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Diagnostic Significance of Fluorocytophotometric DNA Ploidy Analysis of Gastric Cancer with Special Reference to Its Comparison with Clinical and Histopathological Diagnosis

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Abstract

Mithramycin binds stoichiometrically with G-C pairs of nucleotides, thus it is useful for nuclear DNA fluorocytophotometry. In the present study, relationships between clinical features and nuclear DNA ploidy values were explored in 80 gastric cancer cases. For the first time, nuclear DNA ploidy values of tumor tissues even in the same gastric cancer were found to be not always identical, when those were determined at two different sites in the same layer or at two different depths of cancer invasion. The highest DNA ploidy values greater than 9C of the original tumors were significantly more frequently observed in advanced cancer than in early cancer. The highest DNA ploidy values greater than 12°C of the original tumors were significantly more frequently observed in gastric cancer cases with lymph node metastases than in those without lymph node metastases. The highest DNA ploidy values of metastatic tumors in lymph nodes were significantly greater than those of the original tumors. The cell lines with very high nuclear DNA ploidy values, however, usually do not establish new stem lines in metastatic lymph nodes. Borrmann 2 gastric cancer which was clinically diagnosed as the localized type often showed much greater nuclear DNA ploidy values and higher incidence of metastases than Borrmann 3. Therefore, it is not true that Borrmann 2 gastric cancer is less malignant than Borrmann 3. When cancer cells invaded onto the peritoneal surface, through the serosal layer, the highest nuclear DNA ploidy values are significantly reduced for unknown reasons. Therefore, it should be kept in mind that the reduction of highest nuclear DNA ploidy values in these invasive types of gastric cancer patients does not necessarily mean a good clinical sign. Nuclear DNA ploidy analysis is the effective means of cell biological diagnosis for gastric cancer.

Introduction

Recent improvements in postoperative adjuvant chemoimmunotherapy[1,19,24] and surgical procedures for advanced gastric cancer[20,24] gave us much expectations for increasing survival rates. However, the five year survival rate of patients with advanced gastric cancer has not yet been suffi-

Key words: Fluorocytophotometry, Mithramycin, Gastric cancer, Lymph node, Stem line.
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ciently improved[1,19,20,24]. The serosal invasion and lymph node metatasis that influence the prognosis of patients who received curative resection of advanced carcinoma of the stomach are considered to be important factors when treatments are evaluated[24,27].

Nuclear deoxyribonucliec acid (DNA) may be quantitatively determined by various cytophotometric techniques using monolayer smear preparations of dispersed tumor cells. Quantitative cytophotometric DNA analysis has been claimed to be useful as a diagnostic means for malignant tumors[3,8,10,28]. Various dyes and chemicals have been reported for nuclear DNA cytophotometry[2,11,13,14,15,16,25,26,31,32]. Mithramycin is proven to bind stoichiometrically with G-C pairs of nucleotides[13,32], thus it is useful for nuclear DNA fluorocytophotometry.

Considering these facts in the present study, nuclear DNA ploidy analysis was performed in 80 cases of gastric cancer patients and its diagnostic values were evaluated in comparing with histopathological diagnosis and Borrmann classification.

Materials and Methods

Clinical materials:

Surgical specimens of gastric neoplasia were obtained from 80 cases of patients, ranging in age from 25 to 83, at the Kyoto National Hospital between March, 1986 and January, 1988. Clinical diagnosis of these cases are as follows: 1 case of atypia, 16 cases of early cancer, and 63 cases of advanced cancer. Advanced cancer cases are classified into 3 cases of Borrmann 1 type, 25 cases of Borrmann 2 type, 17 cases of Borrmann 3 type, 2 cases of Borrmann 4 type, and 16 cases of unclassified 5 type.

Touch smear preparations:

Surgically excised specimens of gastric tissues and lymph nodes were macroscopically examined. Small portions of tumorous lesions and normal-looking mucosa adjacent to these tumorous lesions were excised for histo-pathological examinations.

The remaining surgical specimens were sectioned as shown in Fig. 1 and each of these freshly cut surfaces was gently touched onto a grease-free clean nonfluorescent microscopic glass slide; thus, fresh touch smears were prepared.

Fixation and Staining:

(A) Fresh touch smears were immediately fixed in 100% methanol for 72 hours at room temperature. To prepare the mithramycin solution for staining, mithramycin (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.05 M Tris-HCl buffer, pH 7.8, containing 0.01 M $MgCl_2$ and 0.154 M NaCl, and the concentration was adjusted to be 50 μg/ml of mithramycin.

Touch smears were covered with the mithramycin solution and mounted by cover slips and sealed by Entellan (Merck Co., Damstadt, BRD) at all four sides. Stained touch smears were kept over night at room temperature, and in the following day the cells contained in touch smears were subjected to nuclear DNA fluorocytophotometry.

(B) Hisotopathological diagnosis of each surgical specimen was undertaken after routine paraffin sectioning and hematoxylin-eosin staining by pathologists at the department of pathology in Kyoto National Hospital.

One case which was clinically suspected as atypia was also histo-pathologically diagnosed as atypia.

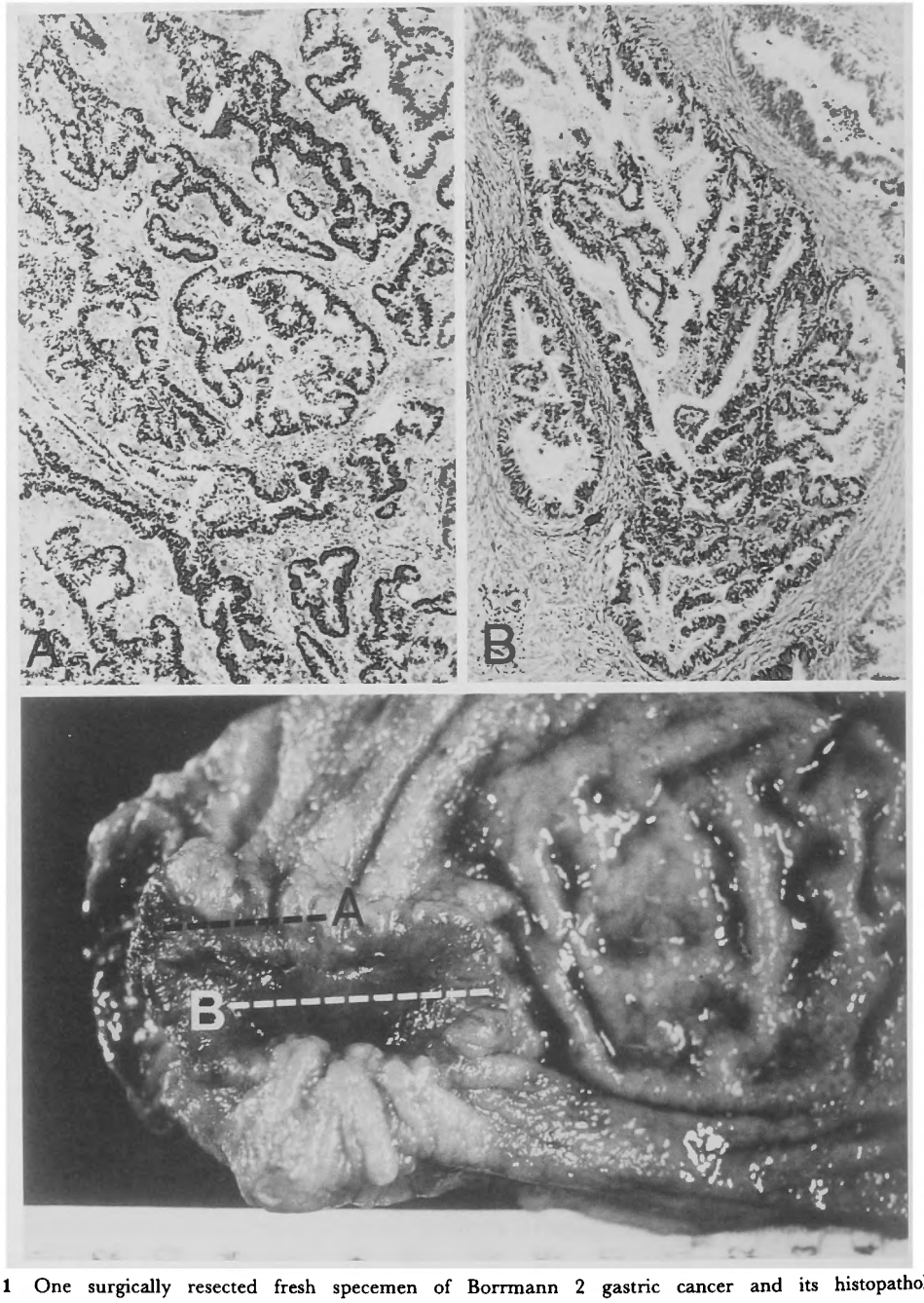

Fig. 1 One surgically resected fresh specemen of Borrmann 2 gastric cancer and its histopathological photomicrographs.
Two photomicrographs (A and B) obtained from cut surfaces of tumor tissues at two separaated locations indicated by A and B sites.

Nuclear DNA fluorocytophotometry:

The nuclear DNA content of each cell was quatitatively measured at 420 nm for excitation and at 630 nm for emission using a fluorocytophotometer (Olympus, BH-MSP-K) interfaced with a YHP 45B desk-top computer that was developed as a prototype of the recent less expensive type[28].

As the control diploid cell, normal lymphocytes located in the same smear were used. Routinely, at least 30 nuclei in control cells and more than 200 nuclei in experimental cells were randomly selected in each smear for nuclear DNA fluorocytophotometric measurements.

The nuclear DNA content of each cell in a smear was expressed by an arbitrary unit of fluorescence intensity. A mean nuclear DNA content of control cells in each smear was designated as its 2C value and was utilized to determine the nuclear DNA ploidy value of experimental cells located in the same smear. In each smear, all the nuclear DNA ploidy values were logarithmically transformed and plotted in a semi-logarithmic histogram based on its 2C value as shown in Fig. 2.

Definition of nuclear DNA content and its ploidy value:

In our laboratory, the term "nuclear DNA content" was defined as follows[8]: "A normal human somatic cell in interphase contains $2 \times 23 = 46$ uncoiled and invisible chromosomes in its nucleus. The DNA content of such a normal euploid set of chromosomes is referred to as euploid or diploid (2C) amount of DNA. Prior to mitosis, the nucleus has to doubled its DNA content. During mitosis, the DNA strands are coiled and microscopically visible as chromosomes. The metaphase plate of a normal human somatic cell thus comprises 46 double-chromosomes which contain altogether a tetraploid (4C) amount of DNA. The karyotype of such a cell, however, is named euploid or diploid or 2n. This must be clearly kept in mind in order to avoid confusion"

In normally dividing human somatic diploid cells, nuclear DNA content begins to increase from the G1 phase (2C) through the S phase to the G2 phase (4C), thus a distinct bimodal histogram is formed with a basic DNA peak at 2C and a concomitant DNA duplication peak at 4C. A cell line showing such a bimodal DNA histogram is defined as a DNA stem line. A DNA ploidy value of a DNA stem line is expressed by the ploidy of nuclear DNA content of the cells belonging to the basic DNA peak, but not by that of the cells in the concomitant duplication peak. Nuclear DNA content values determined from any DNA peaks in a histogram may be referred to as "modal DNA values"

The same principle is applied to tumor cell lines. Thus, the term "DNA stem line of a tumor" is defined as follows: when a histogram of nuclear DNA contents shows a bimodal ploidy pattern, the cell group with its basic ploidy value is the DNA stem line of the tumor, but not that of the concomitant duplication peak. If, for example, there are modal DNA peaks at 2C, 4C, and 8C in a histogram, some cells in the 4C DNA peaks were no longer forming a duplication peak of the diploid stem line but has formed a new basic DNA peak for a tetraploid stem line having an octoploid duplication peak. When atypical mitosis occurs, nuclear DNA amount of a duplication peak is not exactly double of that of the basic DNA value. When such a duplication peak establishes a new stem line, it becomes an aneuploid stem line.

A nuclear DNA content of 1C, a half of that of a diploid cell, is called as a haploid amount. Any nuclear DNA contents equal to those resulting from multiplication of a haploid amount by any integral numbers, such as 2C, 3C, 4C, 5C, 6C and so forth are designated as a polyploid amount. Any nuclear DNA amounts higher or lower than either a haploid or a polyploid amount is called as an aneuploid amount of nuclear DNA.

Plasama carcinoembryonic antigen assay:

Plasma carcinoembryonic antigen (CEA) assay were performed in all cases by enzyme im-

munoassay using the Roche Kit. All blood samples were taken before surgical operations. A plasma CEA level at 5.0 ng/ml was considered as the upper limit of a normal value[30].

Gastric cancer classification:

As clinical macroscopic criteria, Borrmann classification was used[21]. The definitions and categories defined by the Japanese Research Society for Gastric Cancer and reported in the 11th Edition of the General Rules for the Gastric Cancer (March, 1985) were also used in the present study as follows: various structural depths of cancer invasion in the stomach wall were expressed using the following abbreviations, m: mucosal layer; sm: submucosal layer; pm: proper muscle layer; and ss: subserosal layer.

Subdivisions of ss: ssα (the sublayer close to the pm layer), ssβ (intermediate between ssα and ssγ) and ssγ (close to the serosal layer).

The ps (prognostic serosal invasion) classification was added as follows:

ps (−): m, sm, pm, ssα and ssβ ps (+): ssγ, se, si and sei

A term, se, stands for cancer cells invaded onto the serosal surface and exposed to the peritoneal cavity, a term, si, for cancer cells infiltrating into neighboring tissues, and a term, sei, for cancer cells present in both se and si.

Statistical analysis:

Paired t-test, Fisher's exact test and chi square test with Yates correction were used for statistical analysis.

Results

(1) Classification of nuclear DNA modal patterns (Fig. 2):

Nuclear DNA modal pattens of normal-looking gastric mucosa adjacent to cancerous lesions in surgical specimens of gastric cancer patients were unimodal diploid or bimodal diploid patterns with a few cells forming 4C duplication peak.

By analyzing nuclear DNA modal patterns in 130 semi-logarithmic histo-grams prepared from surgical specimens of 80 cases of gastric cancer patients, the following three characteristie stem line groups were recognized.

Type I: diploid (2C) and tetraploid (4C) type,

Type II: triploid type (3C, 6C) and aneuploid type,

Type III: mixed types (Type I+Type II).

(2) Correlations between nuclear DNA modal patterns and Borrmann classification (Table 1):

In general, as clinical features were changed from Borrmann 1 to 4, the frequency of occurrence of types of nuclear DNA modal patterns were shifted from Type I to Type III. However, there were no distinct differences between Borrmann 1 and 2.

One case diagnosed histopathologically as atypia showed Type III of nuclear DNA modal pattern consisting of multiple stem lines at 2C, 2.5C, 3C and 3.7C ploidy values. These data clearly indicate that this case was malignant neoplasia but not atypia.

(3) Correlations between nuclear DNA modal patterns and histopathological classification (Table 2):

In papillary type and moderately and poorly differenciated types of gastric cancer cases. Type II and Type III of nuclear DNA modal patterns were more often observed than Type I. However, in well differenciated adenocarcinoma, no cases of Type I were present.

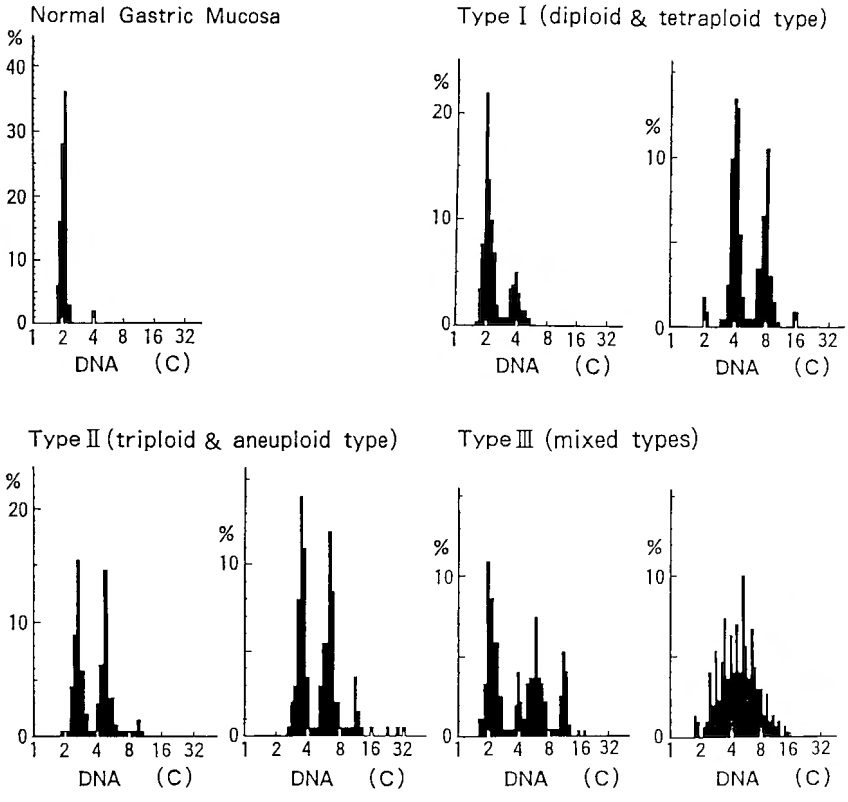

Fig. 2 Classifications of nuclear DNA modal patterns into the following four groups: normal gastric mucosa and Type I, Type II and Type III of gastric cancer.

Table 1. Correlations between nuclear DNA mordal patterns (Type I, II and III) and Borrmann classification.

	Nuclear DNA modal patterns		
	Type I	Type II	Type III
Atypia			1
Early	2	8	6
Borrmann 1	1		2
Borrmann 2	4	15	6
Borrmann 3	1	10	6
Borrmann 4		1	1
Unclassified 5	3	3	10
	11	37	32

Type I: diploid and tetraploid type, Type II: toriploid type and aneuploid type, Type III: mixed types (Type I+Type II)

Table 2. Correlations between nuclear DNA modal patterns (Type I, II and III) and the histopathological diagnosis.

Histopathological diagnosis	Nuclear DNA modal patterns		
	Type Ⅰ	Type Ⅱ	Type Ⅲ
papillary	2	5	6
well differentiated		10	6
moderately differentiated	2	4	4
poorly differentiated	6	15	15
signet-ring	1	1	
mucinous		1	
adenoacanthoma		1	
	11	37	31

Type I: diploid and tetraploid type, Type II: toriploid type and aneuploid type, Type III: mixed types (Type I+Type II)

(4) The occurrence of metastases and elevated plasma CEA in advanced gastric cancer patients (Table 3):

In 63 cases of advanced gastric cancer patients, metastases occurred in 49 cases at lymph nodes, in 8 cases at the liver and in 5 cases at the peritoneum. Metastases, particularly hepatic and peritoneal metastases, occurred predominantly in cases of Borrman 2 and Borrmann 3 types. Elevated plasma CEA levels beyond 5.0 ng/ml were found in 18 cases; among them, 12 cases were in Borrmann 2 type and 5 cases were in unclassified 5 type, but only one case was in Borrmann 3 type. Borrmann 2 cases had more cases of elevated plasma CEA than Borrmann 3 cases.

(5) Correlations between the occurrence of metastases and nuclear DNA modal patterns (Lower parts in Table 3):

The metastases of advanced gastric cancer occurred predominantly in cases whose nuclear DNA modal patterns showed Type II and Type III. The incidence of metastases was very similar to each other between Type II and Type III. In Type I cases, no hepatic metastasis took place.

(6) Comparisons of nuclear DNA ploidy values of stem lines determined at two sites within the mucosal layer of each of 11 cases of advanced gastric cancer (Table 4):

In 9 of 11 cases of advanced gastric cancer, nuclear DNA ploidy values of stem lines determined

Table 3. Correlations between the incidence of various metastases and elevated plasma CEA levels, and Borrmann classification or nuclear DNA modal patterns (Type I, II and III).

	n (+)	h (+)	p (+)	CEA (+)
Borrmann 1	3			
Borrmann 2	21	5	1	12
Borrmann 3	12	3	3	1
Borrmann 4	2		1	
Unclassified 5	11			5
Type I	7		1	1
Type II	21	5	2	9
Type III	21	3	2	8

Type I: diploid and tetraploid type, Type II: toriploid type and aneuploid type, Type III: mixed types (Type I+Type II)

Table 4. Comparisons of nuclear DNA ploidy values of stem lines of tumor tissues determined at two sites within the mucosal layer of each of 11 cases of advanced gastric cancer.

Clinical classification	Nuclear DNA modal patterns		
	Type I	Type II	Type III
Borrmann 2		Ⓐ (5c) Ⓑ (5c)	
Borrmann 2		❶ (2.5c, near 3c, 5c, near 6c)	❷ (2.5c, 4c, 5c)
Borrmann 2		Ⓐ (near 3c) Ⓑ (near 3c)	
Borrmann 2		Ⓐ (near 3c) Ⓑ (near 3c)	
Borrmann 2		❶ (near 3c)	❷ (2c, 2.5c, 3c)
Borrmann 3			Ⓐ (2.5c, 4c, 5c) Ⓑ (2.5c, 4c, 5c)
Borrmann 3			Ⓐ (3c, 4c) Ⓑ (3c, 4c)
Borrmann 3		Ⓐ (2.6c) Ⓑ (2.6c)	
Unclassified 5			Ⓐ (near 3c, 4c) Ⓑ (near 3c, 4c)
Unclassified 5	Ⓐ (2c) Ⓑ (2c)		
Unclassified 5	Ⓐ (2c) Ⓑ (2c)		

( ) indicates nuclear DNA ploidy values of the stem lines

Ⓐ, Ⓑ indicates two sites within the mucosal layer showing identical stem line(s).

❶, ❷ indicates two sites within the mucosal layer showing different stem lines.

Table 5. Comparisons of nuclear DNA ploidy values of stem lines of tumor tissues determined at two sites located in the mucosal and serosal sides of each of 9 cases of advanced gastric cancer.

	Type I	Type II	Type III
Borrmann 2		■ (near 3c) ▲ (2.7c, 3.5c)	
Borrmann 2		■ (3.6c, 7.2c) ▲ (near 3c, near 6c)	
Borrmann 2		□ (near 3c) △ (near 3c)	
Borrmann 3	□ (2c, 4c) △ (2c, 4c)		
Borrmann 4			□ (2c, near 3c) △ (2c, near 3c)
Unclassified 5			□ (2c, near 3c) △ (2c, near 3c)
Unclassified 5			□ (near 3c, 4c) △ (near 3c, 4c)
Unclassified 5		■ (2.5c) ▲ (3.7c)	
Unclassified 5	■ (2c) ▲ (2c, 4c)		

mucosal side □ serosal side △

( ) indicates nuclear DNA ploidy values of the stem lines

■ ▲ indicates two sites showing different stem lines.

at two sites within the same mucosal layer were identical. In the remaining two cases that were both diagnosed clinically as Borrmann 2 type and histopathologically as papillary adenocarcinoma, nuclear DNA ploidy values of stem lines of gastric cancer lesions determined at two different sites. A and B sites, were not same. In one case (Fig. 1), nuclear DNA ploidy values were 2.5C, near 3C, 5C and near 6C at A site and 2.5C, 4C and 5C at B site. In the other case, nuclear DNA ploidy value at A site was near-3C and those at B site were 2C, 2.5C and 3C.

(7) Comparisons of nuclear DNA ploidy values of stem lines determined at two sites located in the mucosal and serosal sides of each of 9 cases of advanced gastric cancer (Table 5):

Nuclear DNA ploidy values of cancer lesions determined at two sites located in the mucosal side and the serosal side of a same gastric cancer were compared in 9 cases. In four cases (two cases of Borrmann 2 type and two cases of unclassified 5 type), nuclear DNA ploidy values of stem lines determined at two sites in each case were found to be different.

(8) Correlations between highest nuclear DNA ploidy values and structural depths of cancer invasion (m•nsm, pm, ssα•nssβ and ps (+) groups) of original tumors (Fig. 3):

In some cases, unusually high highest DNA ploidy values in each case were observed in the levels greater than 9C. Among 80 cases, the highest DNA ploidy values greater than 9C were found in 3 of 17 cases (17.6%) of early cancer and in 40 of 63 cases (63.5%) of advanced cancer as shown in a small inset of Fig. 3 (All cases); the difference in occurrence between early cancer group and advanced cancer group being statistically significant ($p<0.01$).

The number of cases whose highest DNA ploidy values were greater than 9C increased as the structural depth of cancerous invasion became deeper from m•sm to ssα•ssβ through pm in the ps (−) category, but unexpectedly decreased significantly ($p<0.01$) when the category was changed from ssα•ssβ to ps (+) which is clinically supposed to be more malignant group than ssα•ssβ group.

(9) Comparisons of nuclear DNA ploidy values in stem lines determined at the original tumors and the metastatic lymph nodes (Table 6):

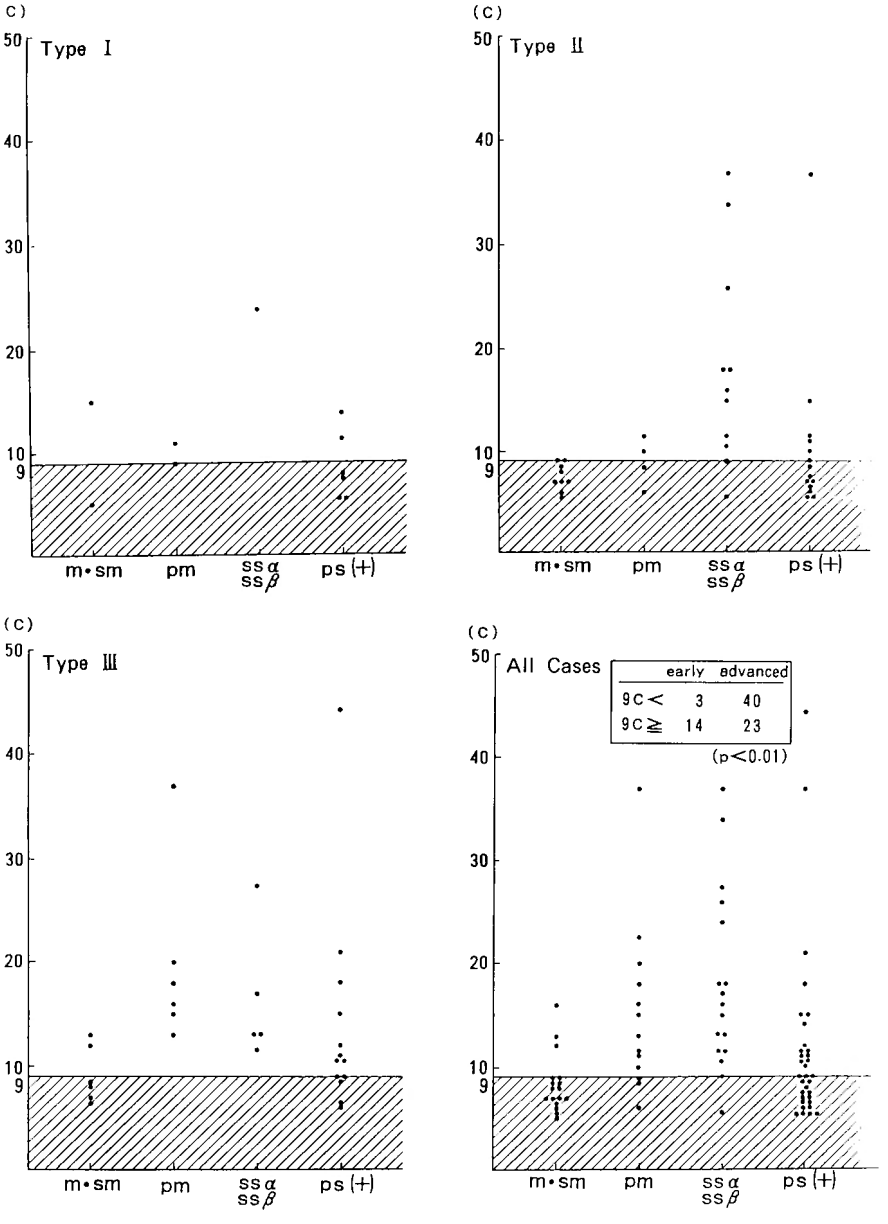

Fig. 3 Comparisons of the highest DNA ploidy values of the original tumors among groups of m•sm, pm, ssα•ssβ and ps (+) in relation to their nuclear DNA modal patterns in Type I, II, III and All cases.
Shaded area indicates the highest DNA ploidy values below 9C, within which differences in the highest DNA ploidy values among m•sm, pm, ssα•ssβ and ps (+) were nonsignificant ($p>0.05$).
Statistical difference ($p<0.01$) by Fisher's exact test (early vs. advanced in All cases).
Statistical difference ($p<0.01$) by Fisher's exact test (ssα•ssβ vs. ps (+) in All cases).

Table 6. Comparisons of nuclear DNA ploidy values of stem lines determined at original tumors and metastasized lymph nodes (n1, n2 and n4).

Clinical classification	Original tumors	Lymph node metastases			
		n1	n2	n3	n4
1. Borrmann 1	[2C], 2.5C	2.5C			
2. Borrmann 2	near3C	near3C near3C near3C	near3C		near3C
3. Borrmann 2	2.5C 3.5C, 4C	2.5C, 3.5C, 4C 2.5C, 3.5C, 4C 2.5C, 3.5C	3.5C		
4. Borrmann 2	[2.5C], 3C	3C, [5C]			
5. Borrmann 2	2C, [3C], 4C, [5C]	2C, 4C 2C, 4C			
6. Borrmann 2	near3C, 4C	near3C, 4C, near[6C] [2C], near3C, 4C			
7. Borrmann 2	7.5C	7.5C			
8. Borrmann 2	3.5C	3.5C			
9. Borrmann 2	near3.5C, near7C	near3.5C, near7C			
10. Borrmann 3	3.5C	3.5C			
11. Borrmann 3	2.5C	2.5C			
12. Borrmann 3	near3C, near6C	near3C, near6C near3C, near6C			
13. Borrmann 3	2C, 4C	2C, [2.5C], 4C	[2.5C], 4C		
14. Borrmann 4	2.5C	2.5C			
15. Unclassified 5	2C	[2.5C]	(2C), [3C], [4C]		
16. Unclassified 5	2C	2C			
17. Unclassified 5	near3C, [4C]	near3C			
18. Unclassified 5	2.5C(mucosa) 3.7C(serosa)	2.5C, 3.7C			

Numbers in [ ] indicate stem lines which are different from those determined at the original tumors.

Nuclear DNA ploidy values in stem lines of the original tumors and the metastatic lymph nodes (n1, n2 and n4) were compared in 18 cases and various differences were observed between stem lines of the original tumors and those of n1 or n2 of metastatic lymph nodes in 7 cases (Cases 1, 4, 5, 6, 13, 15 and 17). In Cases 2, 3, 7, 8, 9, 10, 11, 12, 14, and 16, the nuclear DNA ploidy values of stem lines were identical in the original tumor and metastatic n1 lymph nodes. Particularly in Case 2, nuclear DNA ploidy values were all same 'near 3C' in the original tumor and the metastatic n1, n2 and n4 lymph nodes. In Case 1 and 17, 2C or 4C of the original tumors were lost in the metastatic

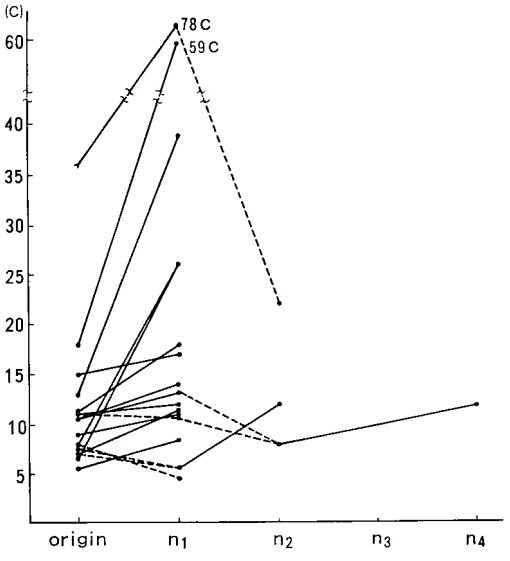

Fig. 4 Comparisons of the highest DNA ploidy values between the original tumors and metastasized lymph nodes (n1, n2 and n4).
Statistical difference ($p<0.05$) by paired t test (origin vs. nl).

Table 7. Correlations between nuclear DNA modal patterns of the original tumors and Borrmann classification with or without lymph node metastases.

	Type I	Type II	Type III
Borrmann 1			
n(−)	0		0
n(+)	1		2
Borrmann 2			
n(−)	1	3	0
n(+)	3	12	6
Borrmann 3			
n(−)	0	3	2
n(+)	1	7	4
Borrmann 4			
n(−)		0	0
n(+)		1	1
Unclassified 5			
n(−)	1	2	2
n(+)	2	1	8

n1 lymph nodes. In Case 4, 2.5C of the original tumor became a new stem line at 5C after metastasis to the n1 lymph node. In Case 5, both 3C and 5C stem lines of the original tumor were lost after metastasis to the n1 lymph node. In Case 6, two stem lines at 2C and near-6C were added in the metastatic n1 lymph node. In Case 13, a new aneuploid (2.5C) stem line was added in the metastatic n1 lymph node and maintained further in the metastatic n2 lymph node. The 2C stem

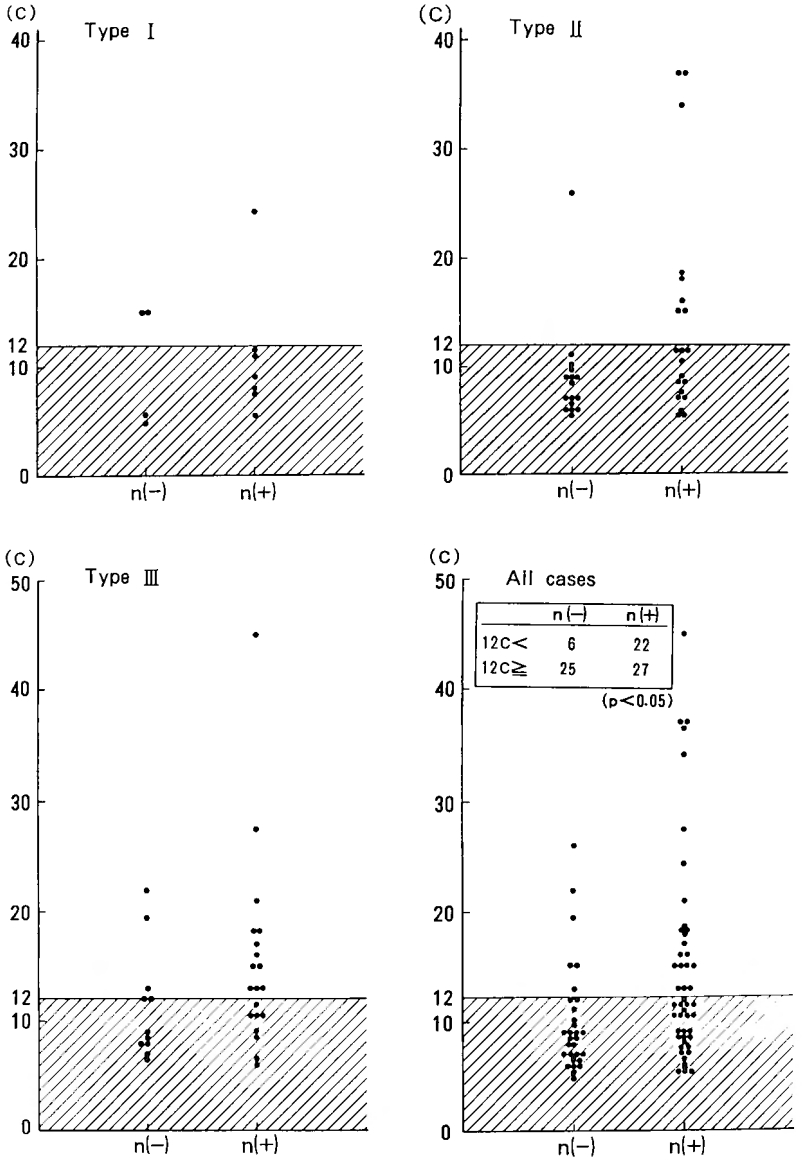

Fig. 5 Comparisons of the highest DNA ploidy values of the original tumors between cases with and without lymph node metastases in relation to nuclear DNA modal patterns (Type I, II, III and All cases).
Shaded area indicates the highest DNA ploidy values below 12C within which differences in the highest DNA ploidy values between n (−) and n (+) were nonsignificant ($p>0.05$).
Statistical difference ($p<0.05$) by Fisher's exact test in Type II.
Statistical difference ($p<0.05$) by chi square test in All cases.

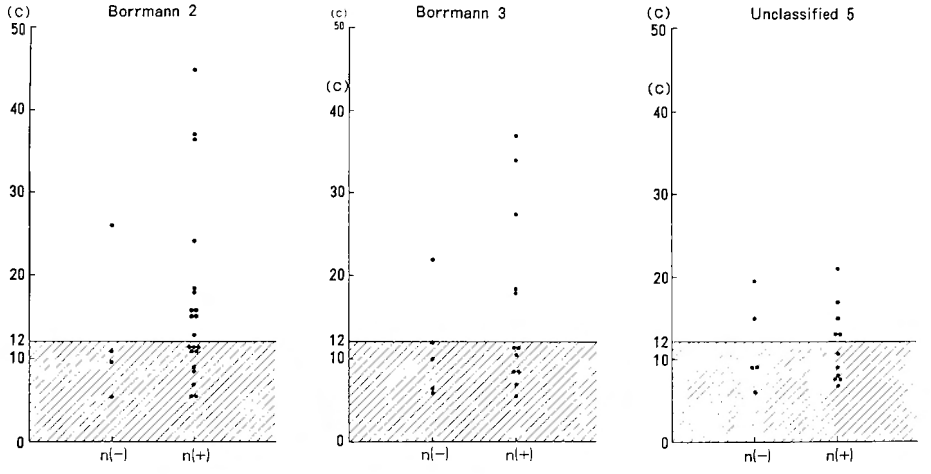

Fig. 6 Comparisons of the highest DNA ploidy values of the original tumors among Borrmann classifications with or without lymph node metastases.

line of the original tumor metastasized into the n1 lymph node, but was lost after it again metastasized into the n2 lymph node. In Case 15, the ploidy values of the stem lines of the original tumor and the metastatic n1 and n2 lymph nodes were (2C), (2.5C) and (2C, 3C, 4C) respectively. In Case 17, the 4C stem line was lost from the metastatic n1 lymph node. In Case 18, the nucler DNA ploidy values of the original tumor examined at two different sites (mucosal and srosal sides) were 2.5C and 3.7C respectively and the cells from both stem lines metastasized to the n1 lymph node.

(10) Comparisons of the highest nuclear DNA ploidy values determined at the original tumors and the metastatic n1 or n2 lymph nodes (Fig. 4):

The highest nuclear DNA ploidy values, instead of the highest stem lines, determined at the orginal tumors and the metastatic n1 or n2 lymph nodes were compared in 18 cases, and in 14 cases the highest nuclear DNA ploidy values significantly ($p<0.05$) increased after metastasizing into the n1 lymph nodes; in particular their increases in 5 cases being remarkably sharp.

(11) Correlations between nuclear DNA ploidy patterns of the original tumors and Borrmann classification with or without lymph node metastases (Table 7):

All of six cases of Type III of nuclear DNA ploidy patterns in Borrmann 2 cases metastasized into the lymph nodes. In 8 of 10 cases of Type III in unclassified 5 cases, metastases occured in the lymph nodes.

(12) Comparisons of the highest nuclear DNA ploidy values of the original tumors between the original tumors with and without lymph node metastases (Fig. 5):

In all cases, lymph node metastases occured significantly more often when the highest DNA ploidy values of the original tumors were greater than 12C ($p<0.05$) (See inset in Fig. 5, All cases).

In 37 cases of Type II, also lymph node metastases occured significantly more often when the highest DNA ploidy values were greater than 12C ($p<0.05$) (Fig. 5, Type II).

(13) Comparisons of the highest nuclear DNA ploidy values of the original tumors between the original tumors with and without lymph node metastases in each groups of Borrmann classification (Fig. 6):

In Borrmann 2 and Borrmann 3 cases, lymph node metastases occured frequently when the

highest DNA ploidy values were greater than 12C.

Discussion

Mithramycin is a fluorescent antibiotic that binds with G-C pairs in double stranded DNA by non-intercalation at the presence of a divalent cation such as magnesium ion[9,11,12,13,22,32]. In our laboratory, the most reliable method utilizing mithramycin for nuclear DNA fluorocyto-photometry has been explored[17] and was used in the present experiments for determination of nuclear DNA contents of gastric cancer cells.

In the present study, one case that was histopathologically diagnosed as atypia was revealed to be actually cancerous, because the nuclear DNA ploidy values of stem lines of this tumor were found to be 2C, 2.5C, 3C and 3.7C. This discrepancy in diagnosis warns us that histopathological diagnosis alone is not sufficient for border-line cases of tumors such as atypia.

For the first time, it was found in the present study that nucclear DNA ploidy values determined at the original tumors may be different from those determined at the metastatic n1 to n4 lymph nodes. Also the nuclear DNA ploidy values determined at two different sites of the same tumor may be different. These results must be taken into serious consideration in estimating prognosis of gastric cancer patients. In the previously reported paper[18], a list of nuclear DNA ploidy values of the metastatic lymph nodes was tabulated, however unfortunately, no detailes were provided. Therefore, 1) whether the ploidy values listed in their table were those of the highest peaks observed in histograms or those of the stem lines in tumors, 2) whether lymph nodes with metastatic tumors belonged to the n1 group or not, and 3) whether or not their fairly complicated and lengthy in vitro procedures before preparing monolayer smears have damaged fragile cancerous cells, are unknown. Without experimental evidences to answer these questions, meaningful comparisons between our present data and their data are not possible.

It was also found in the present study that nuclear DNA ploidy values of the stem lines of metastatic tumors in the n1 lymph nodes are not necessarily same as or greater than those of the original tumors. However, the highest nuclear DNA ploidy values found in histograms of metastatic tumors in lymph nodes were significantly greater than those of the original tumors. These cell lines with very high nuclear DNA ploidy values usually do not establish new stem lines in the metastatic lymph nodes.

In Case 15 (Table 7), the nuclear DNA ploidy values of stem lines were 2C in the original tumor, 2.5C in the n1 lymph node, and 2C, 3C and 4C in the n2 lymph node. There are two possibilities in interpreting how the 2C line was not found in the n1 lymph node, but found both in the original tumor and the n2 lymph node: one possibility may be that tumorous cells in the original tumor may metastasize to the n2 lymph node directly without metastasizing from the n1 lymph node; and another possibility may be that the 2C stem line was lost before metastasizing to the n1 lymph node. A new 2.5C stem line was established in the metastatic n1 lymph node, but this stem line was lost after metastasis to the n2 lymph node. In the metastatic n2 lymph node, three stem lines at 2C, 3C and 4C were newly established.

The nuclear DNA ploidy values in the original tumors were often polyploid, however, those found in the n1 lymph nodes frequently became aneuploid. It is a common belief that prognosis of the patients carrying advanced cancer with metastases are poor[24] and also that aneuploid types of malignant tumors are more malignant than polyploid types[28].

The highest DNA ploidy values of invasive tumors were found to have tendency to become lower after the tumor invaded through the subserosal layer onto the surface of the serosal layer of the stomach and was exposed to the peritoneal cavity. Clinically, it has been well known that the ps (+) cases are more malignant than the ps (−) cases[21]. However, as shown in Fig. 3, the number of ps (+) cases showing greater than 9C of the highest DNA ploidy values became significantly less than that of the ssα•ssβ cases ($p<0.01$). The reasons for this phenomenon are presently unknown. When cancer invasion advanced to the peritoneal surface, outside of the serosal layer, the highest nuclear DNA ploidy values are significantly reduced for unknown reasons. Therefore, it should be kept in mind that the reduction of the highest nuclear DNA ploidy values in these invasive types of gastric cancer patients does not necessarily mean good clinical signs.

The nuclear DNA modal patterns in malignant tumors were found to reflect well the grade of malignancy of tumors and the prognosis of cancer patients[3~8,10]. The analysis of modal DNA values of human lung cancer revealed that lung tumors having the modal DNA values of 4C or higher were more malignant than polyploid tumors with those less than 4C[28]. The incidence of lymphatic invasion, lymph node metastases and advanced cases has been reported to be significantly higher in aneuploid gastric tumors than those in diploid tumors[33]. The present data coincide well with the previous report.

Lung cancer patients whose tumors have already established near-triploid (3C) or near-hexaploid (6C) stem lines showed higher preoperative plasma CEA levels than did patients having tumors with diploid (2C) or tetraploid (4C) stem lines or octoploid (8C) tumor cells[29]. In the present study on the gastric cancer, similar tendency was observed and gastric tumors showing Type II and Type III of nuclear DNA modal patterns were more malignant than Type I.

Because Borrmann 2 gastric tumors are defined macroscopically as the localized type and Borrmann 3 tumors as the infiltrative type[23], Borrmann 3 tumors must be considered more malignant than Borrmann 2 tumors. However, in the present study, Borrmann 2 were found frequently to be more malignant than Borrmann 3, because the higher incidence of the hepatic metastases and elevated plasma CEA levels were found in Borrmann 2 than Borrmann 3 cases. Almost all cases with the hepatic metastases or elevated plasma CEA levels belonged to either Type II or Type III of the nuclear DNA modal patterns. Further, all the cases of Type III in Borrmann 2 cases had lymph node metastases. Almost all cases of Borrmann 2 type whose highest DNA ploidy values were greater than 12C had lymph node metastases. Therefore, the differences between Borrmann 2 and Borrmann 3 types do not reflect meaningful cell biological characteristics of gastric cancer, thus much diagnostic values were not recognized in Borrmann clssifications 2 and 3.

The present study strongly suggests that the nuclear DNA ploidy analysis of the gastric cancer is the effective means of cell biological diagnosis of gastric cancer patients.

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和文抄録

核 DNA 蛍光顕微測光による DNA ploidy 解析の胃癌における診断的意義
——臨床所見及び病理組織学的診断との比較を含めて——

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ミスラマイシンは核酸塩の G-C 塩基に化学的に結合する抗生物質であるため，核 DNA 蛍光顕微測光に有用である．本研究では，臨床像と核 DNA の ploidy 値との関係が胃癌80症例を対象として検討された．同一胃癌病巣内における異なった2点の核 DNA ploidy 値が必ずしも一致しないことが判り，この事実は進行胃癌患者の予後を評価する際の重要な問題となった．胃癌原発巣の核 DNA ploidy 最高値が 9C 以上ならは，その胃癌病巣は有意に進行癌であり，また同様に 12C 以上ならば有意にリンパ節転移陽性症例であった．またリンパ節転移巣の核 DNA ploidy 最高値は有意に原発巣の最高値より高値であった．ボールマン2型胃癌は限局性であり，ボールマン3型胃癌は浸潤型であるため，ボールマン3型の方が悪性度が高いと見なされがちであるが，ボールマン2型胃癌にも予後の悪い症例は少なくない．核 DNA の ploidy 解析によりボールマン2型において悪性度の高い症例とより悪性度の低いと思われる症例に再分類することが可能となった．胃癌細胞が漿膜に向って浸潤して行くに従って，原発巣の核 DNA ploidy 最高値は高くなって行くが，癌細胞が漿膜面に到着すると有意に原発巣の核 DNA ploidy 最高値が低くなることが判明した．この理由は不明であるが，ps (+) の進行胃癌において核 DNA ploidy 最高値が低いからと言って必ずしも臨床的に悪性度が低いと決めることは危険である．核 DNA の ploidy 解析はヒト胃癌の細胞生物学的診断に有用であることが判った．