Evaluation of protein products of cell cycle regulating genes in gastric cancer

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Abstract

Formalin fixed, paraffin embedded tissue samples of 45 gastric carcinomas, resected curatively, were used for the study. An immunohistochemical analysis employed monoclonal antibodies: p53 (No N1581, DAKO) and $p27^{KIP1}$ (NCL-$p27^{KIP1}$, Novocastra). Positive nuclear protein expression was assessed at the 30% level. We found no correlations between the expression of either protein and Lauren's classification, the age of patients and tumour localization. Borderline significance of $p=0.07$ was noted in the association of p53 expression and histological differentiation. However, a decrease of p27 expression and an overexpression of p53 correlated with the presence of lymph node metastases ($p<0.01$). Simultaneously, the expression of p27 protein in main mass of tumour correlated with the lack of p53 expression in the main mass and lymph node metastases.

Key words: p53, p27, gastric cancer.

Introduction

Loss of cell-cycle regulation can play an important role in the process of tumour genesis and strongly correlates with the aggressiveness and progression of many neoplasms [1, 2]. Progression of the cell cycle depends on interactions between many cell-cycle regulators [3]. The cell cycle is controlled by cyclin-dependent kinasae (Cdks) [3]. Those cyclins regulate cell cycle and cell proliferation by checking G1-S and G2-M control points. Cdks are inhibited by many proteins, including p27KIP1, which is representative of the CIP/KIP CDK family of inhibitors [4]. In normal cells, the evaluation of p27 increases before the resting phase and quickly declines, when the cells are ready to enter a subsequent cell cycle [4]. The loss of p27 expression in cells is connected with susceptibility to carcinogenesis. p53 is also a regulator of the cell-cycle, which can stop cell cycle at G1/S and G2/M points. Inactivation of p53 protein by mutation has been found in many different types of neoplasia in humans [5,6]. Neoplastic cells with an overexpression of mutated p53 protein cannot regulate the progression of cell cycle in G0-G1 phase, and these tumours continue to grow. The aim of this study was to evaluate p53 and p27 expression in correlation with selected anatomo-clinical parameters.

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Materials and methods

Forty-five (45) patients with gastric cancer, treated by surgery at the 2nd Department of Surgery, Medical University of Białystok, Poland. were selected for the study. Tissue specimens were collected immediately after tumour removal, fixed in 10% buffered formaldehyde solution and embedded in paraffin. They were then histopathologically examined, using standard haematoxylin-eosin staining, according to the TNM classification and Lauren classification. Immunohistochemistry: Slides of 4μm thick serial sections of the primary tumour were prepared from each patient. Also, slides of metastatic lymph nodes were prepared from each patient. A Standard avidin-biotin immunoperoxidase (Novostain Super ABC Kit universal) method was used for the detection of p27KIP1 (NOVOCASTRA, No NCL-$p27^{KIP1}$, Biokom, Poland) expression. The immunolocalization of p53 protein was performed, using the labelled streptavidin biotin (LSAB) method protocol, described by DAKO (DAKO, LSAB Kit, Dako, Poland). In brief, the slides from each patient were de-waxed, using xylene and transferred to alcohol. They were then placed in citric acid buffer (pH=6.0) and heated in a

Table 1. Expression of p27, p53 proteins and selected parameters.

Parameters		Expression of p27		p	Expression of p53		p
		negative	positive		negative	positive	
Localization	1/3 of up part	3(43%)	4(57%)	0.1	4(57%)	3(43%)	0.3
	1/3 of middle part	8(90%)	1(10%)		2(22%)	7(78%)	
	1/3 of down part	19(70%)	8(30%)		16(60%)	11(40%)	
	All stomach	2(100%)	0(0%)		1(50%)	1(50%)	
Sex	female	12(86%)	2(14%)	0.2	8(57%)	6(43%)	1
	male	20(65%)	11(35%)		18(58%)	13(42%)	
Lymph node metastases	absent	9(45%)	11(55%)	0.001	18(90%)	2(10%)	0.0001
	present	23(92%)	2(8%)		5(20%)	20(80%)	
Lauren's classification	Intestinal type	24(70%)	10(30%)	1	20(59%)	14(41%)	0.1
	Diffuse type	8(73%)	3(27%)		3(27%)	8(73%)	
Histological type	G2	17(81%)	4(19%)	0.2	14(67%)	7(33%)	0.07
	G3	15(63%)	9(37%)		9(37%)	15(63%)	

Table 2. Expression of p53 and p27 proteins in the main mass of tumour and lymph node metastases.

Expression of		p27 in the main mass of tumour		P	p27 in lymph node metastases		p
		negative	positive		negative	positive	
p53 in main mass	negative	11(48%)	12(52%)	0.001	22(96%)	1(4%)	0.4
	positive	21(91%)	1(9%)		19(86%)	3(14%)	
p53 in lymph node	negative	14(54%)	12(46%)	0.003	24(92%)	2(8%)	1
	positive	18(95%)	1(5%)		17(89%)	2(11%)	

microwave oven (700W) for 15 minutes to expose antigens. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide in methanol for 10 minutes. After washing with PBS, the slides were incubated overnight at 4°C with monoclonal antibodies. Anti-human p27 protein monoclonal antibody (Novocastra, No NCL-p27, dilution 1:50, Biokom, Poland) was used for one slide, while for the other slides, mouse anti-human p53 monoclonal antibodies (clone DO-7; M7001; dilution 1:100; Dako, Poland) were applied. The reaction products were visualized with diaminobenzidine DAB (DAKO S3000, Dako, Poland). Nuclear immunostaining was observed for both proteins. p53 and $p27^{KIP1}$ expression was semi quantitatively assessed in neoplastic cells of the primary tumours and lymph node metastases and defined as follows: p53 and p27-negative (lack of reaction and the reaction present in less than 30% of cells) and p53 and p27-positive (the reaction present in more than 30% of cells). The percentage of p53 and p27 positive cells was calculated in, at least, 500 neoplastic cells per sample, using a light microscope (x400). The χ^2 and Fisher's exact test were used for statistical analysis. P values, smaller than 0.05, were considered statistically significant.

Results and discussion

Even though much attention has recently been paid to the evaluation of p53 and p27 expression in different carcinomas, the results and clinical implications remain conflicting [7,8]. Therefore, it was the aim of this study to evaluate the role of these factors after curative surgery solely for gastric cancer. In the present study, we found no associations between the expression of p53, p27 proteins and Lauren's classification, the patients' age and tumour localisation (Table 1). Furthermore, the expression of $p27^{KIP1}$ protein was observed in 13/45 cases, whereas the expression of p53 was detected in 22/45 cases of gastric cancers. It has been found in some studies that the nuclear accumulation of mutated p53 protein is associated with an unfavourable prognosis of gastric cancer [9]. However, other studies have not confirmed this association [8]. In most reports, the loss of $p27^{KIP1}$ expression and the overexpression of p53 were observed in cases with high aggressiveness. In this study, we found no association between the expression of $p27^{KIP1}$ and the histological grade of tumour. However, we observed a strong association between the overexpression of p53 protein in G3 gastric cancers. In our study, we observed that the overexpression of p53 strongly correlated

with the presence of lymph node metastases ($p<0.0001$), where there was a simultaneous loss of $p27^{KIP1}$ protein expression in the main mass of tumour. Our results strongly correspond with the results obtained by other authors [10], who observed an association between a decreased $p27^{KIP1}$ expression and the depth of invasion, lymph node involvement and vascular invasion. Moreover, the loss of $p27^{KIP1}$ expression was the parameter of short survival in patients with oesophageal, prostate, and gastric carcinomas [10, 11, 12]. Li J-Q et al. [13] showed that, in colorectal neoplasms, the loss of $p27^{KIP1}$ promotes lymph node metastasis. At the present study, we also analysed the association between the expression of $p27^{KIP1}$ in the main mass of tumour and lymph node metastases in correlation with p53 expression at the same localization (Table 2). We noted that the loss of $p27^{KIP1}$ expression in the primary tumour was strongly associated with the overexpression of p53 protein in the main mass of tumour and lymph node metastases. Our results suggest that the overexpression of p53 influenced the loss of p27 expression and the presence of lymph node metastases in the investigated gastric cancers.

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