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Comparison of Cell Nuclear DNA Contents between Intraepithelial and Invasive Components of Oesophageal Squamous Cell Carcinoma

Shinichi Tsutsui, Hiroyuki Kuwano, Masaki Mori, Hiroshi Matsuura and Keizo Sugimachi

Detailed cytophotometric analyses of cell nuclear DNA content were performed on 132 different areas from 24 oesophageal squamous cell carcinomas with an intraepithelial component. Measurements of DNA content were performed at the three different areas within each of the intraepithelial and invasive components. There were no differences in the overall incidence of DNA distribution patterns which were classified according to the degree of dispersion and the peak value on the DNA histogram between intraepithelial and invasive components, as well as the average of mean values of the DNA content. In 13 (54%) of 24 cases, there was a variation among the DNA distribution patterns obtained from three different areas of the intraepithelial components, whereas there was a variation in 4 (22%) of the 18 invasive components. In 11 of 13 intraepithelial components with a variation, the same DNA distribution pattern as seen in the invasive component was present. Provided that the DNA content remains stable, variations in intraepithelial components may reflect various tumour cell populations. These findings support the idea of a multicentric carcinogenesis of oesophageal carcinoma, and shed light on the mechanisms in which a single cell population is selected from various cell populations in the progression from an intraepithelial to an invasive lesion.
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INTRODUCTION

PRESENCE OF intraepithelial carcinoma (carcinoma *in situ*) contiguous to the main lesion of oesophageal squamous cell carcinoma is a fairly frequent occurrence [1, 2]. In our study of 205 specimens of oesophagaeal carcinoma, we noted a close relationship between the multiplicity of oesophageal squamous cell carcinoma and the coexistence of intraepithelial carcinoma contiguous to the main lesion [3]. We also found a higher incidence of the coexistence of intraepithelial carcinoma contiguous to the main lesion in earlier than in advanced cases [4]. We considered that these findings support the concept of a multicentric or field carcinogenesis of oesophageal squamous cell carcinoma, although it has been interpreted to be the result of an intraepithelial spread of an invasive carcinoma [5].

In the present work, we compared the cell nuclear DNA contents between the intraepithelial component contiguous to the main lesion and the invasive component of oesophageal squamous cell carcinoma. We directed attention to origin of the oesophageal carcinoma and the mode of progression from the intraepithelial to the invasive carcinoma, from the aspect of DNA content.

MATERIALS AND METHODS

Patients

Measurements of DNA content were performed on 132 different areas from 24 oesophageal squamous cell carcinomas with an intraepithelial component contiguous to the main lesion. These 24 were selected from 290 oesophageal carcinomas surgically resected in the Department of Surgery II, Kyushu University from 1975 to 1989. None of the 24 patients had received preoperative treatment such as radiation or chemotherapy. The depth of invasion of the main lesion was the mucosa, submucosa and the proper muscular layer or beyond it in 6, 10 and 8 cases, respectively.

Histopathological examination

Microscopic sections of the entire resected oesophagus were made from step-sectioned blocks 5 mm in width, following formalin fixation. These sections were evaluated after haematoxylin and eosin (HE) staining and a diagrammatic map was made for each case.

For a diagnosis of intraepithelial carcinoma, we followed the criteria of Suckow *et al.* [2]:(1) absence of cellular differentiation with variations in size and shape and hyperchromatism of the nuclei with increased mitotic activity; (2) that the aforementioned changes must involve the entire thickness of the epithelium and may involve submucous glands and ducts; and (3) intact basement membrane.

Measurement of DNA content

A 10 micron thick section was prepared from the portion just adjacent to the section examined with HE staining, and Feulgen DNA-staining was done by the method of Naora [6]. Nuclear DNA content was measured by the two-wave length method using a microspectrophotometer (MPV3, Leitz, Germany). Data processing was performed with a personal computer (HP-85, Hewlett-Packard, Corvallis, Oregon). The relative DNA content of 100 cancer cells was examined, in accordance with the 2c (diploid) value determined by the mean value of 25 stromal lymphocytes as a control.

DNA measurements were performed at areas shown in Fig.

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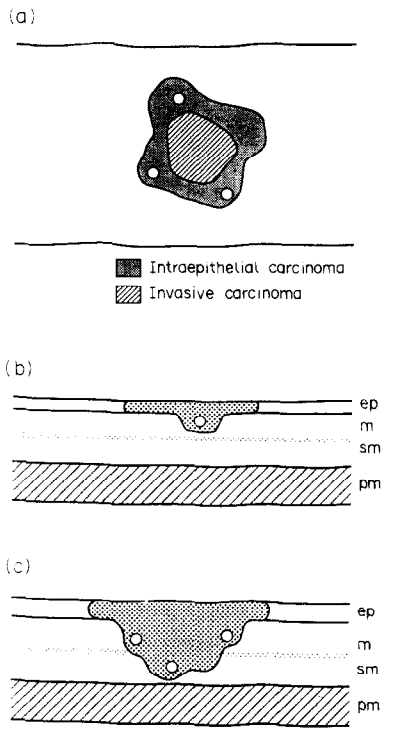

Fig. 1. DNA contents were measured at the three different areas in the intraepithelial component (a). In the invasive component, measurement was done at one area in mucosal carcinoma (b) and at three different areas—proximal, distal and deep margins—in the submucosal carcinoma and carcinoma extending into proper muscular layer (c). Measurement was done at the area "○". ep = epithelium, m = mucosa, sm = submucosa, pm = muscularis propria.

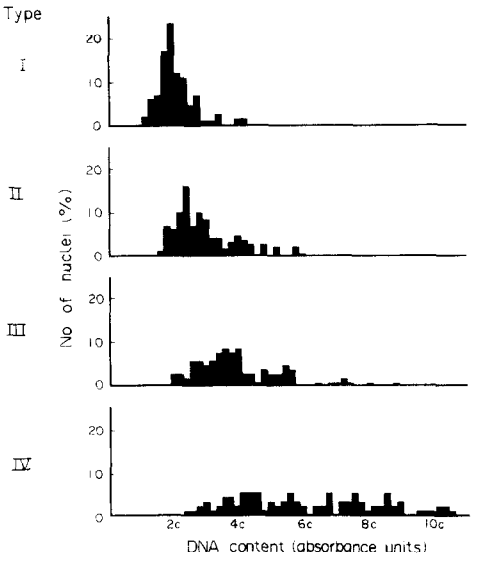

Fig. 2. Classification of DNA distribution pattern in oesophageal carcinoma. Type I: a peak in the 2c region with a dispersion to the 4c region; Type II: a peak in the 2c–3c regions with a dispersion limited up to the 6c region; Type III: a low peak beyond the 3c region and a low proportion of cells (<20%) in >6c; Type IV: a high proportion of cells (>20%) in >6c.

1. Measurements were performed at three different areas within each of intraepithelial component contiguous to the main lesion and invasive component. In the invasive component of the mucosal carcinoma, however, DNA measurements were performed at one area because the invasive component of mucosal carcinoma was too small for measurement at three different areas. In the intraepithelial component, three different areas were selected at random and at the advancing margins—proximal, distal and deep margins—in the invasive components.

Nuclear DNA contents were analysed by the DNA distribution patterns on the DNA histogram and the mean values of the DNA contents of 100 cancer cells. DNA distribution patterns were grouped into four types (Type I–IV) [7–9], according to the degree of the peak and dispersion, as shown in Fig. 2.

Statistical analysis

Analysis of variance [10] was used for comparisons among the mean values of DNA content obtained from three different areas in intraepithelial or invasive components. A variance ratio (F) was calculated from the mean value and standard deviation of the DNA content of 100 cancer cells obtained from three different areas. There is a statistical difference among the three different mean values of DNA content when the variance ratio is over 3.03 ($P < 0.05$). Other statistical analyses were performed using the X^2 and Student's t test.

RESULTS

Results of a DNA study in 24 oesophageal carcinomas with an intraepithelial component are listed in Table 1. The overall incidence of DNA distribution patterns of the intraepithelial and invasive components are summarised in Table 2. There were no differences in the overall incidence of the DNA distribution patterns between the intraepithelial and invasive component. Figure 3 shows the frequency distribution of the mean values of DNA content of 100 cancer cells, respectively for intraepithelial and invasive components. The averages of mean values of the DNA contents of 100 cancer cells in the intraepithelial and invasive component were 3.67 (S.D. 0.80) and 3.67 (0.83) respectively. There were no differences in the averages of mean values of the DNA contents.

Table 1. Combination of DNA distribution patterns obtained from six different areas in 24 oesophageal carcinomas

	Combination of DNA distribution patterns		
	Intraepithelial	Invasive	No. of cases
1.	III, III, III	— III, III, III	5
2.	II, II or III, III	— II, II, II, or II*	3
3.	II, II, II	— II, II, II	2
4.	III, III, III	— II, II, II	2
5.	II, II or III, III	— II, II or III, III	2
6.	II, II or IV, IV	— II*	2
7.	II, II, II	— III, III, III	1
8.	III, III, IV	— IV, IV, IV	1
9.	II, III, IV	— IV, IV, IV	1
10.	II, III, IV	— II, II, III	1
11.	II, II, III	— II, IV, IV	1
12.	IV, IV, IV	— III*	1
13.	II, III, III	— III*	1
14.	II, II, III	— IV*	1

*: DNA measurement was performed at one area in the invasive component of mucosal carcinoma because the invasive components of mucosal carcinoma were too small for measurements at three different areas.

I, II, III, IV = types of DNA distribution patterns.

Table 2. Overall incidence of DNA distribution patterns of the intraepithelial and invasive components

DNA distribution pattern	No. of areas	
	Intraepithelial	Invasive
I	0	0
II	27 (38%)	26 (43%)
III	36 (50%)	25 (42%)
IV	9 (13%)	9 (15%)
Total	72	60

*No statistical difference (χ^2 test).

The combination of DNA distribution patterns obtained from three different areas in each intraepithelial and invasive component is summarized in Table 3. In 13 (54%) of 24 cases, we noted a variation among the DNA distribution patterns obtained from three different areas of intraepithelial component, whereas a variation was found in three different areas of 4 (22%) of 18 invasive components. Moreover, in 18 (75%) of the 24 cases, there was a difference among the mean values of the DNA content obtained from three different areas of the intraepithelial components, with a statistical significance ($P < 0.05$), determined using analysis of variance, whereas there was a difference in 6 (33%) of the 18 invasive components.

The DNA distribution patterns of 4 (Nos 4, 7, 12 in Table 1) of 11 intraepithelial components without a variation differed from those of the invasive components. Therefore, different DNA distribution patterns from those of invasive components were found in 17 (71%) of 24 intraepithelial components. On the contrary, all of the DNA distribution patterns obtained from six different areas were in agreement in 7 (Nos 1, 3 in Table 1) cases.

An example of DNA histograms in the case with a variation in the intraepithelial component and without a variation in the invasive component is shown in Fig. 4. In this case, however, Type II, the DNA distribution pattern of the invasive component was found in the intraepithelial component. As in this case, in 11 (Nos 2, 5, 6, 8, 9, 10, 13 in Table 1) of 13 intraepithelial carcinomas with a variation of the DNA distribution patterns, there existed the same DNA distribution patterns as seen in the invasive component.

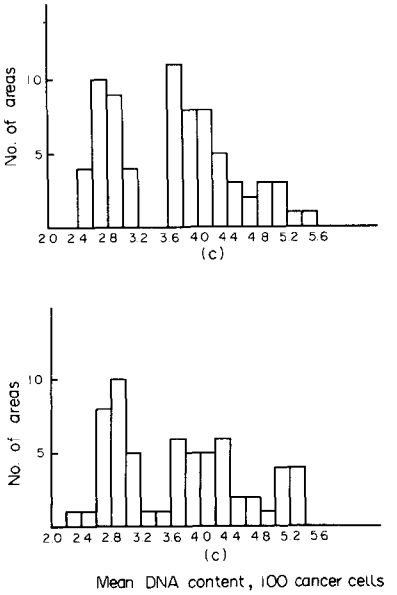

Fig. 3. The frequency distribution of the mean values of DNA contents of 100 cancer cells, respectively, for intraepithelial (upper) and invasive (lower) components.

Table 3. Combination of DNA distribution patterns obtained from three different areas in each intraepithelial and invasive component

Combination of DNA distribution patterns	No. of lesions	
	Intraepithelial	Invasive
Variation (-)	11 (46%)	14 (78%)
II, II, II	3	6
III, III, III	7	6
IV, IV, IV	1	2
Variation (+)	13 (54%)	4 (22%)
II, II or III, III	8	3
II, II or IV, IV	2	1
III, III or IV, IV	1	0
II, III, IV	2	0
Total	24	18

DISCUSSION

Many investigators directed attention to whether or not DNA content remains stable during growth of a malignant tumour. Friedlander *et al.* [11] reported the intratumoral stability of

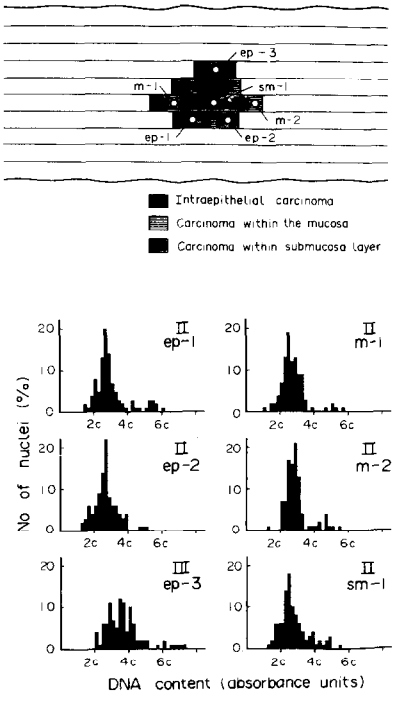

Fig. 4. DNA histograms in the case with a variation in the intraepithelial carcinoma and without a variation in the invasive carcinoma. In this case, however, type II which is the DNA distribution pattern of invasive component is present in the intraepithelial component. m-1 = proximal, m-2 = distal, sm-1 = deep margins.

DNA content in cases of an epithelial ovarian cancer. Frankfurt *et al.* [12] noted the stability of DNA content by means of a comparative study in the primary and metastatic solid tumours. Similar findings were observed in breast [13], thyroid [14] and colon [15] cancer. Korenaga *et al.* [16] reported the consistency of DNA content between primary and recurrent gastric cancers.

Conversely, a considerable variability in DNA content within different areas in the primary tumour or its metastases of lung [17], kidney [18], stomach [19, 20] and colon [21, 22] has been reported. Regarding the cause of the variability in the DNA content, at least two hypotheses are tenable [12, 19, 20, 22, 23]: (1) Variability in the DNA content reflects multifocal carcinogenesis (from the viewpoint of consistency of DNA content) and (2) DNA aneuploidy develops during tumour progression. The oesophageal carcinoma without a variation in the DNA distribution pattern obtained from six different areas may suggest intraepithelial spread from the invasive component. In the present study, however, variability in the DNA distribution patterns and mean value of the DNA content obtained from three different areas of the intraepithelial carcinoma was higher than observed in the invasive components. If it is assumed that the intraepithelial component contiguous to the main lesion is the result of a lateral intraepithelial spread from the main lesion, the development of DNA aneuploidy—clonal evolution—should occur at a higher incidence within the intraepithelial spread than invasion into deeper layers, according to the second hypothesis. Provided that the DNA content remains stable during tumour progression, variations of the DNA content in the intraepithelial components are thought to reflect various cell populations. These findings suggest a multicentric carcinogenesis of oesophageal carcinoma.

In the genesis of oesophageal carcinoma, the sequence from dysplasia through carcinoma *in situ* to the invasive carcinoma has been suggested [24]. The same sequence is suggested for carcinoma of the uterine cervix [25]. Many investigators reported the DNA content of precancerous lesions and invasive carcinoma of the uterine cervix [26–28]. In these sequences, alternations in DNA content precedes the invasive stage of the carcinoma. In our present study, the areas in which DNA content were examined were at the stage of carcinoma *in situ*, not a dysplasia. We found no differences in both the overall incidence of DNA distribution patterns and average of mean values between intraepithelial and invasive components. Similar findings were observed in human oesophageal cancer [29] and oesophageal cancer induced in rats [9]. There are no differences in cellular DNA contents between carcinoma *in situ* and invasive carcinoma. These findings show that intraepithelial carcinoma already has obtained aggressive characteristics as an invasive carcinoma, with regard to DNA contents that reflect proliferative activity.

Böhm and Sandritter [26] examined the DNA content of carcinoma *in situ* and invasive carcinoma of uterine cervix and stated, "When a stem line first develops through selection from this heterogeneous cell population, the basal membrane is broken through and an autonomous, invasive carcinoma with a bimodal DNA histogram develops. This DNA stem line can be hypodiploid, hyperdiploid or even euploid." We found that in 17 (71%) of 24 intraepithelial components, there was a different DNA distribution pattern from that of the invasive component and the variability of DNA content was higher in the intraepithalial than in the invasive component. On the contrary, in 11 (85%) of 13 intraepithelial components with a variation of the DNA distribution pattern, there existed the same DNA distribution pattern as seen in the invasive component. These findings shed light on the mechanisms in which a single cell population is selected from various cell populations in the progression from intraepithelial to invasive lesion.

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A Numerical Prognostic Index for Clinical Use in Identification of Poor-risk Patients with Hodgkin's Disease at Diagnosis

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The aim of this study was to assess the feasibility of using objective data obtained at diagnosis of Hodgkin's disease to predict those patients who were likely to die of progressive disease within 4 years of diagnosis. 92 consecutive patients from one centre (Newcastle upon Tyne) were used to construct a numerical index based on disease stage (Ann Arbor), age, haemoglobin and absolute lymphocyte count. Weight was assigned according to a predictive value in univariate and multivariate analyses based on survival. The index produced was then validated on a separate patient set (455) from other centres within the Scotland and Newcastle Lymphoma Group (SNLG) on whom the same prospective information was available. The index produced provided a useful separation of those patients destined to die of disease. Of 101 patients with index higher than 0.5, 62 (61.4%) were dead at 4 years, whereas with index lower than 0.5, 61 (18%) of 336 patients were dead at 4 years. The index includes Ann Arbor stage but possesses additional practical prognostic value which allows identification of patients with early stage destined to die of disease. Of 149 patients with stage IA and IIA disease 15 patients had index higher than 0.5, and 10 (60%) have died, whereas the remaining patients had survival of 90% and 85% respectively. This numerical index is applicable to all patients at diagnosis and in the SNLG population gives better predictive survival at 4 years than stage alone, and provides a basis for selecting patients for more aggressive therapy.
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INTRODUCTION

PROGRESS IN Hodgkin's disease therapy has been substantial over the last 15–20 years, with 60% of patients surviving disease-free at 10 years. However 30% of patients will die within 4 years of presentation of progressive disease [1–3]. It is possible to recognise a number of those at risk of progressive disease on the basis of clinical observations alone, such as advanced stage [4], older age group [5] and patients with bulky mediastinal disease [3]. In addition to these simple clinical parameters, a substantial literature now exists relating to haematological and biochemical factors which are associated with poor prognosis, a subject reviewed recently by Hagemeister [6]. In the recent past several groups have undertaken reviews of their patient populations and analysed the results of survival relative to various prognostic indicators. In 1985 the British National Lymphoma Investigation (BNLI) produced a prognostic index on patients with stage I and stage II disease [5]. The Manchester Group reviewed patients with advanced disease in stage III and IV [3]. Both these studies applied an index to specific Ann Arbor staging

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