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An analysis of DNA ploidy pattern of hepatocellular carcinoma.

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Abstract

To determine whether a relationship exists between DNA ploidy and the prognosis of hepatocellular carcinoma (HCC), flow cytometric DNA analysis was performed in paraffin-embedded specimens obtained from 44 patients with HCC who underwent hepatectomy. There were 26 diploid (59%) and 18 aneuploid (41%) tumors. No correlation was shown between DNA ploidy pattern and patient age, sex, liver cirrhosis, hepatitis B virus antigen and serum alpha-fetoprotein level. The ploidy pattern had no significant correlation with the presence of vascular invasion or intrahepatic metastasis. Only Edmondson's grade was well correlated with the ploidy pattern. We noted a significant correlation between survival rates and the presence of vascular invasion or intrahepatic metastasis ($p < 0.05$). In contrast, no significant correlation was found between DNA ploidy pattern and the prognosis of HCC. The results of this study indicate that DNA ploidy pattern may not be a useful indicator for the prognosis of HCCs after hepatic resection, unlike the results of gastric and colon cancers.

KEYWORDS: DNA ploidy pattern, hepatocellular carcinoma, hepatic resection, prognosis

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An Analysis of DNA Ploidy Pattern of Hepatocellular Carcinoma

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To determine whether a relationship exists between DNA ploidy and the prognosis of hepatocellular carcinoma (HCC), flow cytometric DNA analysis was performed in paraffin-embedded specimens obtained from 44 patients with HCC who underwent hepatectomy. There were 26 diploid (59 %) and 18 aneuploid (41 %) tumors. No correlation was shown between DNA ploidy pattern and patient age, sex, liver cirrhosis, hepatitis B virus antigen and serum α-fetoprotein level. The ploidy pattern had no significant correlation with the presence of vascular invasion or intrahepatic metastasis. Only Edmondson's grade was well correlated with the ploidy pattern. We noted a significant correlation between survival rates and the presence of vascular invasion or intrahepatic metastasis ($p < 0.05$). In contrast, no significant correlation was found between DNA ploidy pattern and the prognosis of HCC. The results of this study indicate that DNA ploidy pattern may not be a useful indicator for the prognosis of HCCs after hepatic resection, unlike the results of gastric and colon cancers.

Key words: **DNA ploidy pattern, hepatocellular carcinoma, hepatic resection, prognosis**

Following recent advances in hepatic surgery techniques, resectability for hepatocellular carcinoma (HCC) has been increasing, and prognosis has been also improving as well.

The prognosis of HCC after resection, however, is still poor when compared to that of gastric or colon cancer, mainly due to the high rate of recurrence observed in the remnant liver (1, 2). A high incidence of recurrence has been also reported in early cases of HCC (3). Vascular invasion or intrahepatic metastasis is said to be the cause of this increased recurrence. The biological behavior of the tumor seems to be an important factor for prognosis. Recently, DNA ploidy pattern was shown to be correlated with prognosis in gastric and colon cancer (4-8). However, with regard to HCC, the relationship between DNA ploidy pattern and prognosis or degree of malignancy has not yet been clearly investigated. To investigate the relationship between DNA ploidy and survival after hepatic resection in patients with HCC, we measured the DNA ploidy of paraffin-embedded HCC tissues by flow cytometry.

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Materials and Methods

Paraffin-embedded specimens from a total of 44 patients, who underwent curative resection for HCC from January 1986 to April 1990, were available for study by flow cytometry. The mean age of patients was 58 (range 41-75) years; there were 37 men and 7 women. Of these, 34 had liver cirrhosis and 21 were seropositive for hepatitis B surface antigen (HBsAg). Clinical data such as age, sex, and α-fetoprotein (AFP) levels were also recorded for comparative purposes.

Flow cytometry. Flow cytometric DNA analysis was done on nuclei isolated from paraffin-embedded sections by a modified

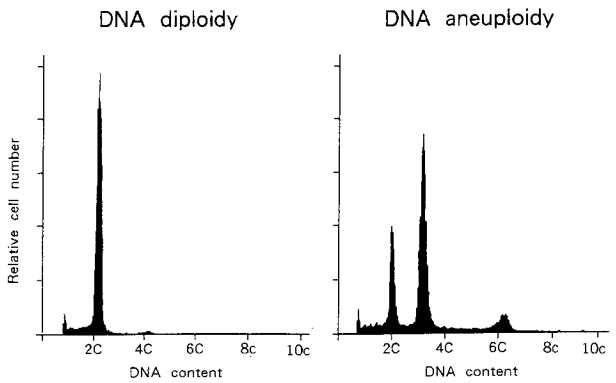

Fig. 1 Examples of DNA histograms from paraffin-embedded tissue: diploid pattern (left) and aneuploid pattern (right).

method of Hedley *et al.* (9). Briefly, 50-μm sections were prepared, deparaffinized in xylene, and rehydrated in a series of graded alcohols. Then, they were digested overnight in 0.25% trypsin solution containing spermine hydrochloride in a shaking water bath at 37°C. The resulting suspension of nuclei was spun at 3,000 rpm for 10 min. and filtered through a 40-μm nylon mesh. The filtered solution was centrifuged at 3000 rpm for 10 min. and the sediment containing the nuclei was stained with 0.05 mg/ml propidium iodide (Calbiochem, San Diego, California, USA) after 0.02 mg/ml RNase A (Sigma, St. Louis, MO, USA) treatment. After refiltration through a 40-μm nylon mesh, the DNA content was measured with a FACS-CAN (Becton-Dickinson, Mountain View, CA, USA).

Histograms of up to 20,000 cells were generated for each sample. If a single G_0G_1 peak was detected, the tumor was considered to be diploid, and if two G_0G_1 peaks could be identified, the tumor was considered to be aneuploid (Fig. 1). The DNA index was calculated by the ratio of channel numbers of the aneuploid and diploid peaks. Tumors with a DNA index between 0.95 and 1.05 were recorded as diploid and all others were recorded as aneuploid. Coefficients of variation (CV) of up to 7% were adopted as evaluable.

Statistical analysis. Statistical comparisons were carried out using the chi square test and Student's t-test where appropriate. Cumulative survival rates were obtained by the Kaplan-Meier method. The generalized Wilcoxon's test was used to determine the significance of the differences between the curves.

Results

Of a total of 44 primary HCCs, 26 (59%) presented a diploid pattern and 18 (41%) had an aneuploid pattern. When the DNA ploidy pattern was compared with preoperative clinical data, no significant correlation was demonstrated between the ploidy pattern and patient age, serum HBsAg, liver cirrhosis, or serum AFP level (Table 1). Pathologic parameters of HCC were also compared with DNA ploidy pattern. Edmondson's grade (10) I through IV accounted for 2, 22, 2, and nil in diploid tumors, and nil, 9, 9, and nil in aneuploid tumors, respectively. The DNA ploidy pattern was closely correlated with Edmondson's histologic classification ($p < 0.05$). The aneuploid pattern was detected more

Table 1 Relationship between DNA ploidy pattern and clinical characteristics of the 44 patients with hepatocellular carcinoma

Patient characteristics	Diploid (n = 26)	Aneuploid (n = 18)
Mean (S.D.) age (years)	56(7)	61(7)
Sex		
Male	23	14
Female	3	4
HBsAg		
Negative	18	14
Positive	8	4
Liver cirrhosis		
Negative	6	4
Positive	20	14
AFP (ng/ml)		
< 20	6	3
20-200	8	7
> 200	12	8

AFP: α fetoprotein; S.D: standard deviation
HBsAg: hapatitis B surface antigen.

Table 2 Relationship between DNA ploidy pattern and histological characteristics

Tumor characteristics	Diploid (n = 26)	Aneuploid (n = 18)
Mean (S.D.) tumor size (cm)	4.2(2.5)	5.5(3.5)
Capsule invasion		
Negative	11	7
Positive	15	11
Venous invasion		
Negative	16	8
Positive	10	10
Intrahepatic metastasis		
Negative	14	9
Positive	12	9
Edmondson's grade*		
I	2	0
II	22	9
III	2	9
IV	0	0

Other variants showed no statistical significance. S.D: standard deviation
*$p < 0.05$

often in larger tumors. There was no correlation between DNA ploidy pattern and capsule invasion, the presence of portal invasion, or intrahepatic metastasis (Table 2).

The DNA ploidy pattern was compared with the prognosis of patients with HCC who underwent curative resection. The 4-year cumulative survival rates of diploid and aneuploid cases were 53 % and 68 %, respectively, and the differences were not statistically significant (Fig. 2). The 4-year cumulative survival rate of the cases without vascular invasion (70 %) was significantly higher than cases with vascular invasion (40 %, $p < 0.05$) (Fig. 3). The 4-year cumulative survival rate of the cases without intrahepatic metastasis (70 %) was significantly higher than cases with intrahepatic metastasis (40 %, $p < 0.05$) (Fig. 4).

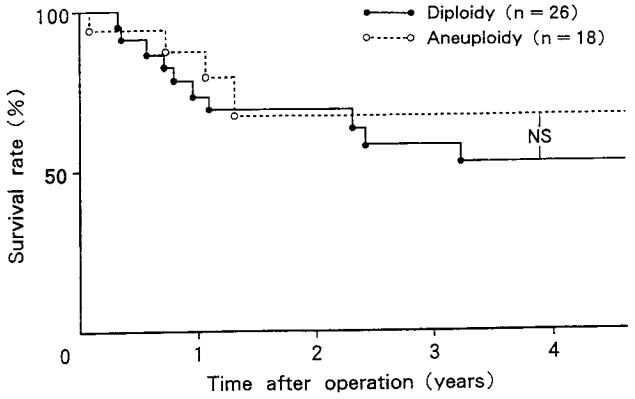

Fig. 2 Survival of patients with diploid and aneuploid DNA pattern: no statistically significant difference in survival rates between the two curves was found. N.S.: not significant

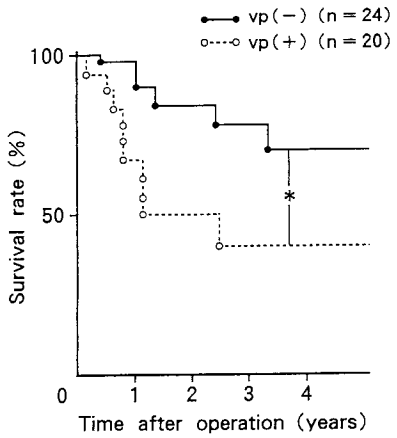

Fig. 3 Survival of patients with and without the histological presence of vascular invasion (vp): a significant difference in survival rates between two curves was found ($p < 0.05$).

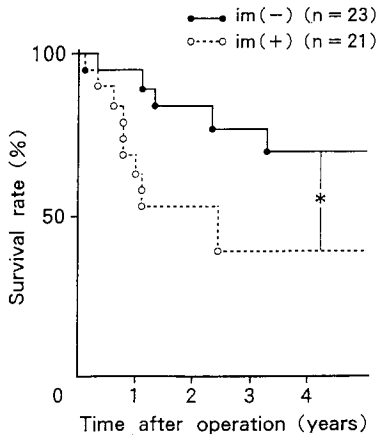

Fig. 4 Survival of patients with and without histological intrahepatic metastasis (im): A significant difference in survival rates between the two curves was found ($p < 0.05$).

Discussion

Hepatocellular carcinoma is one of the most malignant of the solid tumors. The Liver Cancer Study Group in Japan in 1990 reported that the 5-year survival rate for HCC was 28.5 % in resected cases, which is worse than the corresponding rates for gastric or colon cancer (1). One of the main reasons for this poor prognosis is intrahepatic tumor recurrence, which is high even after curative resection of relatively small HCC.

The DNA content in lesions of various organs has proved to be a useful prognostic indicator (11). However, regarding HCC, the clinical significance of DNA ploidy remains controversial (12–17). To clarify this, we retrospectively investigated the effect of DNA ploidy on the clinicopathological features and prognosis of HCC.

A diploid pattern was observed in 59 % of the cases in this series. The distribution rate of diploid, however, varied considerably among previous reports, ranging from 22 % to 58 % (12–17). An aneuploid pattern was detected more frequently in larger tumors in our study, but lacked statistical significance. Ezaki *et al.* (13) and Fujimoto *et al.* (15) reported that the incidence of aneuploidy increased with the size of the tumor. These and our results strongly indicate that DNA ploidy pattern

changes during tumor growth. The main reason for this variation was thought to be due to the difference in tumor size.

Another reason may be that all our results were taken from paraffin-embedded material. The disadvantages of using paraffin-embedded samples are well known; they seem to cause a wider CV that fails to detect near-diploid aneuploid population. Thus, we only used those specimens which demonstrated a CV of less than 7 % to avoid confusing results.

We found no correlation between DNA ploidy pattern and patient age, sex, serum AFP level, and serum HBsAg, or pathologic findings. The only correlation we observed was with Edmondson's grade. These results are similar to those of Nagasue *et al.* (12) and McEntee *et al.* (17).

On the other hand, Fujimoto *et al.* (15) observed a significant correlation between DNA ploidy pattern and tumor size, the presence of vascular invasion, and intrahepatic metastasis. As in the present study, these authors used paraffin-embedded tissues and flow cytometry. So, it is difficult to ascribe the cause of this variation to the materials and methods used. Thus, the variation of these findings may have been caused by differences in patient populations inherent to HCC, in addition to the DNA ploidy patterns.

Since the first report of Ezaki *et al.* (13), several authors reported the relationship of DNA ploidy pattern and prognosis. Fujimoto *et al.* (15) examined 149 patients with HCC (76 diploid and 73 aneuploid), and reported that the 5-year survival rate of diploid cases was 54 %, which was significantly higher than that of the patients with aneuploid tumors (12 %). In contrast, Chen *et al.* (16) reported that the 3-year survival rate was 43 % in 11 patients with diploid tumor and 44 % in 39 patients with aneuploid tumors, which shows no significant difference. We also found no difference in survival rates between patients with diploid and aneuploid tumors. However, we noted a significant correlation between the prognosis and the presence of vascular invasion. Portal invasion of HCC is known to be a cause of intrahepatic recurrence that leads to a dismal prognosis (2, 18), although metachronous multicentric origin has to be considered as a cause of recurrence in some patients.

We conclude that, although the clinical outcome of gastric and colon cancers is well reflected by the DNA ploidy pattern, DNA ploidy pattern in HCCs has no significant prognostic value.

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