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Flow Cytometric DNA Analysis of Gastric Cancer

— correlation with histology and clinical outcome —

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Nuclear DNA content was analysed by means of flow cytometric measurements in 103 patients with gastric carcinomas, using paraffin-embedded archival tissue. DNA aneuploidy was found in 40 cases(38.8%). The mean DNA index of aneuploid tumors was 1.45(range 1.13 to 2.37). No significant association between ploidy and either age, sex, tumor location, size, stage, growth pattern, or histologic type was found. However, the incidence of aneuploidy was higher in high grade carcinomas than in low grade carcinomas; the incidence of aneuploidy was 10%, 68.8%, and 45.8% for Grade II, III, and IV carcinomas, respectively, as compared with Grade I carcinomas which were all diploid. On statistical analysis, Abnormal cellular DNA content was significantly correlated with high histologic grade(P<0.005). Patients with aneuploid cancer(39.2%) had a poorer prognosis than those with diploid cancer(70.0%) based on (P<0.01). The 2-year survival rate for advanced gastric carcinoma. Therefore, DNA ploidy might be a useful prognostic factor in cases of advanced gastric cancer.

Key Words: *DNA content, flow cytometry, gastric carcinomas, ploidy level*

INTRODUCTION

The incidence of gastric carcinoma is high in Korea with a cancer mortality rate ranking first for both men and women. The prognosis for patients with operable gastric carcinoma is generally poor; the overall 5-year survival rate is approximately 10% (Bizer, 1983). In order to improve prognosis, the diagnosis at an early stage, when the tumor is confined to the mucosa or submucosa, is very important because the most accurate prognostic factor is stage(Curtis et al., 1985; Tosi et al., 1988; Filipe et al., 1991). However, the rate of early detection is still low in Korea. In advanced gastric carcinoma the effect of morphologic parameters used to predict biologic behavior , such as cancer subtype, histologic grade, and growth pattern, is not obvious and seems sometimes to be futile(Tosi et al.,1988; Filipe et al., 1991).

There is increasing evidence that tumor cell DNA content and/or proliferative activity, as analyzed by flow cytometry, is of considerable prognostic value with regard to survival in a variety of human tumors, including breast, colorectal, and ovarian (Friedlander et al., 1984; Armitage et al., 1985; Kallioniemi et al., 1988; Hedley, 1989). A few studies on flow cytometric DNA analysis of gastric carcinoma have been published, but consistent results on the significance of DNA content as an independent prognostic indicator have not yet been established(Hattori et al., 1984; Korenaga et al., 1988; Sasaki et al., 1989; Filipe et al., 1991). In spite of the high incidence of gastric cancer in Korea, flowcytometric studies of DNA ploidy of advanced gastric carcinomas are rare(Kim et al., 1991).

This study was conducted to analyse correlations between DNA ploidy patterns, histopathologic characteristics of different types of gastric canc-

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ers, and clinical prognosis based on flow cytometric measurements of nuclear DNA.

MATERIALS AND METHODS

Patients and histopathologic specimens

One hundred and three patients with gastric cancers resected at Chungnam National University Hospital in Daejeon, Korea during the years 1988 through 1990, for which paraffin block specimens of the tumor were still available, were selected for investigation. Surgical procedures performed included either subtotal, or total gastrectomy with radical lymph node dissection, or palliative gastrectomy. Clinical information, such as age, sex, extent of tumor, treatment, and outcome, was collected from pathologic and hospital records. Determination of clinical stage was done according to the modified AJCC (The American Joint Committee on Cancer) system(Curtis et al., 1985), into stages I, IIA, IIB, III, or IV.

Gross pathological findings and hematoxylin eosin-stained sections were reviewed. Tumor locations in the stomach were grouped as gastroesophageal(GE)/cardiac, body-antral, or diffuse carcinomas. Gastric carcinomas were divided into expanding and infiltrative types based on patterns of tumor growth and invasiveness, according to Ming's classification(Ming, 1977). Histological classification was performed according to the criteria of the World Health Organization(WHO) (Watanabe et al, 1989). The degree of differentiation was graded as I to IV, based on the degree of tubule formation and cytologic atypia.

Flow cytometric analysis

Paraffin-embedded samples of normal stomach tissue from the same patients were processed, along with the tumor blocks, as a control. For flow cytometric analysis, five to six 50um thick paraffin sections were prepared, dewaxed in xylene, then rehydrated with a serial alcohol treatment and distilled water using the technique described by Hedley et al.(1983). The pellet was incubated in 3ml of a 0.5% pepsin-Hcl solution at pH 1.5 in a water bath at 37°C with intermittent vortex mixing for 30 minutes. Pepsin digestion was stopped by adding pepstatin A(1.51mg/ml), then samples were filtered through a 37um nylon mesh, yielding supensions of bare nuclei. Samples were then treated with ribonuclease(180ug/ml) to remove RNA. Finally, suspensions were stained with fluorochrome propidium iodide(50ug/ml), and the DNA content of 10,000 to 20,000 nuclei was analysed using a flow cytometer (FACSTAR, Becton-Dickinson, C.A.).

The normal diploid DNA value was established by analysis of normal gastric tissue. The quality of the results was estimated by calculating the coefficient of variation(CV) for each G0/G1 peak, based on the half maximum peak height. The CV of the G0/G1 peak was usually less than 8% in this study. If the CV was greater than 8%, the case was excluded from the study.

A tumor with a single G0/G1 peak was considered to be diploid, while evidence of a second G0/G1 peak indicated aneuploidy. The DNA index (DI) for aneuploid specimens was calculated from the ratio of the mean channel value of the aneuploid G0/G1 population, divided by the mean channel value of the diploid G0/G1 population. Cell proliferation was expressed as an S-phase fraction(SPF). SPF was only calculated for diploid tumors. Diploid tumors with a G2/M fraction two times larger than SPF, and constituting over 15% of the total cell count, were regarded as aneuploid because of difficulties in the distinction between a diploid tumor with a prominent G2/M fraction, and an aneuploid tumor with a DI of around 2.0 (Kallioniemi et al., 1988).

Statistical analysis

Relationships between DNA ploidy/SPF and clinicopathologic parameters potentially predictive of biologic behavior were investigated using the chi-square method or Student's t test. The correlation between DNA ploidy level and prognosis was evaluated using the Kaplan-Meier method and compared using the log-rank test.

RESULTS

Clinicopathologic findings

The distribution of the study population, based on clinicopathologic parameters, is summarized in Table 1. The mean age was 55.9 years(range, 29 to 70); the male to female ratio was 2.2:1. Eighty seven lesions(84.5%) were body-antral, 12 (11.7%) were cardiac or fundic, and four(3.9%) were diffuse. The mean tumor size was 5.5cm. Of the 103 cancers examined, 7 cases(6.8%) were early cancers in which neoplastic invasion was confined to the mucosa and the submucosal layer. The other 96 cases (93.2%) were advanced cancers. Based on Ming's classification(1977), 40 cases (38.8%) were expanding type, 44 cases (42.7%)

Table 1. Clinicopathogic parameters of stomach carcinomas in relation to ploidy

Parameters	No(%)	Diploid (n=63)	Aneuploid (n=40)	P value	Test
Age (yr)				N.S.	t-test
<45	19(18.4)	12	7		
>45	84(81.6)	51	33		
Mean SD		55.5 11.9	56.5 11.6		
Sex				>0.1	X^2test
Male	71(68.9)	47	24		
Female	32(31.1)	16	16		
Ratio(M:F)	2.2:1	2.9:1	1.5:1		
Location				>0.1	X^2test
GE-cardia	12(11.7)	9	3		
Body-antrum	87(84.5)	51	36		
Diffuse	4(3.9)	3	1		
Tumor size				N.S	t-test
1-3cm	14(13.6)	6	8		
3-5cm	41(39.8)	28	13		
>5cm	48(46.6)	29	19		
Mean SD		5.6 2.3	5.5 2.3		
Stage				>0.05	X^2test
I	7(6.8)	4	3		
IIA	17(16.5)	13	4		
IIB	16(15.5)	12	4		
III	61(59.2)	34	27		
IV	2(1.9)	0	2		
Growth pattern				>0.05	X^2test
Expanding	40(38.8)	28	12		
Infiltrative	44(42.7)	25	19		
Mixed	19(18.4)	10	9		
Histologic type				>0.05	X^2test
Papillary	2(1.9)	2	0		
Tubular	45(43.7)	32	13		
Signet-ring	34(32.0)	20	14		
Mucinous	6(5.8)	1	5		
Mixed	16(15.5)	8	8		

showed an infiltrative growth pattern, and 19 cases (18.4%) showed a mixed pattern. Based on the WHO classification(Watanabe et al., 1989), 45 cases(43.7%) were tubular type, and 34 cases(33.0%) were signet-ring cell type including immature round cells. The degree of differentation was Grade I, 6 cases (5.8%); Grade II, 22 cases(21.4%); Grade III, 27 cases (26.2%); and Grade IV, 48 cases (46.6%).

Flow cytometric data

In this study 63 cases(61.2%) of gastric carcinoma were considered to be diploid cancers, and 40 cases (38.8%) showed abnormal DNA content. The mean DNA index of aneuploid tumors was 1.45 (range, 1.13 to 2.37). SPF values could be determined for diploid cancers in 63 cases. SPF values ranged from 1.4% to 17.7% with a mean of 8.7%.

The distribution of various clinicopathologic parameters, based on DNA ploidy, is shown in Table

Table 1. Clinicopathologic parameters of stomach carcinomas in relation to ploidy "(cont)"

Tumor grade			<0.005 X^2test
I	6(5.8)	6	0
II	22(21.4)	20	2
III	27(26.2)	11	16
IV	48(46.6)	26	22

* N.S. : not significant, GE : gastroesophageal

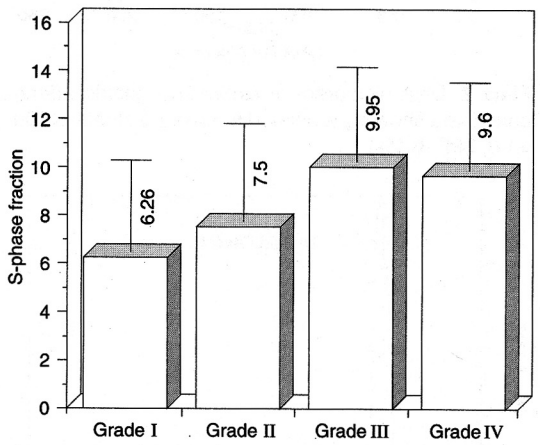

Fig. 1. Comparison of SPF among grade groups of diploid carcinomas (p > 0.05).

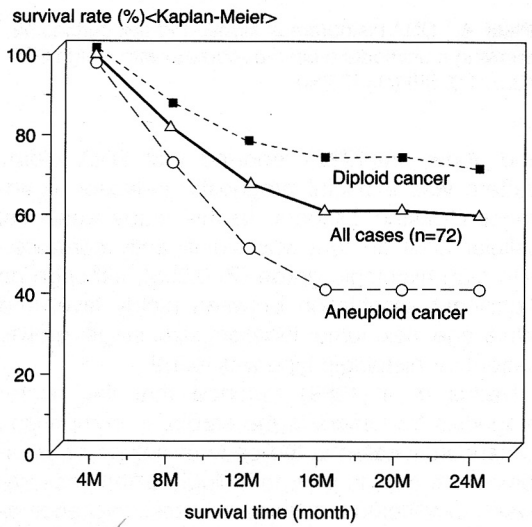

Fig. 2. DNA ploidy pattern and prognosis (p < 0.01).

1. No significant association between ploidy level and either age, sex, tumor location, size, stage, growth pattern, or histologic type was found. However, the incidence of aneuploidy was higher in high grade carcinomas than in low grade carcinomas; the incidence of aneuploidy was 10%, 68. 8%, and 45.8% for Grade II, III, and IV carcinomas, respectively, as compared with all Grade I carcinomas which were diploid. On statistical analysis, abnormal cellular DNA content was significantly correlated with high histologic grade(P<0.005). Among diploid carcinomas, SPF values were compared according to histologic grade (Fig. 1). The mean SPF value of Grade I cancers was 6.3% 3. 7%, Grade II was 7.9% 4.0%, Grade III was 9.9% 3.8%, and Grade IV was 9.6% 3.6%. The difference among the grade groups was not statistically significant(P>0.05), although high grade diploid cancers showed somewhat higher SPF values than low grade diploid cancers.

Clinical follow-up data

At least 2 years of follow-up data were available in 72 patients allowing a survival analysis, except for Stage I early gastric cancer. The 2-year survival rate of all the patients was 58.2%. The 2-year survival rates by DNA ploidy were 70.0% for diploid cancers and 39.2% for aneuploid cancers, showing that patients with aneuploid cancers had a poorer prognosis than those with diploid cancers(P<0.01)(Fig. 2). The relationship between SPF values and the mortality rate of diploid patients was not significant.

DISCUSSION

There is increasing evidence that cancers with abnormal nuclear DNA content, as analyzed by flow cytometry, are characterized by a more clinically aggressive course than cancers with diploid DNA content(Friedlander et al., 1984; Kallioniemi et al., 1988; Hedley, 1989). However, a favorable clinical outcome has been demonstrated in neuroblastomas associated with an aneuploid stem line(Gansler et al., 1986).

The incidence of aneuploidy in gastric carcinoma differs widely, from 27% to 89%, among different study groups(Deinlein et al., 1983; Hattori et al., 1984; Teodori et al., 1984; Macartney et al., 1986; Ballantyne et al., 1987; Odegaard et al., 1987; Tosi et al., 1988; Sasaki et al., 1989; Filipe et al., 1991; Kim et al., 1991). In this study about 61. 2% of gastric cancers were considered to be

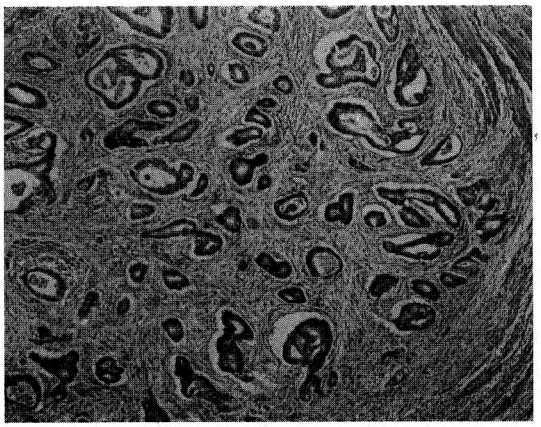

Plate 1. Tubular adenocarcinoma, expanding type, grade I (H&E, x100).

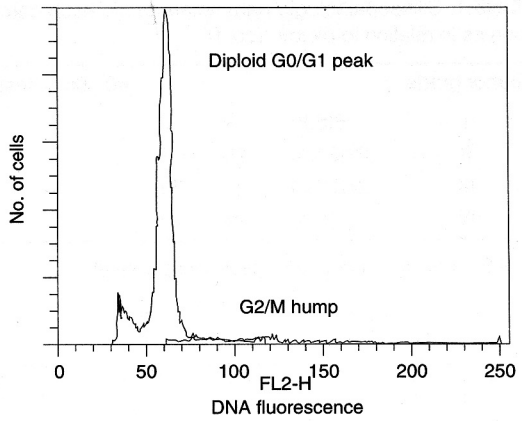

Plate 2. DNA histogram of expanding gastric adenocarcinoma showing a unimodal diploid distribution(D.I. =1.0, SPF=6.1%).

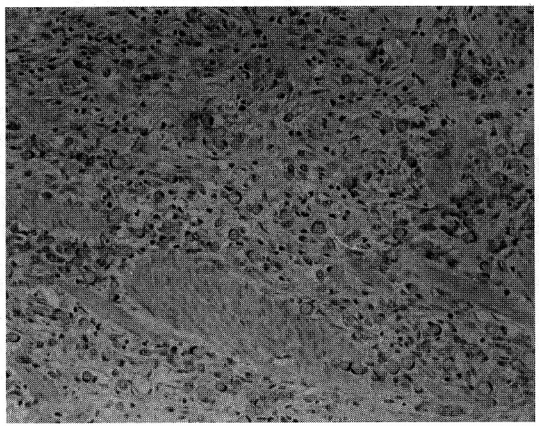

Plate 3. Signet-ring cell adenocarcinoma, infiltrative type, grade IV (H&E, x100).

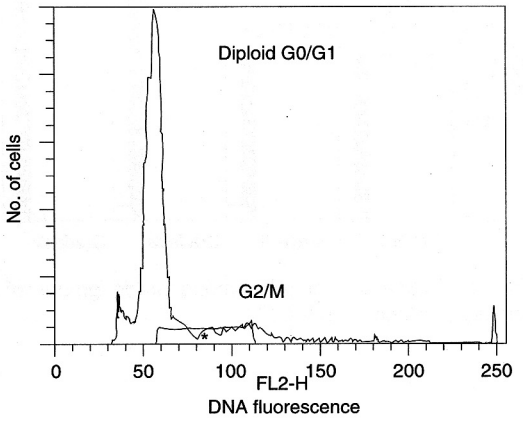

Plate 4. DNA histogram of signet-ring cell carcinoma showing a unimodal diploid population with a high SPF (D.I.=1.0, SPF(*)=17.7%).

diploid. Overall aneuploid incidence was 38.8%.

The significance of DNA ploidy as an independent prognostic factor has not yet been established in advanced gastric carcinomas, and most flow cytometric analyses of gastric carcinomas have not demonstrated a relationship between ploidy and tumor stage, histological tumor type, or patient survival(Hattori et al., 1984; Macartney et al., 1986; Ballantyne et al, 1987). However, DNA ploidy appears to be valuable as an independent predictor of recurrence, metastatic potential, and survival in early gastric carcinomas(Inokuchi et al, 1983; Korenaga et al., 1986). Hattori et al.(1984) suggested a possible association between aneuploidy and high histological tumor grade. Kimura and Yonemura(1991) reported that DNA ploidy pattern was a useful prognostic indicator in advanced gastric cancers. In this study abnormal cellular DNA content was significantly correlated with high histologic grade ($P<0.005$), although no significant association between ploidy level and either age, sex, tumor location, size, stage, growth pattern, or histologic type was found.

Nadus et al.(1989) reported that the poorer prognosis for tumors in the cardia, in comparison to cancers located in the body or antrum of the stomach, is due, in part, to biologic differences between carcinomas of these two sites, because aneuploidy is strongly, correlated with site. Ninety six percent of cardia, carcinomas were aneuploid,

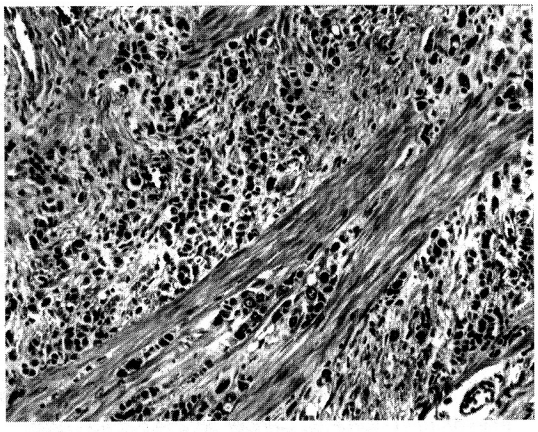

Plate 5. Poorly differentiated adenocarcinoma composed of immature round cells, infiltrative type, grade IV (H&E, x100).

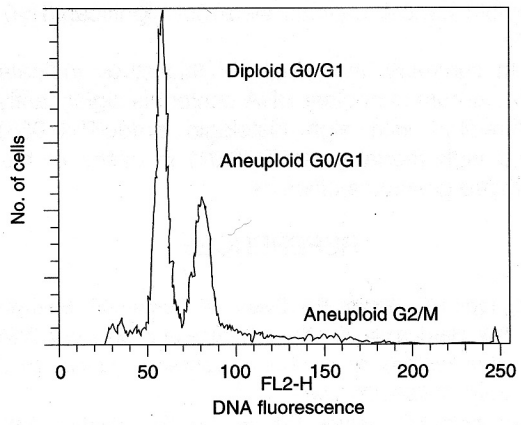

Plate 6. DNA histogram of poorly differentiated adenocarcinoma demonstrating a bimodal distribution of the nuclear DNA content with a diploid peak and an extra-peak at the aneuploid level (D.I.=1.40).

as compared with only 48% of body-antrum cancers (P=.0008). In this study, however, no correlation was found between ploidy and tumor location.

From a histogenetic point of view, gastric cancers are divided into two major types, an expanding (including intestinal/well differentiated) type, and an infiltrative (diffuse/poorly differentiated) type(Ming, 1977). The expanding type has a higher survival rate than the infiltrative type and is prevalent in high-risk countries(Ming, 1977). In this study about 42.7% were of an infiltrative type and about 38.8% were of an expanding type. Hattori et al.(1984) observed that the ploidy patterns of early and advanced intestinal type cancers were essentially the same, whereas advanced cancers tended to contain more polypoid cells than early cancers. Deinlein et al.(1983) demonstrated that diffuse carcinomas showed a higher rate of aneuploidy, although intestinal type and diffuse carcinomas could not be distinguished by proliferation kinetics. It has been proposed that poorly differentiated adenocarcinomas are often mosaic cancers of several different ploidies, compared to differentiated adenocarcinomas(Sasaki et al., 1989). In this study no significant association between ploidy level and growth pattern was found. However, abnormal DNA content was correlated with high histologic grade($P<0.005$).

These results are in contrast to observations that there is a tendency for tumours with an infiltrating growth pattern to be diploid(Macartney et al, 1986), and that there is a higher incidence of aneuploidy in differentiated vs. undifferentiated adenocarcinomas(Sasaki et al., 1989). Ohyama et al. (1990) commented that the DNA ploidy pattern showed no relation to clinicopathologic findings.

Hattori et al(1984) postulated that signet-ring cells filled with mucin could be degenerated cells which are out of the normal cell cycle, because signet-ring cells are rarely labeled with ^{3}H-thymidine indicating that this cell group no longer retains a proliferative activity. The signet-ring cell type carcinomas in our study showed the trend that most of the diploid cancers were composed of mucin-filled signet-ring cells, whereas aneuploid cancers consisted of immature round cell types. Therefore, it would be appropriate that signet-ring cell types should be sub-classified into mucin-filled signet-ring cell types, and immature round cell types.

Kimura and Yonemura(1991) assumed that the DNA ploidy pattern was a useful prognostic indicator of advanced gastric cancer because the 5-year-survival rate of diploid patients was significantly higher than that of aneuploid patients ($P<0.01$). In this study the patients with aneuploid cancer had a significantly poorer prognosis than those with diploid cancer($P<0.01$).

Ohyama et al.(1990) reparted that patients with an SPF over 10% had a poorer prognosis than those with an SPF below 10%, even in cases of diploid cancers. In this study high grade diploid cancers showed somewhat bit higher SPF values than low grade diploid cancers, but the differences between the grade groups and the relationships between SPF values and the mortality rate of

diploid cancer patients were not significant($P>0.05$).

In summary, the results of this study indicate that abnormal nuclear DNA content is significantly correlated with high histologic grade($P<0.005$) and high mortality rate($P<0.01$) in cases of advanced gastric carcinomas.

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