Evaluation of proliferating markers Ki-67, PCNA in gastric cancers

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Abstract

Tumours from 45 patients with advanced gastric cancer were assessed by immunohistochemistry. Tissue sections were fixed in 10% buffered formaldehyde solution, embedded in paraffin and stained immunohistochemically with anti-human Ki-67 and PCNA antibodies. No correlation was found between Ki-67, PCNA protein expression, the age of patients and the localization of tumour. A significant, positive association was observed between the expression of Ki-67, PCNA and tumour differentiation and Lauren's classification. Also a strong correlation was found between lymph node involvement and the expression of Ki-67 protein. These data suggest that the expression of Ki-67, PCNA proteins were closely connected with the high grade of tumour malignancy.

Key words: Ki-67, PCNA, gastric cancer.

Introduction

Recent studies suggest that proliferating activity may provide important prognostic information in different types of tumours. Ki-67 and PCNA, the two most frequent used cell proliferation markers, recognized nuclear antigens as associated with all the phases of the cell-cycle expect G0 [1]. Antigen Ki-67, was originally discovered by Gerdes [1, 2]. The proliferating cell nuclear antigen (PCNA), the level of which increases in the nucleus, achieved the maximal level during the S phase (nearby before the beginning of DNA synthesis) [3].The aim of this study was to evaluate the expression of Ki-67, PCNA antigens in cases of gastric cancer in correlation with chosen clinico-pathological parameters.

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Material and methods

The 45 patients with gastric cancer, treated by surgery at the 2nd Department of Surgery, Medical University of Białystok, Poland, were selected for this study. Tissue specimens were collected immediately after tumour removal, fixed in 10% buffered formaldehyde solution and embedded in paraffin. Then, they were histopatologically examined, using standard haematoxylin-eosin staining, according to the TNM classification and Lauren's classification.

Immunohistochemistry: Slides of 4µm-thick serial sections of the primary tumour were prepared from each patient. The immunolocation of Ki-67 (M7240, DAKO) and PCNA (M0879, DAKO) was performed, using the labelled streptovidin biotin (LSAB) method protocol, described by DAKO (LSAB+HRP Kit, DAKO, Poland). In brief, the slides from each patient were dewaxed, using xylene and transferred to alcohol. Then, they were placed in citric buffer (pH=6.0) and heated in a microwave oven (700W) for 10 minutes to expose antigens. Endogenous peroxidase activity was blocked by incubating the section with 3% hydrogen peroxide in methanol for 10 minutes. After washing with PBS, the slides were incubated at 20°C for one hour with monoclonal antibodies. Anti-human Ki-67 protein monoclonal antibody (M7240, DAKO, dilution 1:100) was used for some slides and mouse anti-human PCNA monoclonal antibody (M0879, DAKO, dilution 1:200) was used for other slides. The reaction products were visualised with diaminobenzidine DAB (DAKO S3000, DAKO, Poland). Nuclear immunostaining was observed for both proteins (Fig. 1 and Fig. 2). Ki-67 and PCNA expression was semi quantitatively assessed in neoplastic cells of the primary tumour and defined as follows: Ki-67 and PCNA-negative (the lack of reaction or a reaction, present in less than 20% of cells) and Ki-67 and

Table 1. Expression of Ki-67, PCNA proteins and chosen parameters in gastric cancers.

Parameters		Expression of Ki-67		P value	Expression of PCNA		P value
		negative	positive		negative	positive	
Sex	Female	2(14%)	12(86%)	0.9	9(64%)	5(36%)	1
	Male	14(45%)	17(55%)		21(67.7%)	10(32,3%)	
Localization of tumour in the stomach	1/3 of up part	2(28.5%)	5(71.5%)	0.14	5(71.4%)	2(28.6%)	0.1
	1/3 of middle part	1(11%)	8(89%)		5(55.5%)	4(44.5%)	
	1/3 of down part	13(48%)	14(52%)		20(74%)	7(26%)	
	All stomach	0(0%)	2(100%)		0(0%)	2(100%)	
Lauren	Intestinal type	15(44%)	19(56%)	0.07	30(88.2%)	4(11.8%)	0.0001
	Diffuse type	1(9%)	10(91%)		0(0%)	11(100%)	
pN	absent	14(70%)	6(30%)	0.0001	16(80%)	4(20%)	0.1
	present	2(8%)	23(92%)		14(56%)	11(44%)	
Tumour differentiation	G2	14(66.7%)	7(33.3%)	0.0001	18(85.7%)	3(14.3%)	0.01
	G3	2(8.3%)	22(91.7%)		12(50%)	12(50%)	

Figure 1. Nuclear expression of Ki-67 in gastric cancer cells.

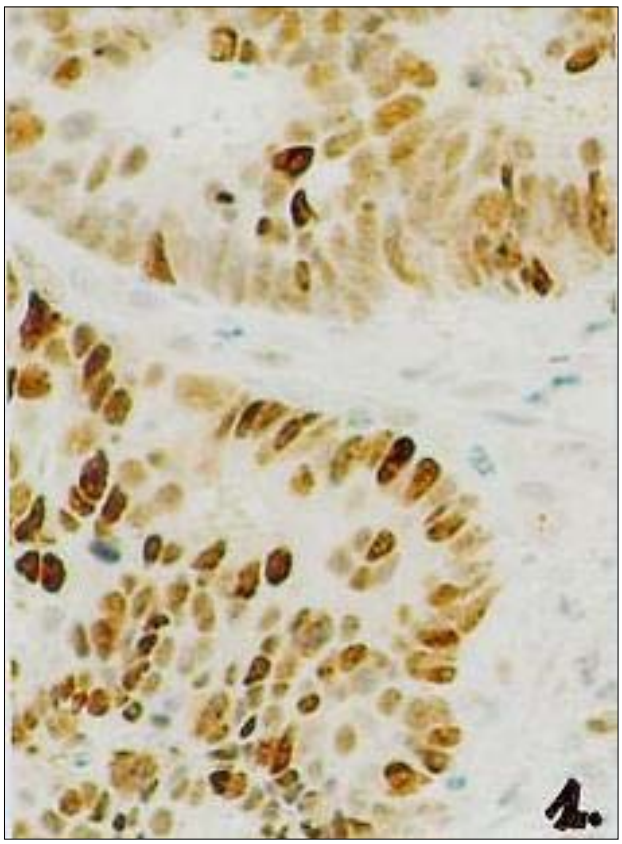
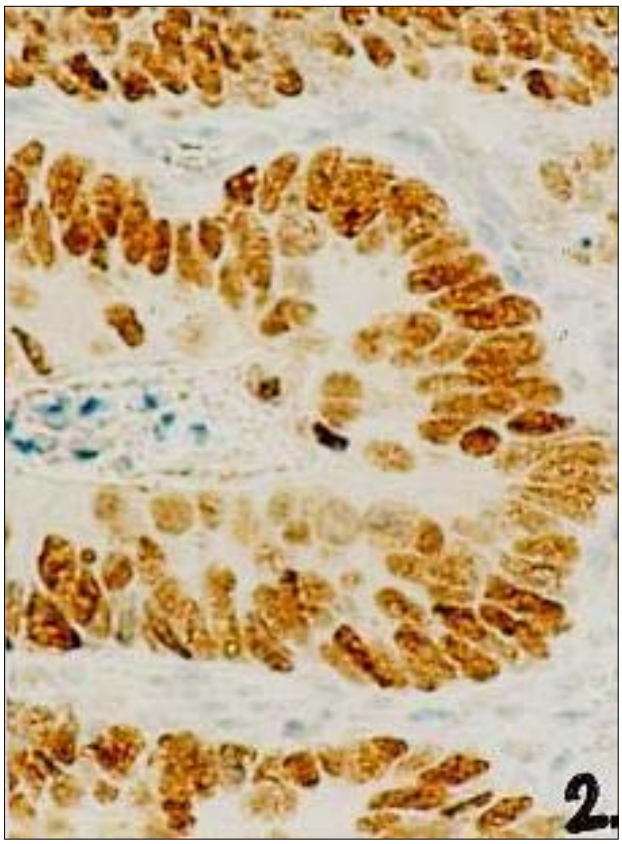


Figure 2. Nuclear expression of PCNA in gastric cancer cells.



PCNA-positive (a reaction present in more than 20% of cells). The percentage of Ki-67 and PCNA positive cells was calculated in, at least, 500 neoplastic cells per sample, using a light microscope (x400). The χ^2 and Fisher's test were used for statistical analysis. P-values, smaller than 0.05, were considered statistically significant.

Results and discussion

Statistical analysis showed correlations between the expression of Ki-67, PCNA proteins and tumour differentiation and the tumour type (Lauren's classification). A strong expression was observed between the presence of lymph node metastasis and the expression of Ki-67 (p=0.0001). However, we did not find any of such correlation for PCNA (Table 1).

Maedera et al. [4] showed that, in patients with invasion, deeper than the muscle layer, the labelling index of PCNA was significantly higher than that in patients with only mucosal or submucosal invasion. The PCNA labelling index became higher as the histological stage increased. Similary, Elpek et al. [5] showed that the PCNA levels increased with histological stages and with lymph node involvement. Oya et al. [6] observed a sig-

nificantly higher value of Ki-67, PCNA in patients with lymph node metastasis. In our study, 23/25 patients with lymph node involvement showed Ki-67 expression higher than 20%. Műller et al.[7] showed a correlation between cell proliferation and Lauren's classification. The carcinomas of intestinal type showed a statistically significantly higher value for LI Ki-67 than signet-ring cell carcinomas. No correlation was found in the study between the proliferative activity and the depth of invasion (pT), lymph node involvement (pN) and the grade of differentiation [7]. Lee et al. [8] showed a positive correlation between the PCNA index and increasing age, male gender, larger tumour size, the type of tumour, according to Borman's classification (type I/II) and tumour differentiation. They also observed that, according to the Lauren classification, the intestinal type of gastric cancer had a more positive PCNA expression than the diffuse type of gastric cancer. We found the opposite: 30/34 cases of intestinal type were negative for PCNA expression. Xu et al. [9] showed an association between the Ki-67 expression in gastric cancer with peritoneal metastases to the liver, the ovary and the adrenals, but there was no correlation between the histological type, the grade of tumour growth, the depth of invasion and lymph node involvement. Yonemura et al. [10] described that large tumours, with diameter >6 cm, showed a higher Ki-67 expression than those with diameter <6 cm. However, there was no association between Ki-67 expression and peritoneal metastases, serosal invasion or macroscopic type. These results indicate that the high levels of expression of Ki-67 and PCNA were associated with tumour malignant parameters, such as lymph node involvement, or the grades of differentiation in gastric cancer.

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