Malignant Potential in the Analysis of DNA Ploidy Pattern in Patients with Colorectal Cancer

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Recently the measurement of cellular DNA content has been focused on knowing the extent of cancer extension and biological behaviour of the tumor cells as well as on producting occurrence of recurrence and the survival time in patients with carcinomas of various organs.

And also it is well known that tumor markers such as CEA, CA19-9 and CA72-2 are of great value in predicting recurrence in the follow-up study. It is common that colorectal cancers show well differentiated carcinoma which demonstrates relatively fair prognosis. However, some revealed aggressive and rapid extension of carcinoma, indicating a poor prognosis.

The purpose of this study is to certify the significance of the measurement of cellular DNA content for assessing biologic behavior of colorectal cancer in comparison with clinicopathologic factors which have been used for assessment of their prognosis.

Development of flow cytometer enabled us to measure the nuclear DNA content with ease, speed and producibility. Furthermore, prevalence of flow cytometric technique makes it possible to know more accurate outcome. When assessed biologically aggressive behavior of tumor cells, potent chemotherapy and extensive surgery are mandatory for improvement of the outcome.

It is necessary to search for the method of the accurate assessment of the outcome for patients with carcinomas.

The purpose of this study is to clarify the validity of nuclear DNA measurement for assessment of the prognosis in patients with colorectal cancer in comparison with clinicopathologic factors.

Material and Methods

During the period from 1980 to 1987, 226 patients with colorectal cancer were operated upon at the First Department of Surgery, Nagasaki University School of Medicine. The 5-year survival time was compared with the result of DNA analysis.

The surgical specimens were histologically examined. A paraffin block containing tumor extending through muscu-laris propria was selected. A 5μm slide was cut and stained with hematoxylin and esosin. Examination of the slide allowed identification of regions where viable tumor tissues were separated. The corresponding area was indented into the paraffin block.

A nuclear suspension was prepared using the technique described by Hedley et al in 1983.

The sample were analysed by using an FACS IV flow cytometer. Their coefficient of variation had to be less than 8%.

A DNA index was calculated by equation of mean channel number of this second population divided by the channel number of the first population, CEA level were measured by using IMX Dinamic Kit. The cut-off value of CEA was regarded as 2.5ng/ml.

Results

The surgical specimens from 226 patients with colorectal cancer were subjected in this study. As a result of DNA analysis, diploid pattern included 83 patients (61 men and 22 women) and aneuploid contained 14 3patients. (Table 2)

Between the two groups in the DNA analysis, there was no significant difference in the histologic findings such as depth of cancer, node metastasis, peritoneal dissemination, hepatic metastasis, histologic cell differentiation and vascular invasion of lymphatic and blood vessels as shown in Table 1 and 3.

As for surgical curability, relative curability is somewhat frequent in aneuploid patients in reflection of advances in cancer infiltration in spite of not statistically significant difference.

With respect to the depth of cancer infiltration, deeply invasive cancer extension and node involvement were more often in patients with aneuploid than diploid pattern. It is indicated that aneuploidy pattern is dominant for the depth of invasion and node metastasis. However, they were not statistically significant. Histologic parameters such as peritoneal dissemination, hepatic metastasis and vascular invasion of lymphatic and blood vessels were almost equivant

in the both groups without a significant difference.

**Table 1.** Peripheral and portal plasma CEA according to Dukes' classification

Dukes	peripheral	portal CEA
A	2.6 ± 1.9	2.7 ± 2.2
B	4.2 ± 4.9	6.0 ± 7.5
C	8.3 ± 12.1	9.9 ± 12.1
D	23.0 ± 30.7	45.2 ± 64.7
	8.0 ± 10.2	13.9 ± 32.3

Table 2. DNA ploidy patterns and clinical features

		S55-62	(226)
		diploid	Aneuploid
		83	143
male		61 (73.5%)	82 (57.3%)
female		22 (26.5%)	61 (42.7%)
mean age		62.2 ± 10.9	60.9 ± 11.2
Curability		69	98
		14	45
invasion	m	2	1
	sm	6	3
	pm	13	6
	ss (a1)	32	58
	s (a2)	18	43
	si (ai)	12	32
n	n0	54	52
	n1	18	41
	n2	9	35
	n3	2	11
	n4		4
P	P0	79	139
	P1	2	3
	P2	1	1
	P3	0	0
H	H0	81	134
	H1	1	6
	H2	1	3
	H3	0	0
well		69	108
moderate		11	31
poor		2	4
mucinous		1	0
ly0		37	32
1		35	65
2		11	38
3		0	8
v0		48	78
1		32	53
2		3	9
3		0	3
Stage			
I		17	4
II		37	48
III		18	41
IV		9	35
V		2	15

Table 3. DNA ploidy patterns and cell differentiation

| differentiation	Diploid	Aneuploid
well	69 (83.1%)	108 (75.5%)
moderate	11 (13.3%)	31 (21.7%)
poor	2 (2.4%)	4 (2.8%)
mucinous	1 (1.2%)	
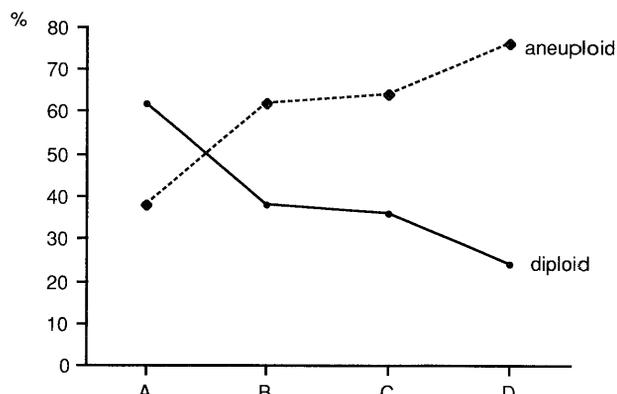
Fig. 1. Relationship between DNA analysis and clinical stages by Dukes classification

According to Dukes classification, a decrease in distribution of diploid patients proportionated to progression of the tumor in reverse proportion to aneuploid one as shown in Fig. 1.

The survival times were compared by DNA index (DI) as shown in Fig. 2.

The prognosis of patients with diploid DNA was very fair. However, the survival times were proportionally shortened with an increase in DI in reflection of a satisfactorily prognostic parameter. In particular, more than 1.5 of DI indicated a poor prognosis.

Among aneuploid patterns of DNA analysis, it is shown that less than 1.5 of DI provides a longer survival time after surgery for patients with colorectal cancer.
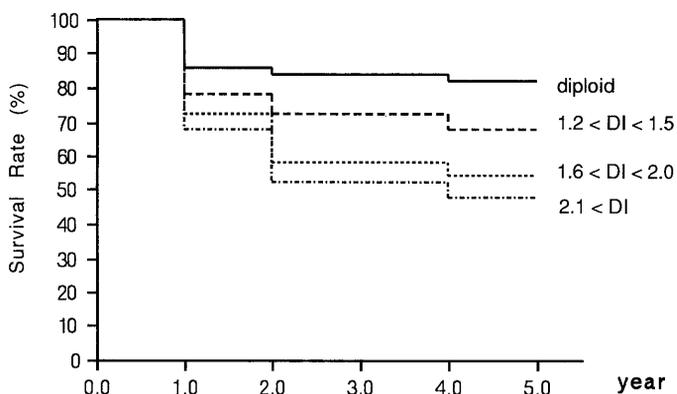
Fig. 2. The survival curve according to DI

On the other hand, the AgNOR counts averaged from 1.6 to 8.2 and correlated well with the DI values as shown in Fig. 2.

The tumor marker levels of CEA were compared with the DI values as shown in Fig. 3. High levels of CEA of more than 2.5ng/ml tend to be kept with an increase in the DI values. It was common that high abnormal CEA levels were seen in over DI values of 1.4.
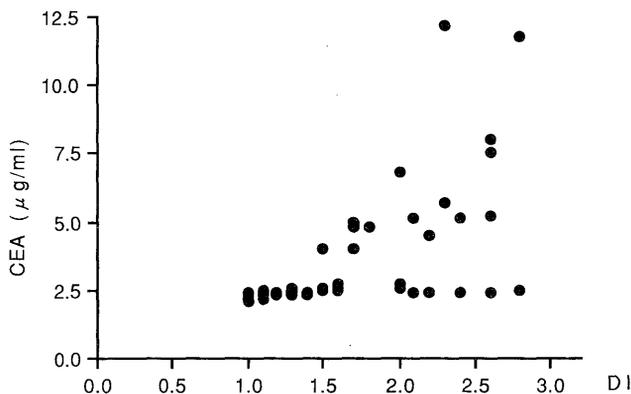

Fig. 3. Correlation between the serum CEA levels and DI

Discussion

Since Hedley[1] reported the measurement method from paraffin-embedded block, an attempt to assess the outcome for patients with carcinoma has been made by nuclear DNA measurement.[2, 3]

The correlation with DNA ploidy pattern and histologic factors were evaluated. As a result, it is defined that the outcome for patients with aneuploid colon cancer is worse than that for diploid one.[1, 11, 12]

It is common that colorectal cancer with aneuploidy is far advanced. There are some reports that lost colon cancers of Dukes C include aneuploidy pattern,[13] in contrast. There is no close correlation with histologic factors. Armitage[4] reported that most well-differentiated carcinomas revealed diploidy pattern and moderately differentiated carcinomas tend to show aneuploidy pattern. It is generally accepted that DNA pattern is independent parameter to assess the outcome of patients with colorectal cancers except for histologic types.[5, 6] In this series, aneuploidy pattern correlated with node metastasis and the depth of tumor invasion as reported by Funai.[7] The higher the histologic cell differentiation, the more aneuploidy is frequent.

The relation between ploidy pattern and CEA in the serum was reported by many investigators in gastric cancers. These are consistent with the fact that the tissue generation of CEA is enhanced in proportion to the degree of aneuploidy pattern.

On the other hand, Rognum[10] clarified that when the CEA scores in patients with colorectal cancer were figured out by factors of the localization of CEA and the serum CEA values, these in aneuploid group were higher than those in near-diploid one. It is suggested that CEA generated by aneuploid cells is prone to liberation to the blood stream and the serum CEA values correlate with ploidy patterns of DNA analysis.

On the contrary, there is a report[8] that the serum CEA levels are not in association with ploidy pattern. The incidence of aneuploidy pattern in colorectal cancers is reported to be 60 to 68 percent. In advanced cancer, DNA ploidy is of great value in assessing the prognosis for the reason of surgically complete excision of the tumor in early cancer.

On the other hand, Wolley[15] pointed out that most of the patients with colon cancer of Dukes C classification revealed non-diploid pattern with a poor prognosis.

The relation between CEA levels and ploid pattern was also evaluated and there is a high tendency of revealing non-diploidy pattern in patients with an abnormal CEA level.

And also it is indicated, as reported by Sugiyama,[9] that aneuploidy pattern well correlates with generation of tumor markers such as CEA, AFP and HCG. It is also investigated as to whether cell cyle is suitable for CEA generation or not. Kuki[14] reported that extra-cellular CEA content was increased at the phase of cell proliferation although intracellular CEA content was reduced at that time. It is recognized that CEA generates at the time of G_0 /G_1 phase to early s stage.

An increase in CEA level is attributed to intracellular CEA content, localization of CEA in cancer cells, vascular invasion and the depth of tumor invasion.

It is a fact that CEA production reflects cell proliferation and biologic behavior of malignant cells. And also CEA is a valuable marker of knowing occurrence of recurrence in follow-up study.

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