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Nuclear DNA Analysis in Hepatocelular Carcinoma in Comparison with Clinicopathologic Factors

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ABSTRACT : Nuclear DNA patterns were analyzed in the 33 patients with primary hepatocellular carcinoma on the basis of clinicopathologic standpoint. It is concluded that biologic behavior of hepatocellular cancer cells in patients with DNA aneuploidy pattern displayed highly malignant potential, indicating severe atypism, rapid growth of rupturing, the presence of cancer invasion into the wall of portal vein and distant metastasis. Furthermore, the survival time in patients with DNA aneuploidy pattern in hepatocellular carcinoma was shorter than that in patients with diploidy one.

INTRODUCTION

According to advance in diagnostic techniques, echosonography is now prevalent in clinical use to detect the tumor mass in the liver. Early excision is recommended to improve its prognosis. However, early detection is not enough for aggressive tumors. The goal of satisfactory results requires combined adjuvant therapy with potent chemotherapy for prevention of recurrent or metastatic tumor growth.

On the other hand, there is nothing to exactly assess and compare with the degree of tumor malignancy and to expect as to whether or not metastasis and/or recurrence appear sooner or later.

The aim of this study is to clarify the validity to assess the malignant potential of carcinoma of the liver by means of measurement of cellular DNA and RNA in comparison with clinicopathologic factors.

MATERIALS AND METHODS

Thirty-three patients with primary hepatocellular carcinomas were eligible for this study in clinicopathologic and nuclear DNA analysis. The ratio of men and women was 28 : 5. According to the disease stages, seven were in Stage I, 12 in Stage II, 11 in Stage III and 3 in Stage IV. For the measurement of nuclear DNA and RNA content, paraffin-embedded materials were prepared. These were sliced at 30μm sections, dewaxed by xylene, rehydrated by using 100%, 95%, 70%, and 50% ethanol and washed in distilled water. Moreover, these were immersed into trypsincitrate buffer and incubated overnight at 37°C. Thereafter, these were sequentially moved to stain in the order of Solution A (trypsin), Solution B (trypsin inhibitor, RNase A), Solution C (speramine tetrahydrochorolide propidium iodide). Finally fluorescence measurement by using FACS IV was made to analyse the nuclear DNA and RNA content. The DNA index was calculated as the

following fomula: Peak channel No of cancer cells/Peak chennel No of normal cells.

RESULTS

The result of nuclear DNA and RNA was divided into the two categories, diploidy (DI 0.9–1.1) and aneuploidy (DI<1.1) respectively. Seventy percent of the patients were shown aneuploidy as shown in **Table 1.** There was no difference in distribution of diploidy and aneuploidy between both sexes. In advanced disease of stage IV, aneuploidy was dominant rather than diploidy despite a few cases of stage IV.

Fig. 1 showed distribution of DNA index. Most distributed in the area from 1.0 to 2.3, although some were in DI of 2.5 to 2.9. Relationship between tumor sizes and DNA patterns was shown in **Table 2.** The more the tumor sizes increase, the higher the incidence of aneuploidy has become step by step. It was more likely that the tumors without capsula-formation indicated aneuploid pattern of nuclear DNA rather than diploid one.

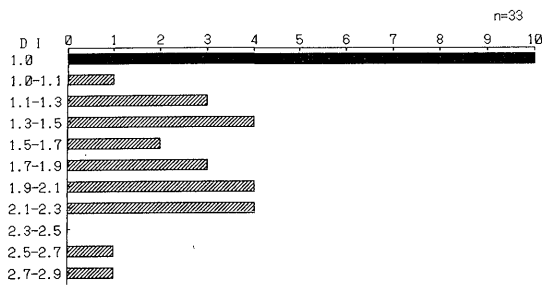

Fig. 1. Distribution of DNA Index

Table 1. Disease stage and DNA pattern in patients with hepatocellular carcinomas

	n	Diploidy (%)	Aneuploidy (%)
Age (mean)	54.7±10.4	54.1±8.3	55.0±11.3
Sex M	28	9 (32)	19 (68)
Sex F	5	1 (20)	4 (80)
Stage I	7	2 (29)	5 (71)
II	12	4 (33)	8 (67)
III	11	4 (40)	7 (60)
IV	3	0 (0)	3 (100)
Total	33	10 (30)	23(70)

Table 2. Tumor sizes and ploidy

Size (cm)		Diploidy (%)	Aneuploidy (%)
~ 2	8	34 (37)	5 (63)
2~ 5	12	4 (33)	8 (67)
5~10	8	2 (25)	6 (75)
10~	5	1 (20)	4 (80)
	33	10 (30)	23 (70)

Table 3. Capsular invasion and ploidy

		Diploiy (%)	Aneuploidy (%)
fc (−)	5	2 (40)	3 (60)
(+)	28	8 (29)	20 (71)
fc-inf (−)	11	5 (45)	6 (55)
(+)	22	5 (23)	17 (77)
	33	10 (30)	23 (70)

Table 4. Growth modes and ploidy

		Diploiy (%)	Aneuploidy (%)
eg	29	8 (28)	21 (72)
ig	4	2 (50)	2 (50)
	33	10 (70)	23 (70)

However, the finding of capsular infiltration of the tumor is more definitively recognized in cases of displaying aneuploid patterns than diploid one as shown in **Table 3.** As for the growth modality of the tumor as shown in **Table 4,** the majority cases showed a mode of *eg,* demonstrating aneuploid pattern of nuclear DNA except for 4 cases with ig of growth modality, although the mode of ig showed aneuploidy pattern in half.

As for cellular atypism according to Edmondson' classification. Severe Edmondson III and IV was more frequently seen in patients with aneuploid pattern of cellular DNA than diploid one as shown in **Table 5.** In view of portal vein invasion, the patients with aneuploid were susceptible to portal vein invasion regardless of the degree of cancer invasion into the wall of the portal vein which was represented as vp1-3 as shown in **Table 6.** From the standpoint of hepatic metastasis, there was no tendency that hepatic metastasis increases in patients with DNA aneuploid pattern rather

Table 5. Cellular atypism and ploidy

			Diploiy (%)	Aneuploidy (%)
Edmondson	I, II	23	8 (32)	15 (68)
	III, IV	10	2 (20)	8 (80)
		33	10 (30)	23 (70)

Table 6. Portal invasion and ploin

		Diploiy (%)	Aneuploidy (%)
vp 0	16	6 (38)	10 (62)
1	8	1 (12)	7 (88)
2	8	3 (37)	5 (63)
3	1	0 (0)	1 (100)
	33	10 (30)	23 (70)

Table 7. Hepatic metastasis and ploidy

		Diploiy (%)	Aneuploidy (%)
im 0	24	8 (33)	16 (67)
1	7	2 (29)	5 (71)
2, 3	2	0 (0)	2 (100)
	33	10 (30)	23 (70)

Table 8. Relationship between the presence of rupture or distant metastases and ploiy

		Diploiy (%)	Aneuploidy (%)
rupture	4	0 (0)	4 (100)
distant metastasis	3	0 (0)	3 (100)

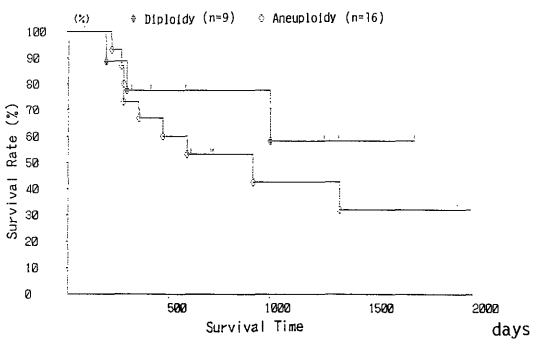

Fig. 2. Comparison of Survival Rate between Diploid and Aneuploid in Curative Cass

than in those with diploid pattern as shown in **Table 7.**

In this series, we encountered 4 patients with rapid growth of rupture and 3 patients with distant metastasis.

Patient had an aneuploid pattern of nuclear DNA as shown in **Table 8,** indicating that aneuploid pattern implied highly clinical malignant potential. The survival time between patients with diploidy and aneuploidy patterns was compared. Fig. 2 showed that survival time in patients with diploid was longer than that in patients with aneuploid in analysis of DNA patterns.

DISCUSSION

It is common that until recently the degrees of malignancy for cancer cells have been evaluated by Edmondoson's histologic classification and/or mitotic index[1)2)]. However it is difficult to assess how malignant cancer cells behave biologically. Few reports can be found regarding assessment of malignancy for hepatic cell carcinoma by analysis of DNA ploidy pattern which closely relates to the grade of malignancy of cancer cells and prognosis. Many reports clarify that DNA ploidy patterns closely relate to biologic malignancy of cancer cells and are suggestive of its prognosis of tumor-bearing host with respect to esophageal cancer[3)], breast cancer[4)5)6)] and colon cancer[7)].

In conclusion, aneuploidy pattern of DNA in carcinoma of hepatocellular carcinoma was in accordance with the severity of cellular atypism and portal vein invasion and also suggestive of biologic behavior of highly malignant potential such as rupturing and distant metastasis. Moreover, the survival time in patients with DNA aneuploidy pattern was apparently shorter than that in patients with DNA ploidy one. The longevity of survival time for patients with hepatocellular carcinoma may be anticipated by analysis of DNA pattern and meticulous postoperative cares should be managed by using potent anticancer drugs, aiming at improvement of surgical outcome.

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