CL-134

(18)

Eur Surg Res 1990;22:365–370

© 1990 S. Karger AG, Basel
0014–312X/90/0226–0365$2.75/0

Assessment of Tumor Cell Kinetics by Monoclonal Antibody Ki-67

Y. Yonemura, S. Ohoyama, H. Kimura, T. Kamata, A. Yamaguchi, I. Miyazaki

Department of Surgery II, School of Medicine, Kanazawa University, Kanazawa, Japan

Key Words. Ki-67 Proliferative activity Gastric cancer

Abstract. The expression of Ki-67 antigen in 71 patients with advanced gastric cancer was studied by immunohistochemical technique. Immunohistochemical staining with Ki-67 produced clear labeling of a portion of tumor cell nuclei, and the nucleoli stained intensely. The Ki-67 labeling rates of the 71 specimens ranged from 7.7 to 70.5% (mean: 29.2%; standard deviation: 12.9%). There was no significant association between Ki-67 labeling rates and macroscopic type, peritoneal metastasis, or serosal invasion. The tumors showing high Ki-67 labeling rates (> 25%) are more likely to have liver metastasis and lymph node involvement. Larger tumors, with a diameter > 6 cm, more frequently showed high Ki-67 labeling rate than those with a diameter < 6 cm. When the Ki-67 labeling rate and 9 clinicopathologic parameters, as conventional prognostic factors, were entered simultaneously into the regression model, nodal status and Ki-67 labeling rate emerged as independent prognostic factors. These results indicate that the in situ determination of the growth fraction by Ki-67 antibody may be a reliable prognostic marker of advanced gastric cancer.

Introduction

Advanced gastric cancer continues to carry a dismal prognosis despite great efforts to improve the radical treatment of this disease, with 5 year survival rates ranging from 10 to 30% [1]. Many attempts to identify factors predictive of survival among patients with this type of neoplasia have been made [2–5]. Numerous clinical and pathologic features of gastric cancer are known to be associated with prognosis [6–8]. Recently, measurements of cell cycle kinetics have been found to correlate with clinical course [9–12]. We previously reported that patients with gastric cancer with high bromodeoxyuridine labeling indices (BrdU LI) have lower survival rates than patients with low BrdU LI [13]. However, BrdU labeling requires in vitro incubation of viable tumor tissues. Recently, extensive literature has developed on the prognostic efficacy of flow

cytometry in the cell kinetics of malignant tumors [10–12, 14]. S-phase fractions of breast carcinomas determined by flow cytometry closely correlated with results obtained by thymidine labeling [9, 15], and many investigators have reported the prognostic value of S-phase fraction by flow cytometry. However, the S-phase fraction obtained by flow cytometry reflects not only the proliferative activity of tumor cells, but also the proportion of nonproliferating stromal cells in a tumor. In addition, the overlapping cell cycle distribution from DNA aneuploid clones as well as from nuclear debris may also interfere with the analysis of S-phase fractions. Volm et al. [12] reported that cell cycle analyses were possible in only 65% of cases by flow cytometry. More recently, a mouse monoclonal antibody (Ki-67) has become available defining a nuclear antigen present in proliferating cells throughout the cell cycle [16]. Ki-67 enables the immunocytochemical detection of cycling cells without the need of external administration of BrdU or ^{3}H-thymidine. The antigen is present in the late G_1, S, G_2, and M phase [17]. The present study was aimed at determining whether proliferative activity determined by Ki-67 monoclonal antibody was associated with histopathologic findings and the clinical outcome of patients with surgically resectable gastric cancer.

Materials and Methods

Tumors from 71 patients with advanced gastric cancer were analyzed using the monoclonal antibody Ki-67 (Dakopatts, Copenhagen, Denmark), and the results compared with respect to Ki-67 labeling rates and histopathologic features. Tissue samples were frozen in liquid nitrogen and stored at –70 °C until use. The frozen specimens were sectioned 6 μm thick, mounted on albumin-coated slides, and immediately fixed with aceton/ether (60%/40%) for 20 min. The sections were first washed in 0.5 *M*-Tris-buffered saline (TBS), pH 7.2 for 2 min and then incubated at room temperature for 45 min alternately with a 1:10 dilution of Ki-67 antibody, biotinylated goat anti-mouse IgG (Vector Laboratories, Burlingame, Calif. USA) and avidin-biotinylated horseradish peroxidase complex (ABC) (Vector Laboratories).

Between the incubations the slides were washed with TBS. The tissue sections were then incubated with 0.05% 3-3′-diaminobenzidine and H_2O_2. Finally, the slides were lightly stained with hematoxylin. All labeled nuclei, demonstrated by the use of Ki-67 antibody, were regarded as positive. Between 1,000 and 2,000 cells were counted in each of 10–15 microscopic fields to determine the average Ki-67 labeling rates. Ki-67 labeling rates were counted by two observers independently, and the areas of the sections with the highest labeling rates were used for counting. The mean of the two percentages of Ki-67-labeled nuclei was calculated and used in the statistical analyses described below.

The data are presented as mean ± standard deviation of mean. Statistical analyses were performed by χ^2 test. The outcome of different groups of patients was compared by generalized Wilcoxon test. The Cox proportional hazard model was used in the multivariate regression analyses of survival data. Throughout this report, information of a previous publication is used for the description and classification of the variables [18]. The cut-off point for low or high fraction of cell cycle was determined by a Cox proportional hazard model [19]. This is an efficient graphic tool for determining those levels of quantitative prognostic factors at which the most pronounced deterioration of prognosis takes place.

Results

Immunohistochemical staining with Ki-67 produced clear labeling of a portion of tumor cell nuclei in each section (fig. 1), and the nucleoli stained intensely. The Ki-67 labeling rates of the 71 specimens ranged from 7.7 to 70.5% (mean: 29.2%; standard deviation: 12.9%). The correlation between histo-

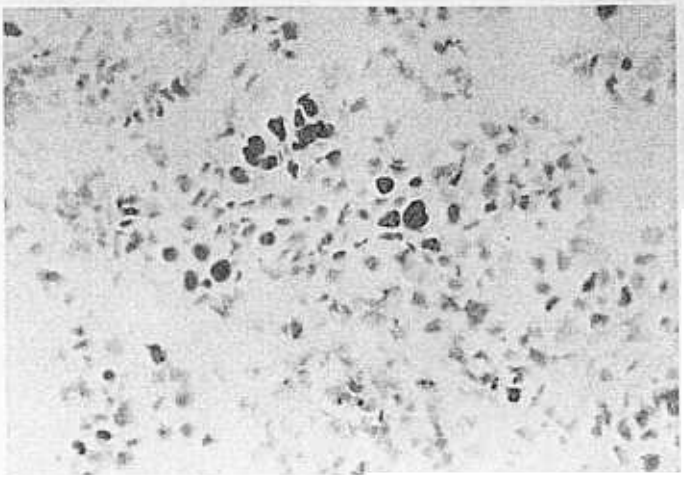

Fig. 1. Photomicrograph of gastric cancer stained with Ki-67 monoclonal antibody.

logic findings and Ki-67 labeling rates is summarized in table 1. The tumors were divided into two groups by the Ki-67 labeling rates. The tumors named 'high Ki-67 labeing' had Ki-67 labeling rates $\geq 25\%$, and those with Ki-67 labeling rates $< 25\%$ were named 'low Ki-67 labeling'. The tumors showing high Ki-67 labeling rates are more likely to have liver metastasis and lymph node involvement. Regarding the histological type, 81% (21/26) of the well-differentiated cancers showed Ki-67 labeling rates $> 25\%$, whereas 52% of poorly differentiated cancers revealed Ki-67 labeling rates $> 25\%$. Two mucinous cancers were classified as poorly differentiated carcinoma. There was a significant difference between these two groups. Larger tumors with a diameter > 6 cm more frequently showed high Ki-67 labeling rates than those with a diameter < 6 cm. However, there was no correlation between Ki-67 labeling rates and peritoneal dissemination, serosal invasion, or macroscopic type. Ki-67 labeling rates and prognosis indicated that the patients whose tu-

Table 1. Correlation of Ki-67 labeling rate and clinicopathologic findings

Variables	Ki-67 labeling rate		p
	$< 25\%$	$\geq 25\%$	
Hepatic metastasis			
Negative	27	38	
Positive	0	6	
Peritoneal metastasis			
Negative	20	27	NS
Positive	7	17	
Nodal status			
Negative	9	6	
Positive	18	38	
Serosal invasion			
Negative	11	14	NS
Positive	16	30	
Histologic type			
Differentiated	5	21	< 0.05
Poorly differentiated	22	23	
Macroscopic type			
Localized	8	15	NS
Infiltrating	19	29	
Tumor size			
> 6 cm	16	13	< 0.05
≤ 6 cm	11	31	

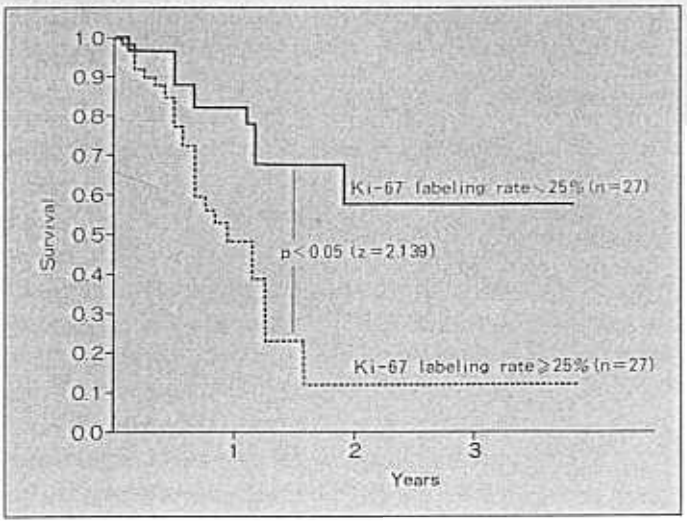

Fig. 2. Survival curves of patients with gastric cancer, subdivided according to the Ki-67 labeling rate.

Table 2. Clinicopathologic findings and Ki-67 labeling rate as prognostic factors in 71 patients with advanced gastric cancer

Variable		Cases	Univariate analysis		Multivariate analysis	
			Z value	p value	F value	p value
Hepatic metastasis	negative	65	1.421	0.1553	1.137	0.2906
	positive	6				
Peritoneal metastasis	negative	47	3.702	0.0002	3.701	0.0591
	positive	24				
Nodal status	negative	15	3.408	0.0001	4.831	0.0316
	positive	56				
Serosal invasion	negative	25	2.750	0.0060	2.297	0.1346
	positive	46				
Histologic type	differentiated	26	0.095	0.9241	0.039	0.8451
	poorly differentiated	45				
Macroscopic type	localized	23	2.271	0.0231	1.119	0.2942
	infiltrating	48				
Tumor size	>6 cm	29	2.536	0.0112	0.532	0.4686
	≤ 6 cm	42				
Ki-67 labeling rate	$\geq 25\%$	27	2.139	0.0325	4.431	0.0393
	$<25\%$	44				
Age	>60 years old	15	0.182	0.8559	0.659	0.4201
	≤ 60 years old	56				
Sex	male	39	0.142	0.8871	1.170	0.2836
	female	32				

mors had high Ki-67 labeling rates died significantly earlier than those with lower proliferative activity (fig. 2). When the Ki-67 labeling rates and 9 clinicopathologic parameters, as conventional prognostic factors, were entered simultaneously into the regression model, nodal status and Ki-67 labeling rate emerged as independent prognostic factors (table 2). However, serosal invasion, tumor size, histologic type, age, sex and macroscopic type were of little independent prognostic value.

Discussion

There have been no reports on the expression of Ki-67 antigen of gastric cancer by immunohistochemistry. The antigen recognized by Ki-67 monoclonal antibody is known to be present in the G_1, S, G_2, and M phase of tumor cells, and the Ki-67 labeling rate substantially provides an estimate of the proliferative activity of the tumor. In general, high proliferative activity of tumors is associated with poor prognosis and survival [10–14]. In breast cancer, high numbers of Ki-67-positive cells are found in tumors with high mitotic rates, high nuclear grade, and high histologic grade [20]. Carpin et al. [21] described that Ki-67 immunostaining was related to vascular invasion, and the presense of axillary lymph node metastases, and that the Ki-67 immunocytochemical assay provides relevant information on selecting subgroups of patients at higher risk for relapse. In our study, tumors showing high Ki-67 labeling rates were more likely to have liver metastasis and lymph node involvement. In addition, tumors with low Ki-67 labeling rates were associated with favorable prognosis, whereas those with high Ki-67 labeling rates were related to poor prognosis. Patients with tumors showing high Ki-67 labeling rates had a fivefold higher relative risk of death, as compared to those with tumors showing low Ki-67 labeling rates. In addition, the Cox proportional hazard model revealed that the Ki-67 labeling rate is a powerful independent prognostic indicator of gastric cancer. The high malignant potential of tumors with high Ki-67 labeling rates may be related to the potential of hematogenous and lymphatic metastases. These results indicate that the in situ determination of the growth fraction by Ki-67 antibody may be a reliable prognostic marker of advanced gastric cancer. Furthermore, the immunohistochemical assay of Ki-67 labeling rates obtained by biopsied tissues may be useful in designing the operative procedure and multimodal therapy for the individual patients with advanced gastric cancer preoperatively. We are now trying to measure the proliferative activity of biopsied materials using Ki-67 monoclonal antibody.

References

1 Nishi M, Nakajima T, Kajitani T: The Japanese Research Society for Gastric Cancer – The general rules for the gastric cancer study and an analysis of treatment results based on the rules; in Preece PE, Cuschieri A, Wellwood JM (eds): Cancer of the Stomach. London, Grune & Stratton, 1986, pp 107–122.
2 Kajitani T: Results of surgical treatment of gastric cancer. Gann Monogr Cancer Res 1968;3:245–251.
3 Inokuchi K, Kodama Y, Sasaki O, et al: Differentiation of growth patterns of early gastric carcinoma determined by cytophotometric DNA analysis. Cancer 1983;51:1138–1141.
4 Kennedy BJ: TNM classification for stomach cancer. Cancer 1970;26:971–983.

5 Cady B, Ramsden DA, Stein A, et al: Gastric cancer. Am J Surg 1977;133:423–429.
6 Hoerr SO: Prognosis of carcinoma of the stomach. Surgery Gynec Obstet 1973;137:204–209.
7 Kaibara N, Nishidoi H, Kimura O, et al: Analysis of results of surgical treatment of gastric cancer. Dig Surg 1988;5:124–127.
8 Hattori T, Hosokawa Y, Sugihara H: Analysis of DNA ploidy patterns of gastric carcinomas of Japanese. Cancer 1984;1541–1597.
9 McDivitt RW, Stone KT, Meyer JAA: A method of dissociation of viable human breast cancer cells that produces flow cytometric kinetic information similar to that obtained by thymidine labeling. Cancer Res 1984;44:2628–2633.
10 Kallioniemi OP, Punnonen R, Mattila J, et al: Prognostic significance of DNA index, multiploidy, and S-phase fraction in ovarian cancer. Cancer 1988;61:334–339.
11 Tribukait B, Hammerberg C, Rubio C: Ploidy and proliferation patterns in colorectal adenocarcinomas related to Duke's classification and to histopathological differentiation. Acta Pathol Microbiol Immunol Scand [Sect. A] 1983;91:89–95.
12 Volm M, Hahn EW, Mattern J, et al: Five-year follow-up study of independent clinical and flow cytometric prognostic factors for the survival of the patients with non-small cell lung carcinoma. Cancer Res 1988;48:2923–2928.
13 Yonemura Y, Sugiyama K, Fujimura T, et al: Correlation of DNA ploidy and proliferative activity in human gastric cancer. Cancer 1988;62:1497–1988.
14 Sledge G, Eble JN, Roth BJ, et al: Relation of proliferative activity in patients with advanced germ cell tumor. Cancer Res 1988;48:3864–3868.
15 Tibiana M, Pejovic MJ, Ranaud A, et al: Kinetic parameters and course of the disease in breast cancer. Cancer 1981;47:937–943.
16 Gerdes J, Lemke H, Baisch H, et al: Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67 J Immunol 1984;133:1710–1715.
17 Sasaki K, Murakami T, Kawasaki M, et al: The cell cycle associated change of the Ki-67 reactive nuclear antigen expression. J Cell Physiol 1987; 133:579–584.
18 Japanese Research Society for Gastric Cancer: The general rules for the gastric cancer study in surgery and pathology. Jpn J Surg 1981;11:127–139.
19 Cox DR: Regression models and life table. J R Stat Soc B 1984;34:187–220.
20 Lelle RJ, Heidenreich W, Stauch G, et al: The correlation of growth fractions with histologic grading and lymph node status in human mammary carcinoma. Cancer 1987;59:83–88.
21 Carpin C, Andrac L, Vacheret H, et al: Multiparametric evaluation (SAMBA) of growth fraction (monoclonal Ki67) in breast carcinoma tissue sections. Cancer Res 1988;48:4368–4374.

Received: March 26, 1990
Accepted: June 14, 1990

Yutaka Yonemura, MD
Department of Surgery II
School of Medicine
Kanazawa University
Takara-Machi 13–1
Kanazawa City, Ishikawa (Japan)

Author

The Author
appears in

(A) = Abst