Expression of P16 and PDGFR-Beta in gastric adenocarcinoma

Expressão do P16 e do PDGFR-Beta no adenocarcinoma gástrico

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A B S T R A C T

Objective: To detect immunohistochemistry expression of p16 and PDGFR-beta on gastric adenocarcinoma. **Methods:** Thirty six patients submitted to surgery for gastric adenocarcinoma between 1998 and 2002 at Santa Casa de Porto Alegre Hospital have been studied. Variables investigated were: age, gender, tumour size and localization, number of dissected and metastatic nodes, histological type, surgical resection extension and pathological staging. **Results:** No expression of PDGFR-beta has been detected on surgical specimens. Concerning to p16, loss of expression lower than 10% and 1% has been detected respectively on 89% and 79% of the specimens studied. **Conclusion**: There has been no correlation among p16 loss and variables studied.

Key words: Stomach Neoplasms . Adenocarcinoma. Genes p16. Immunohistochemistry.

INTRODUCTION

Gastric adenocarcinoma has been the main cause of cancer death during most of the 20th century, now overcame by lung cancer. Annually 750,000 new cases are diagnosed. Great geographic variations are seen and highest incidences can be found in Japan, South America, Eastern Europe and Middle East[1]. It is twice as frequent in men as in women[1,2], has a low incidence before the 4th decade with a peak incidence in the 7th.[1] In Brazil 23,000 new cases and 11,000 deaths are estimated to occur in 2005[3].

The prognosis of gastric adenocarcinoma is poor, mainly because lack of symptomatology and late diagnosis, with an overall survival of 5-15% in five years[1,4]. In Japan, where this disease is endemic but diagnosis is usually done at an early stage due to wide endoscopic availability, survival rate is 50% in five years[1]. Complete resection of all gross and microscopic disease is the only potentially curative treatment. However, disease recurs in 80% of patients even after curative resection[1].

Oncogene p16 is implicated in pathogenesis of many human tumours and even in regulation of normal cellular growth, together with cycling, tyrosine kinases and tumour transforming and growth factors, like TGF-alpha and -beta and platelet derived growth factors (PDGF) ligands and receptors (alpha and beta). Inherited mutations of p16 are associated with hereditary melanomas. Deletions and acquired inactivation of p16 are found in 75% of pancreatic carcinomas, 40-70% of glioblastomas, 50% of oesophageal carcinomas and 20% of non-small cell lung cancer[5]. Recent papers have demonstrated relation between p16 inactivation and development of stomach cancer. Forty to ninety per cent of gastric adenocarcinomas show inactivation of p16,[6-11] appearing to have relation with cellular differentiation[7,10,12]. Expression of p16 is decreased in node metastasis[6]. Apparently there is no difference in expression between intestinal and diffuse-type gastric adenocarcinoma[12].

Platelet derived growth factor receptor-beta (PDGFR-beta), a tyrosine kinase surface receptor, is important in growth, differentiation and cell death controls[13,14]. PDGFR has been found activated and mutated in gastric stromal tumour where c-KIT, the most commonly marker found, is in wild type[15,16]. PDGF receptors act over stromal origin cells and are not expressed in epithelial cells under normal physiologic conditions[17]. Expression of PDGFR has been described in dermatofibromiosarcoma, chronic myelocytic leukemia and in gastrointestinal stromal tumours (GISTs),[18,19] besides other solid tumors like glioblastomas and prostate cancer[13,18]. PDGF-beta and its receptor have not been studied concerning expression and response to cellular growth inhibitors on non-stromal gastric tumours.

METHODS

Thirty six patients submitted to surgery for gastric adenocarcinoma between 1998 and 2002 at Santa Casa de Porto Alegre Hospital have been studied with the aim to determine prevalence of p16 and PDGFR-beta.

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None of the 36 patients had past history of any other malignant tumours (except to skin squamous and basal cell tumours), of pre-operative chemo or radiation therapy, which would exclude them from the study.

Variables investigated were: age, gender, tumour size and localization, number of dissected and metastatic nodes, histological type, extent of surgical resection and pathological staging. Clinical and pathological data were collected from patient charts as well as from surgical reports.

Surgical specimens analysis (haematoxilin-eosin, HE) included assessment of depth of tumour invasion on the gastric wall, nodal metastasis, histological grade and histological type (intestinal or diffuse, according to Lauren's classification).

Immunohistochemistry

Specimens were processed according to routine of Pathology Department of Clinicas Hospital of Porto Alegre. Mouse monoclonal antibody anti-p16^{ink4a} (DakoCytomation, Carpinteria, California, USA) and rabbit polyclonal antibody anti-PDGFR beta Ab-1 (DakoCytomation, Carpinteria, California) were used to IH valuation, diluted on PBS saline solution at 1:100 and 1:75, respectively. Positivity was determined with ABS method (streptavidin-biotin-peroxidase complex; LABS+System HRP, DakoCytomation, Carpinteria, California, USA).

Slide sample analysis

Up to 20 high-power fields (400X) of each sample were captured to computer (Image Pro-Plus, v 4.5.1.2.2, Media Cybernetics). On computer screen, after insertion of a grid over pictures, total number of cancer cells and total number of immunoreactive cancer cells were counted. Nuclear brown staining over cytoplasmic staining was considered to be positive for p16. Lung cancer samples were used as outer positive controls. Percentage of p16 positivity was calculated dividing the number of stained cancer cells by the total number of cancer cells, and then, multiplied by 100. Two thresholds were regarded as loss of expression: <10% and <1%. A qualitative scale was determined to PDGFR-beta, according to staining intensity: (1) as no staining, (2) weak, (3) moderately and (4) strong staining. Breast cancer samples were used as outer positive controls. To be considered positive the sample should have at least 10% of moderately or strong stained cancer cells.

Statistical analysis

Qualitative data was described as media and standard deviation. Percentage and frequency were used as categorical variables. Fischer's exact test was used comparing p16 expression to gender and histological type. Student's t test was used comparing p16 to size, age and number of metastatic nodes. Pathological stage was compared to p16 using Chi-Square Test (P value <0.05 to all tests). Statistical analysis was performed with Excel Microsoft Office 2003.

RESULTS

Thirty six patients with gastric adenocarcinoma were studied; 20 (55.5%) males and 16 (44.5%) females (Table 1). Overall median age was 59.2 (13.2); men median age was 59.2 (10.7) and women 59.3 (16.6). The difference was not statistically significant.

Tumour location was as follows: 2 (5.5%) cardia, 4 (11.1%) cardia and fundus, 5 (13.9%) body and fundus, 1 (2.8%) body, 2 (5.5%) body and antrum, 19 (52.8%) antrum and 3 (8.3%) pylorus. Twenty three (63.9%) patients were submitted to partial gastrectomy, 11 (33.3%) to total gastrectomy and 2 (5.5%) to oesophago-gastrectomy. The median (sd) number of lymph nodes dissected was 15.9 (10.4), among this, 5 (5.2) showed metastatic disease (30.8%). Analysis of extent of lymph node dissection data was not possible due to lack of this information in most of surgical records (Table 2).

Concerning to Lauren classification 64% was diffuse type and 36% was intestinal type. Histological grade included 91.6% of poorly differentiated, 5.6% moderately and 2.8% well-differentiated tumours. Pathological staging, according to TNM 6th edition demonstrated 13.9%, 5.5%, 8.3%, 27.8%, 27.8%, and 16.7% for IA, IB, II, IIIA, IIIB and stage IV, respectively.

P16 analysis showed positivity lower than 10% in 32 (89%) patients and lower than 1% in 28 (78%). Positivity to p16 was compared (10% and 1%

Table 1 - Patient clinical and pathological characteristics.

Features	n	Percentage
Age (median, sd)	36	59,2 (13,2)
Sex male:female	20:16	55:45
Type		
Intestinal	13	36
Diffuse	23	64
Grade		
Well	1	2,8
Moderately	2	5,6
Poorly	33	91,6
Lymph nodes (sd)	573	15,9 (10,4)
Metastic lymph nodes (sd)	178	4,9 (5,2)
Staging		
IA	5	13,9
IB	2	5,5
II	3	8,3
IIIA	10	27,8
IIIB	10	27,8
IV	6	16,7

Data presented as mean and standard deviation or frequency (percentage) unless otherwise specified.

respectively) to age, gender, tumour size, metastatic nodes, pathological staging and Lauren histological type and these results are presented on table 2. There was no statistical difference in all but age and tumour stage for a p16 loss when comparing >1% to <1% expressions (Table 2).

DISCUSSION

It was a random decision to study simultaneously p16, which loss of expression has been exhaustively studied in gastric tumours and PDGFR-beta, which association to gastric adenocarcinoma has not yet been found in medical literature. Although there is not a close relation between both markers, it could be speculated that overexpression of PDGFR-beta in cancer cells, increasing mitogenic stimuli to cell could be associated to loss of activity of p16, which is one of the important cell cycle inhibitors. Besides, promising results in treatment of these patients with tyrosine kinase inhibitors, such as imatinib (former known as STI571, Gleevec, Novartis Pharmaceutical Corp, East Hanover, NJ, USA) have been reported for gastrointestinal stromal tumours (GISTs) in which PDGFR is overexpressed[13,15,20,21]. In a gastric carcinoma animal model, it was demonstrated increase in antitumour and cytotoxic effects of 5-FU and paclitaxel when combined with imatinib[22].

In this study, 89% of cases had loss of expression of p16 (positivity lower than 10%) which is similar to literature data of 40%-90% loss[3,6,7]. Seventy eight percent of the cases showed positivity lower than 1%. This lower threshold was chosen to determine the power of p16 loss expression and the studied variables.

Expression of PDGFR-beta was undetected in all of the 36 cases studied. The association between this marker and gastric adenocarcinoma have not been found in medical literature, although its expression (PDGFR alpha and beta) has been found in other epithelial tumours such as cholangiocarcinoma[23], ovary[24] and breast cancer[25].

There was no statistical difference between median ages for both expressions, in contrast to american reports, where women tend to be older.[1] Like other reports,[6] this paper did not find relation of p16 expression with either gender or age.

Concerning to Lauren histological type, against expectations the diffuse type was predominant,[1,26-28] no statistical difference between Lauren histological type and p16 expression in the two thresholds studied was found as previously reported,[12] although no agreement on this issue have been reached[7,27].

Although it is known that tumours with p16 loss tend to have a higher metastatic potential immunohistochemistry to p16 in lymph nodes was not carried out in this study, since overall p16 expression is much lower in metastatic lymph nodes than in primary lesion[6].

Table 2 - P16 loss according to clinical and pathological features.

	p16		p16	
Features	**<1% (n=28)**	**>1% (n=8)**	**<10% (n=32)**	**>10% (n=4)**
Male: Female	16:12	4:4	19:13	1:3
	P=1 (*ns*)*		*P*=1 (*ns*)*	
Age (sd) years				
	57,3 (13,6)	66,1 (9,1)	59 (13,6)	61,2 (9,8)
	P=0,05 †		*P*=0,7 (*ns*) †	
Tumour size (sd) cm	6,7 (3,8)	5,2 (3)	6,5 (3,6)	5,2 (3,6)
	P=0,28 (*ns*) †		*P*=0,55 (*ns*) †	
Histological type				
Diffuse	9	4	11	2
Intestinal	19	4	21	2
	P=1 (*ns*)*		*P*=1(*ns*)*	
Metastatic lymph nodes (sd)	4,5 (5)	6,6 (6)	44 (4,7)	9,5 (7,8)
	P=0,37 (*ns*) †		*P*=0,28 (*ns*) †	
Staging				
IA	5	0	5	0
IB	2	0	2	0
II	3	0	3	0
IIIA	5	5	8	2
IIIB	10	0	10	0
IV	3	3	4	2
	P=0,02 ‡		P=0,22 (ns) ‡	

*ns: not significant; *Fischer's Exact Test; † student's t Test ; ‡Chi-Square Test.*

In pathological staging 72% of patients was at advanced stage III or IV as a result of late diagnosis, though this was not significant (p16 loss and staging) in this study.

A great loss of p16 expression on both studied thresholds was demonstrated, although there were no statistical differences among variables. Further investigation is needed in these cases to establish its association to lymph nodes metastasis and survival, as well as for the mechanism involved on the p16 inactivation.

R E S U M O

Objetivo: *Detectar a expressão imunoistoquímica do p16 e do PDGFR-beta no adenocarcinoma gástrico.* ***Método:*** *Foram estudados 36 pacientes submetidos a cirurgia para adenocarcinoma gástrico entre 1998 e 2002 no Hospital da Santa Casa de Porto Alegre. As variáveis investigadas foram: idade, sexo, tamanho e localização do tumor, número de linfonodos dissecados, número de linfonodos metastáticos, tipo histológico, extensão da ressecção cirúrgica e estadiamento patológico.* ***Resultados:*** *Não foi detectada expressão do PDGFR-beta nas peças cirúrgicas. Em relação ao p16, detectou-se perda de expressão menor que 10% e menor que 1% respectivamente em 89% e 79% das peças estudadas.* ***Conclusão:*** *Não houve correlação entre a perda de p16 e as variáveis estudadas.*

Descritores: *Neoplasias gástricas. Adenocarcinoma. Genes, p16. Imunoistoquímica.*

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