patients with HCC according to their age distribution, and thus, we divided the patients into three age groups. We named the younger patients (20–49 yrs) the Eastern type, middle-aged patients (50–69 yrs) the Transitional type and elderly patients (over 70 yrs) the Western type. We then discussed different steps or mechanisms of hepatocarcinogenesis in each of three types.

PATIENTS AND METHODS

During a 12-year period, from 1978 to 1990, 428 patients were diagnosed with HCC at our hospitals. There were 349 men and 79 women; a male:female ratio of 4.4:1. The age distribution of these patients is shown in Figure 1. Diagnosis of HCC was histopathologically or clinicopathologically confirmed from a biopsy specimen or by combined examinations of selective angiography, computed tomography, ultrasonography, and serum alpha-fetoprotein (AFP) assay. The patients were classified according to the TNM classification of the *Manual for Staging of Cancer*,[7] based on diagnostic images. In short, the staging of HCC was defined by the number and size of the primary tumor and by the presence or absence of regional lymph node or distant metastasis. Laboratory examinations were performed during the patients' initial visit to our outpatient clinics. Hepatitis B surface antigen
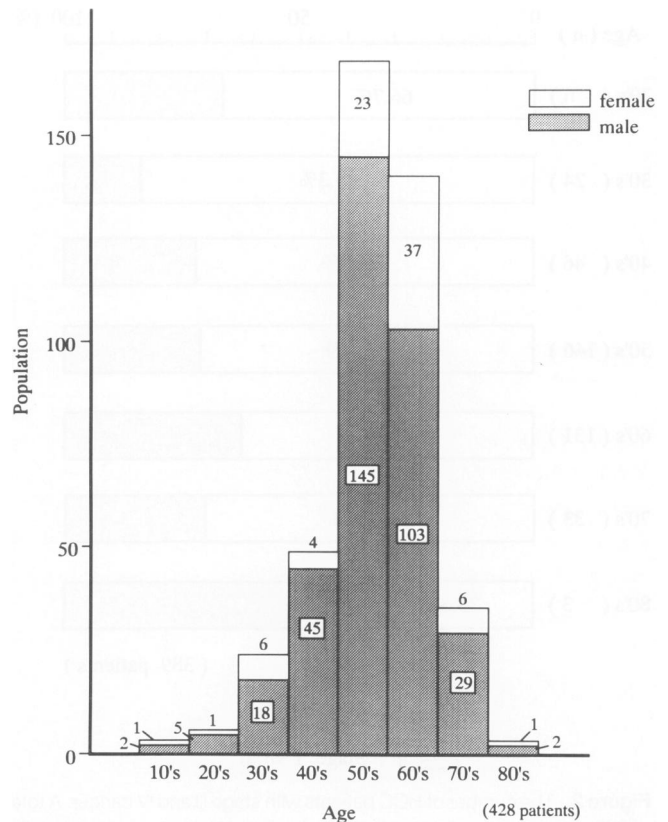
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Figure 1. Age distribution of the patients with HCC. A total of 428 patients were diagnosed as suffering from HCC: 349 men and 79 women. All the patients were grouped according to age distribution.

was measured with a commercially available radioimmunoassay kit (Abbott Laboratories, Abbott Park, IL). Serum AFP values were determined by means of a radioimmunoassay (Amersharm Corp., Amersharm, UK) and a passive hemagglutination method. The retention rate of indocyanine green dye at 15 minutes ($ICGR_{15}$) was tested for after 0.5 mg of indocyanine green dye/kg body weight had been injected intravenously. The biopsy or resected hepatic specimen was stained with hematoxylin-eosin and was examined histopathologically for accompanying liver cirrhosis. The data obtained were arranged according to the age distribution of the patients for a comparative study. For statistical analysis, the chi square test was employed, and a p value of < 0.05 was considered significant.

RESULTS

Incidence

All the patients with HCC were grouped according to age distribution, as shown in Figure 1—i.e., 349 men and 79 women, with a total male:female ratio of 4.4:1. The peak age of onset was in the group of patients in their

Age (n) 0   50   100 (%)

20's (6)   66.7%

30's (24)   83.3%

40's (46)   71.7%

50's (146)   71.2%

60's (131)   62.5%

70's (33)   70.0%

80's (3)   33.3%

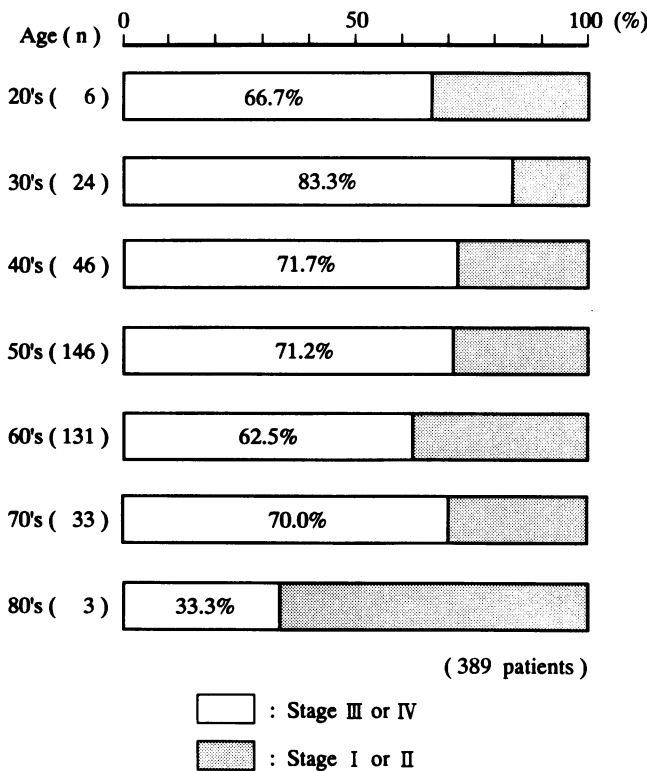(389 patients)

☐ : Stage III or IV

▨ : Stage I or II

Figure 2. The number of HCC patients with stage III and IV cancer. A total of 389 patients were clinicopathologically divided into stages I through IV, according to TNM classification. The ratio of the patients with stage III and IV cancer to those with stage I and II cancer, according to age distribution, is shown.

50s, followed by those in their 60s. When we classified younger patients as ranging in age from 20 to 49 years of age, middle-aged patients as ranging from 50 to 69 years of age, and elderly patients as those older than 70 years, the number of patients with HCC was 79 (18.5%) in the younger patients, 308 (72.0%) in the middle-aged patients, and 38 (8.9%) in the elderly patients, respectively; the number of HCC cases was the highest in the middle-aged patients, followed by the younger group.

TNM Staging

A total of 389 patients were diagnosed accurately with various images and classified according to TNM staging. Figure 2 shows the ratio of stage III and IV cases to stage I and II cases, according to age distribution, because the former stage is more advanced than the latter. Over 60% of the cases in each decade were stage III or IV, except in the group in their 80s. The number of stage III or IV cases was 57 (75%) in the younger patients, 186 (67.1%) in the middle-aged patients, and 24 (66.7%) in the elderly patients, respectively, with no significant difference among the groups.

HBsAg Carrier

A total of 338 patients were examined preoperatively for HBsAg. As shown in Figure 3, the incidence of HBsAg carriers had a characteristic distribution—i.e., the incidence decreased with age. The incidence was 59.4% in the younger patients, 25.0% in the middle-aged patients and 9.1% in the elderly patients, respectively, showing a significant difference (p < 0.001) between the younger patients and the middle-aged or elderly patients, and a significance (p < 0.05) between the middle-aged and the elderly patients.

Serum Alpha-Fetoprotein

A total of 399 patients were examined preoperatively for serum AFP. The number of patients with more than 2000 ng/mL of serum AFP value is represented in Figure 4, according to age distribution. The incidence was very high in the groups of patients in their 20s and 30s, abruptly decreased with age, and was lowest in the group in their 50s. The respective ratio was 46.2% in the younger patients, 28.9% in the middle-aged patients, and 35.1% in the elderly patients, showing a significant difference (p < 0.01) between the younger and the mid-
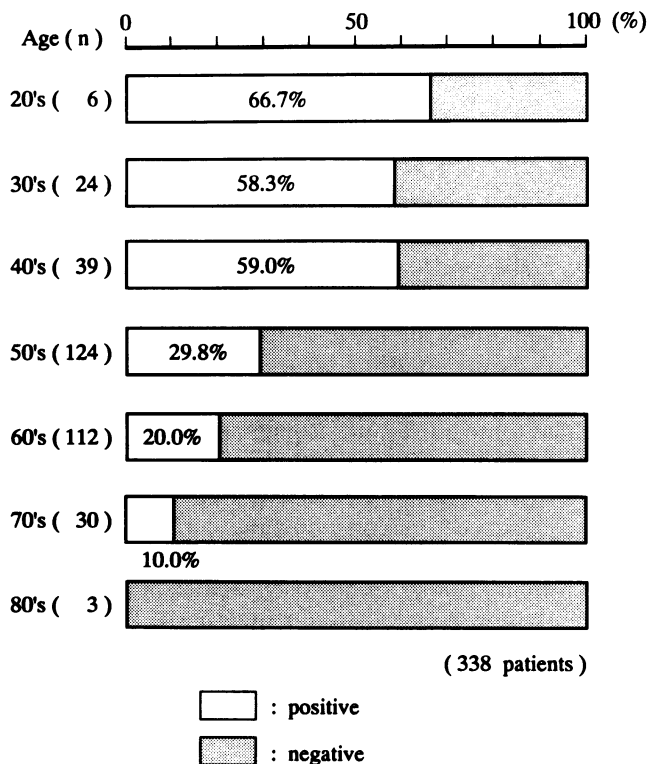
Age (n) 0   50   100 (%)

20's (6)   66.7%

30's (24)   58.3%

40's (39)   59.0%

50's (124)   29.8%

60's (112)   20.0%

70's (30)   10.0%

80's (3)

(338 patients)

☐ : positive

▨ : negative

Figure 3. Incidence of HBsAg-positive patients. A total of 338 patients were examined. The ratio of HBsAg-positive patients to HBsAg-negative ones, according to age distribution, is shown.
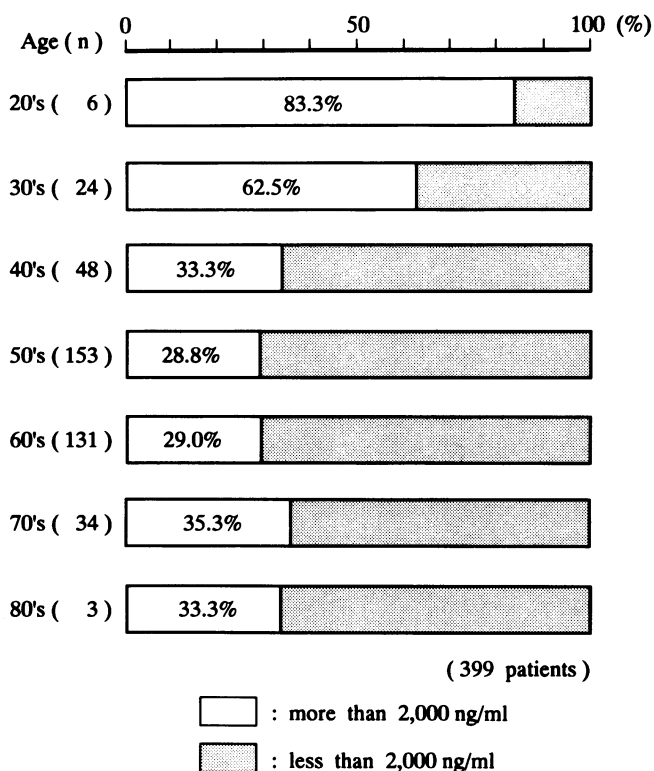
Figure 4. The number of patients with more than 2000 ng/mL of serum AFP. A total of 399 patients were examined. The ratio of patients with more than 2000 ng/mL to those with less than 2000 ng/mL of serum AFP, according to age distribution, is shown.

dle-aged patients, with no significant difference between the younger and the elderly patients or between the middle-aged and the elderly patients.

Indocyanine Green Dye Retention Rate (ICGR$_{15}$)

A total of 324 patients were examined for ICGR$_{15}$. The number of patients with more than 15% ICGR$_{15}$ is shown in Figure 5 according to age distribution. The number of positive-testing patients increased with age through the 70s. The incidence was 23.5% in the younger patients, 61.1% in the middle-aged patients, and 70.6% in the elderly patients, respectively, showing a significant difference (p < 0.001) between the younger patients and the middle-aged or elderly patients, but no significant difference between the middle-aged and the elderly patients.

Liver Cirrhosis

A total of 285 patients were examined histopathologically for cirrhosis using biopsy or resected hepatic specimens. As shown in Figure 6, the incidence of accompa-

nying liver cirrhosis had a distribution similar to that shown in Figure 5. Again, the incidence increased with age through the 70s. The respective incidence was 31.7% in the younger patients, 71.2% in the middle-aged patients, and 70.0% in the elderly patients, respectively, showing a significant difference (p < 0.001, 0.05) between the younger patients and the middle-aged or elderly patients, respectively, but no significant difference between the middle-aged and the elderly patients.

DISCUSSION

Hepatocellular carcinoma is an important cause of cancer-related morbidity and mortality in geographic regions where hepatitis B virus (HBV) infection is prevalent. The incidence and age distribution of patients with HCC are higher and earlier, respectively, in high prevalence areas, and most are older in low prevalence areas of HBV infection.[1-6] In Japan, the peak age of incidence of HCC is in the 50s,[5] supported by the present study. However, the incidence of HCC varied considerably with age distribution as shown in Figure 1, and a correlation between the clinicopathologic features and the age distribution of patients with HCC has, to date, not been
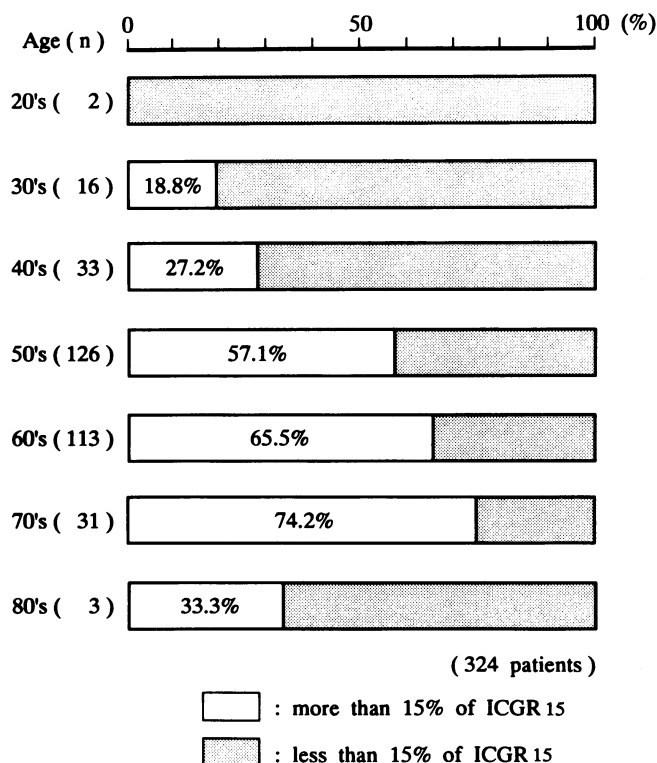

Figure 5. Frequency of patients with more than 15% of ICGR$_{15}$. A total of 324 patients were examined. The ratio of patients with more than 15% to those with less than 15% of ICGR$_{15}$, according to age distribution, is shown.

Age (n)

20's (6)

30's (24) 33.3%

40's (30) 36.7%

50's (113) 69.0%

60's (102) 73.5%

70's (8) 75.6%

80's (2) 50.0%

(285 patients)

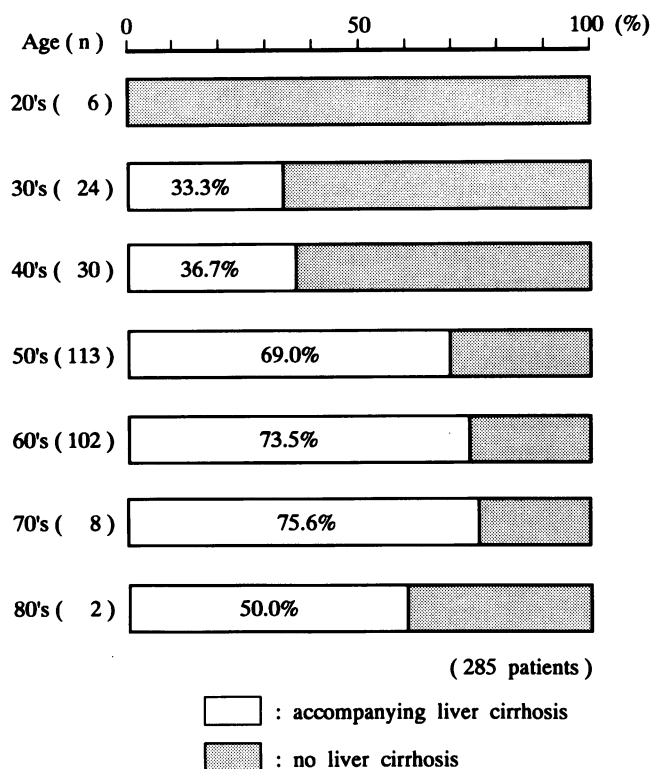☐ : accompanying liver cirrhosis

▨ : no liver cirrhosis

Figure 6. Incidence of accompanying liver cirrhosis. A total of 285 patients were examined histopathologically. The ratio of patients with liver cirrhosis to those without liver cirrhosis, according to age distribution, is shown.

well established. We have, therefore, reviewed features such as positivity tests for HBsAg carriers, serum AFP levels, $ICGR_{15}$, and histopathologic examinations for liver cirrhosis in patients with HCC, according to age distribution, and we divided the patients into three age groups for a comparative study. The ratio of stage III and IV to stage I and II cases of the TNM system[7] was not significantly different among the three age groups, revealing that most patients were diagnosed as suffering from HCC when it already had reached an advanced stage. This study demonstrated that there was a positive correlation between the HBsAg carrier rate and the frequency of a high serum AFP level or between the degree of liver damage represented by $ICGR_{15}$ and the incidence of liver cirrhosis according to age distribution, and the former correlation was inversely related to the latter. In addition, the former and the latter features were frequent and low, respectively, in the younger patients, and not frequent and high, respectively, in the elderly patients, whereas the features of the middle-aged patients were transitional between both age groups.

Epidemiologic, case control, and molecular biologic studies have reported that most patients with HCC suffer from chronic HBV infection,[1,8–10] and the prevalence of

HBV carriers is related closely to the incidence in the HCC.[2] This study revealed an age-related difference in the HBsAg carrier rate, with significant differences among the three age groups. Molecular biology showed that HBV-DNA is integrated in both adult and pediatric patients[11–13]; however, the integration of HBV-DNA into a single site within the host cellular DNA appears to be common in HCC tissues obtained from children, but uncommon in tumor tissues obtained from adults,[13] causing speculation that the development of HCC caused by chronic HBV infection varies according to the patients' age or the carrier status of HBV or HBsAg. In addition, a comparative study of patients with HCC in high, moderate, or low prevalence areas for HCC incidence showed that Asian patients generally are younger, with 82% being positive for HBsAg, and the average Western patients are older by more than a decade, with 25% positive for the same antigen.[14] The positivity rate for HBsAg was 59.4% in the younger patients and 25.0% in the middle-aged patients in this study, indicating that the prevalence of HBV is less in this region (Japan) than in other Asian countries or Africa, and more than in the United States or Europe. These observations raise the possibility that the mechanisms of hepatocarcinogenesis may be different between younger patients and middle-aged or elderly patients, and present the following epidemiologic problems: geographic barriers; environmental factors, such as the workplace; materials or organisms; age, food, smoking, or drinking habits; and diagnostic abilities. It has been reported that hepatocarcinogenesis can be mainly divided into hepatitis virus-related or alcohol-related mechanisms, and that mechanisms of hepatocarcinogenesis in patients in high prevalence areas of HBV infection are correlated strongly with HBV and those in low prevalence areas are correlated strongly with alcohol consumption[14,15]; however, such a concept is too simple, as shown in this study. Because the HBsAg carrier rate in the middle-aged patients was the same as that in Western patients with HCC,[14] conversely, the rate was significantly higher in the younger patients. The pathogenetic effect of HBV on hepatocarcinogenesis is likely to be different according to the age of patients. This study and a previous report[15] give rise to the possibility that hepatocarcinogenesis appears to be caused by a variety of initiators or promotors according to the age distribution of patients.

The percentage of the patients with a serum AFP value of more than 2000 ng/mL varied according to age distribution. This tendency was similar to that of the positivity rate for HBsAg. Kew and Maccrollo[16] and other investigators[17,18] also found that HCC cells secrete more serum AFP in younger patients than in elderly patients, suggesting that the biologic heterogeneity of cancer cells at different ages may influence the

serum AFP secretion. Because the sources of serum AFP are HCC cells or regenerating hepatocytes that originate from cholangiole cells and are in cell differentiation,[19-21] the question remains whether the main source of serum AFP is different with the age of patients. Because the degree of liver damage represented by $ICGR_{15}$ and the incidence of accompanying liver cirrhosis were significantly low in the younger patients, their serum AFP seemed to be generated mainly by cancer cells, indicating that in younger patients, the integration of HBV-DNA into hepatocytes may cause carcinogenesis because the disease progresses with high secretion of AFP. In contrast, because the degree of liver damage and the incidence of liver cirrhosis were significantly high in the middle-aged and the elderly patients, and the regenerating response of hepatocytes to liver damage is likely to be poor because of cirrhotic changes, their serum AFP also seemed to be generated by cancer cells; however, their ability of secreting AFP is thought to be relatively poor. These observations indicate that the mechanisms for hepatocarcinogenesis probably are different between younger patients and elderly patients, and a clue to resolve this issue may exist in the pathosis of middle-aged patients, because the pathosis was likely to be a transitional one between that of the two other age groups. From the aforementioned discussion, hepatocarcinogenesis seems to be different according to age distribution, and we present the following notion: there may be three mechanisms of hepatocarcinogenesis, and we have tentatively named them the Eastern type, the Transitional type, and the Western type. The Eastern type is contributed mostly to by HBV infection with extremely high values of serum AFP and accompanied by low-grade liver damage. The Western type is contributed mostly to by consumed liver damage, with relatively low values of serum AFP and accompanied only by a slight contribution of HBV. The Transitional type is intermediate between both. Even in high prevalence areas for HBV infection, HCC patients also were divided into three types. Younger patients belonged to the Eastern type, middle-aged patients to the Transitional type, and elderly patients to the Western type. What cause these differences between the three age-groups remains to be solved, and we speculate that HBV may act during hepatocarcinogenesis as a promotor and disease progression-enhancer through a specific integration of virus genomes in host hepatocytes and thereby, induce insertional mutations,[22,23] chromosomal deletions or translocation, or both,[24] especially in the Eastern type. Chronic and progressive liver damage may be followed by a serial phenomenon of necrosis, inflammation, and regeneration in the liver, eventually inducing a malignant transformation

of hepatocytes, especially in the Western type. There is no indication of the mechanism for hepatocarcinogenesis in the Transitional type. However, we can imagine that the contribution of the hepatitis C virus may be involved in this type of hepatocarcinogenesis because the percentage of HCC patients with positive HBsAg recently has been decreasing in Japan and, in contrast, the number of hepatitis C virus carriers is increasing.[25] The C type hepatitis has a chronic course leading to liver cirrhosis after approximately 20 years, probably without hepatitis C virus-DNA integration into hepatocyte DNA because it is an RNA virus,[26,27] speculating that hepatocarcinogenesis associated with hepatitis C virus infection can possibly be grouped in the Transitional type. We need to make further investigations to study the possible mechanisms of hepatocarcinogenesis, including the etiologic role of hepatitis-related viruses, habits of food and drink, and environmental factors.

References

1. Beasley RP. Hepatitis B virus as the etiological agent in hepatocellular carcinoma: epidemiological considerations. Hepatology 1982; 2(suppl):21-26.
2. London WT. Primary hepatocellular carcinoma—etiology, pathogenesis, and prevention. Hum Pathol 1981; 12:1085-1097.
3. Anthony PP. Primary carcinoma of the liver: a study of 282 cases in Ugandan Africans. J Pathol 1972; 110:37-48.
4. Kew MC, Geddes EW. Hepatocellular carcinoma in rural Southern African blacks. Medicine 1982; 61:98-108.
5. Tobe T, Endo Y, Hattori N, et al. The Liver Cancer Study Group of Japan—clinicopathologic features and results of surgical treatment. Ann Surg 1990; 211:277-287.
6. Sandler DP, Sandler RS, Horney LF. Primary liver cancer mortality in the United States. J Chron Dis 1983; 36:227-236.
7. American Joint Committee on Cancer. Manual for Staging of Cancer. 3rd ed. Philadelphia: JB Lippincott, 1988, pp 87-92.
8. Szmuness W. Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. Prog Med Virol 1978; 24:40-69.
9. Popper H, Gerber MA, Thung SN. The relation of hepatocellular carcinoma to infection with hepatitis B and related viruses in man and animals. Hepatology 1982; 2(suppl):1-9.
10. Harrison TJ, Chen JY, Zuckerman AJ. Hepatitis B virus and hepatocellular carcinoma. Clin Trop Med Commun Dis 1986; 1: 395-409.
11. Yaginuma K, Kobayashi H, Yoshida E, et al. Direct evidence for the expression of integrated hepatitis B virus DNA in human hepatoma tissue. Jpn J Cancer Res 1984; 75:743-746.
12. Tanaka T, Miyamoto H, Hino O, et al. Primary hepatocellular carcinoma with hepatitis B virus—DNA integration in a 4-year-old boy. Hum Pathol 1986; 17:202-204.
13. Chang MH, Chen PJ, Lai MY, et al. Hepatitis B virus integration in hepatitis B-related hepatocellular carcinoma in childhood. Hepatology 1991; 13:316-320.
14. Poenaru D, Szilagyi A, Zabad F, et al. Hepatocellular carcinoma: comparison of clinical features among ethnic groups in a area of low prevalence. Am J Gastroenterol 1991; 86:487-494.
15. Chen CJ, Lang KY, Chang AS, et al. Effects of hepatitis B virus,

alcohol drinking, cigarette smoking and familial tendency on hepatocellular carcinoma. Hepatology 1991; 13:398–406.

16. Kew MC, Maccrollo P. Effect of age on the etiologic role of the hepatitis B virus in hepatocellular carcinoma in blacks. Gastroenterology 1988; 94:439–442.

17. Vogel CL, Primack A, McIntire KR, et al. Serum alpha—fetoprotein in 184 Ugandan patients with hepatocellular carcinoma. Cancer 1974; 33:959–964.

18. Chen DS, Sung JL. Serum alphafetoprotein in hepatocellular carcinoma. Cancer 1977; 40:779–783.

19. Ogawa K, Minase T, Onoe T. Demonstration of glucose 6-phosphatase activity in the oval cells of rat liver and the significance of the oval cells in azo dye carcinogenesis. Cancer Res 1974; 34:3379–3386.

20. Dempo K, Chisaka N, Toshida Y, et al. Immunofluorescent study on α-fetoprotein-producing cells in the early stage of 3-methlyl-4-dimethylaminoazobenzen carcinogenesis. Cancer Res 1975; 35:1282–1287.

21. Okuda K, Kubo Y, Obata H. Serum α-fetoprotein in the relatively early stages of hepatocellular carcinoma and its relationship to gross anatomical types. Ann NY Acad Sci 1975; 259:248–252.

22. Dejean A, Bougueleret L, Grzeschik KH, et al. Hepatitis B virus DNA integration in a sequence homologous to v-erb-A and steroid receptor genes in a hepatocellular carcinoma. Nature 1986; 322:70–72.

23. Benbrook D, Lernhardt E, Pfahl M. A new retinotic acid receptor identified from a hepatocellular carcinoma. Nature 1988; 333:669–672.

24. Hino O, Shows TB, Rogler CE. Hepatitis B virus integration site in hepatocellular carcinoma at chromosome 17;18 translocation. Proc Natl Acad Sci U S A 1986; 83:8338–8342.

25. Tanaka K, Hirohata T, Koga S, et al. Hepatitis C and hepatitis B in the etiology of hepatocellular carcinoma in the Japanese population. Cancer Res 1991; 51:2842–2847.

26. Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A non-B viral hepatitis genome. Science 1989; 244:359–362.

27. Esteban JI, Esteban R, Viladomiu L, et al. Hepatitis C virus antibodies among risk groups in Spain. Lancet 1989; i:294–296.