Clinical Value of Flow Cytometric DNA analysis in colorectal cancers

Masao TOMITA, Hiroshi ISHIKAWA, Tone WAKAGOE,
Teruhisa SHIMIZU, Hiroyoki KUSANO, Koji TAKAHIRA,
Tatsuo HIRANO, Takatoshi SHIMOYAMA and Toshio MIURA

First Depertment of Surgery
Nagasaki University School of Medicine

ABSTRACT :   The distribution of DNA in colon cancers and its relationship to factors related to clinical features of cancer invasion and noncurative operation are evaluated by using paraffin-embedded specimens.

The survival in patients with DNA diploid tumor is apparently much more satisfactory than that in patients with aneuploid tumor in stage B and C patients in accordance with advances in the disease stage. The deeper the depth of cancer invasion reaches, the more the number of DNA aneuploid tumor increased in the distribution.

Non-curative operation was more frequently made in patients with DNA aneuploid tumor rather than in patients with DNA diploid one.

In conclusion, analysis of the distribution of DNA contributes to assessment of the prognosis of colorectal carcinoma in combination with conventional prognostic parameter of clinicopathologic variables.

INTRODUCTION

Colon cancers are now increasing in number in accordance with improvement of dietary life. In general, the prognosis following surgery for colon cancers is not so unsatisfactory as does gastric cancers reveal. It is reasoned that most of colon cancers are well differentiated carcinoma, which demonstrates a localized growth. Therefore, local control including involvement of regional nodes is mainly made by surgical treatment as far as it may be diagnosed at early stage. Curative operation is essential to obtain satisfactory results. Clinical analysis of major contributing factors to non-curative operation for the treatment of colon cancers is of great value to get surgical outcome much more improved.

The aim of this study is to clarify the correlation between prognosis and/or non-curative operation and nuclear DNA patterns and assess as to whether DNA patterns is adequate for the indicators of its prognosis or not.

MATERIAL AND METHOD

Two hundred sixty-six patients with colon cancers were resected at the First Department of Surgery, Nagasaki University School of Medicine from January 1978 to December 1986. Among of them, 59 (22.2%) underwent non-curative operation. The locations of the tumors were cecum (C), ascending colon (A) and transverse colon (T) in 16, descending colon (D) and sigmoid colon (S) in 16 and rectum(R) in 27 respectively. According to the

location of the tumors, the incidences applying for non-curative operation for colon cancers were 27.6% in CAT, 18.8% in DS and 22.0 in R respectively as shown in Table 1.

Table 1. Patinents with non-curative operation

location of the tumor	resected patients	non-curative operation (%)
C. A. T	58	16 (27.6)
D. S	85	16 (18.8)
R	123	27 (22.0)
合　計	266	59 (22.2)

From the standpoint of clinical findings, the main factor related to non-curative operation based on liver metastasis which contributed to a pessimistic prognosis.

The nuclear DNA content were measured from formalin-fixed and paraffin-embedded tissue blocks according to schutte B' method[1]. After sampling the adquate part selected by microscopic examination, three or four pieces of 50 m sections were dewaxed, and rehyhdrated. The sample was incubated in 0.05% collagenase (type IV sigma) overnight, washed with PSB (−) and filtrated with nylon mesh and then single cell suspension was prepared. Vindel V[2] method was used for PI staining, and DNA peak values were obtained by using FACS-IV.

Nuclear DNA ploidy patterns were divided into diploidy and aneuloidy in accordance with DNA index (DI). Diploid corresponds to DI = 1.0 and Aneuploid includes DI over 1.0.

RESULT

The survival curve was compared between patients indicating nuclear DNA diploidy and aneuploidy patterns. Fig. 1 showed that the prognosis for patients with nuclear DNA diploidy pattern was still better than those with aneuploidy pattern. It was a statistically significant difference ($p < 0.001$).

According to advances in Dukes' classification, the survival curves between ploidy and aneuploidy patterns were clearly different. These were statistical significances in Dukes
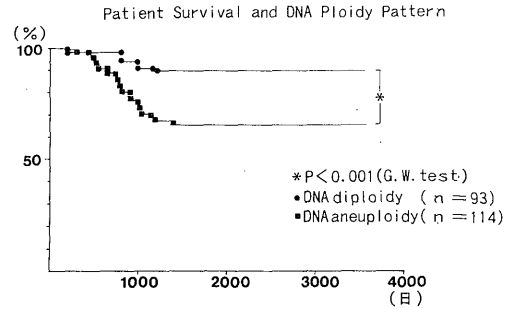

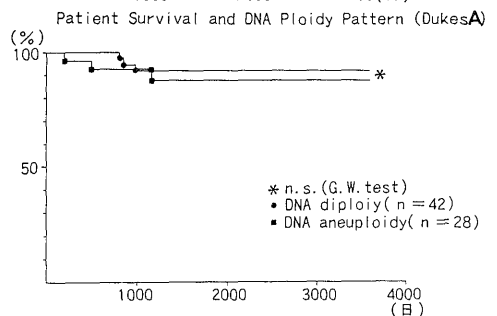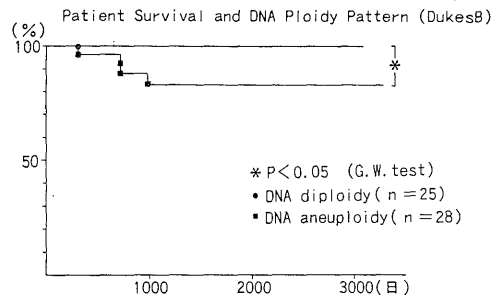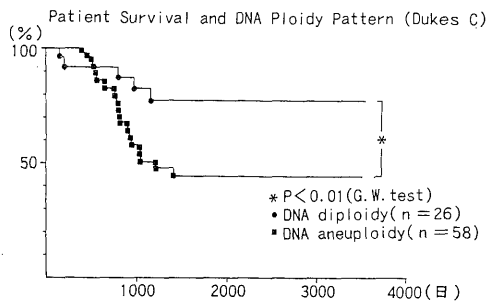Fig. 1. Relationship in survival time between DNA dipoidy and aneupoidy patterns.

Fig. 2. Relationship between survival time and DNA ploidy patterns according to Dukes classification

C($p < 0.01$) and B($p < 0.05$) as shown in Fig 2.

The relationship between the depth of cancer infiltration and DNA patterns was assessed. The deeper the degree of cancer infiltration,

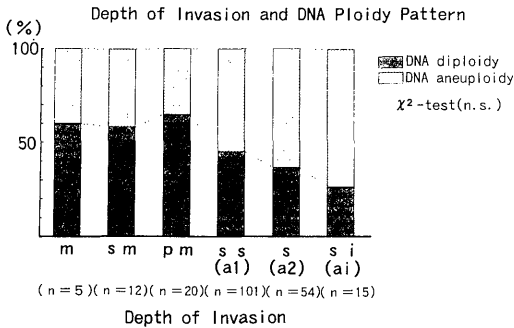the more frequently aneuploidy appeared as shown in Fig. 3.

Fig. 3. Relationship between depth of cancer inf iltration and DNA ploidy pattern

On the other hand, in 59 cases with non-curative operation, 42 (71.9%) revealed an aneuploidy pattern and 17 (28.8%) showed a diploidy pattern while half of the cases with curalive operation displayed an aneuploidy pattern as shown in Table 2.

Table 2. Distribution of DNA ploidy and aneupoidy patterns in patients with non- and curative operations

	diploidy	aneuploidy
curative op.	93 (44.9)	114 (55.1)
non-curat. op.	17 (28.8)	42 (71.2)

In the patients with non-curative operation, the prognosis of the survival rate was much worse than that in the patients with curative operation and also the differences in the survival rate between those who have shown diploidy and aneuploidy patterns were a few in each period as shown in Fig. 4.

DISCUSSION

Assessment of the intensity of malignancy of malignant cells is not so easy because of multiple aneuploid DNA stemlines.

The distribution of DNA in human colon carcinoma and its relationship to clinical behavior was first reported in 1982 by Wolley and his co-workers[3]. Since the development of a method for DNA flow cytometric analysis
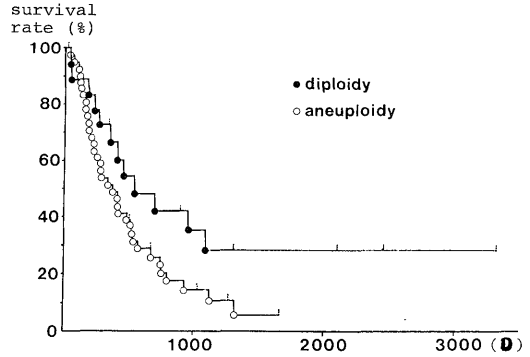

Fig. 4. Comparation in survival rates between patients with DNA dipoidy and aneup-loidy

of paraffin-embedded specimens, a number of reports on the correlation between DNA ploidy of tumor cells and the survival of colorectal cancer patients were made.

The simpler the ploidy in the distribution of DNA, the longer the survival period may be expected[4][5] QUIRKE et al[6] reported a similar result that the 5-year survival of patients with DNA aneuploid tumors was only 35 per cent as compared with 57 per cent of those with diploid tumors. He also noted that DNA ploidy was significantly related to survival in stage A, B and C patients but not in stage D patients.

In this study, the survival in patients with DNA diploid tumor was longer than that in patients with aneuploid tumor and this was a definitive difference in the survival between stage A and stage B, C patients.

In contrast, FINAN and colleagues[7] reported that there was no difference in the survival between 27 patients with aneuploid tumors and 19 patients with diploid tumors. Melamed and associates[8] found a similar result in 15 patients with near--diploid tumors and 18 with aneuloid tumros.

In general, a result of clinicopathological study did not certify significant correlation between DNA ploidy and conventional prognostic variables. In this series, the deper the depth of cancer invasion, the more aneuploid tumor increases. One of the pathologic pattern of depth of cancer invasion as the advanced stage disease correlated with DNA aneuploid tumor

of colorectal cancer cells. It, however, is not so clear that DNA analysis may help us to assess the prognosis in combination with conventional parameters as a suggestion cited by Goh and associates[9].

Needless to say, patients with non-curative operation used to have poor prognosis. An analysis of DNA ploidy in patients with non-curative operation showed a pattern of aneuploid tumor in thirds fourth. It is strongly suggested that aneuploid tumor is one of the contributing factors to non-curative operation.

Further investigation of DNA analysis will make it possible to precisely assess the progrosis by analysis of the fraction of cells in the $S + G_2$ M phase of the cell cycle$>$20-30 per cent of total tumor cells[6].

REFERENCE

1) Schutte, B. Reynders, MM, J. Bosman, FT *et al* : Flow cytometric determination of DNA ploidy level in nuclei isolated from paraffin-embedded tissue cytometry **6** : 26-30, 1985.

2) Lars, L., Vindel V. Christensen J. : A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis cytometry **3** : 323-327, 1983.

3) Wolley RC, Schreiber K, Koss LG, Karas M Sherman A. DNA distribution in human colon carcinomas and its relationship to clinical behavior. *JNCI* **69** : 15-22, 1982.

4) Scott NA, Rain water LM, Wieand HS, Weiland LH, Pemberton JH, Beart RW, Lieber MM. The relative prognostic value of flow cytometric DNA analysis and conventional clinicopathologic criteria in patients with operable rectal carcinoma. *Dis Colon R ectum* **30** : 513-520, 1987.

5) Chang KJ, Enker WE, Melamed M. : Influence of tumor cell DNA ploidy on the natural history of rectal cancer. *Am. J. Surg* **153** : 184-188, 1987.

6) Quirke P. Dixon MF. Claydon AD Durdey P. Dyson JD. William NS Bird C : Prognostic significance of DNA aneuploidy and cell proliferation in rectal adenocarcinomas. *J Pathol.* **151** : 285-291, 1987.

7) Finan PJ, Quirke P, Dixon MF, Dyson JED Giles GR, Bird CC : Is DNA aneuploidy a good prognostic indicator in patients with advanced colorectal canec ? *Br. J. Cancer* **54** : 327-330, 1986.

8) Melamed MR Enker WE Banner P. Janov AJ. Kessler G. Darzynkiewicz Z : Flow cytometry of colorectal carcinoma with three-year follow-up. Dis Colon Rectum. **29** : 184-186, 1986.

9) Goh HS, Jass JR, AtkinWS, Cuzick J. Northover JMA : Value of flow cytometric determination of ploidy as guide to prognosis in operable rectal cancer : A multivariate analysis. *Int. J. Colorect. Dis* **2** : 17-21, 1987.