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Original Article

Expression of p16 predicts poor outcome for patients with gastrointestinal stromal tumors

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Abstract: Gastrointestinal stromal tumors are the most common mesenchymal tumors found in the gastrointestinal tract. Their biological behavior is still predicted by a consensus scheme proposed by the U.S. National Institutes of Health. In this study, we investigated the prognostic significance of p16 protein expression in gastrointestinal stromal tumors. Expression of p16 protein was observed in 42.4% (92/217) of tumors and was significantly associated with a high mitotic count, tumor necrosis, recurrence or metastasis, and a higher-risk group. Patients with p16-expressing gastrointestinal stromal tumors showed a shorter overall survival and disease-free survival than those without p16 expression; however, p16 expression was not an independent prognostic factor. The risk of malignant behavior and the presence of recurrence or metastasis were independent prognostic factors. Expression of p16 protein predicts poor outcome and can be a useful marker to predict relapse or metastasis and aggressive behavior in gastrointestinal stromal tumors.

Keywords: Gastrointestinal stromal tumor, p16, risk, survival, immunohistochemistry

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract and characterized by oncogenic mutation in the *KIT* (80-85%) and platelet-derived growth factor receptor alpha (*PDGRFA*; 5-7%) gene [1, 2]. The majority of GISTs occur in adults and respond to targeted tyrosine kinase therapy. However, approximately 10-15% of GISTs are *KIT/PDGFRA* wild-type (WT) GISTs, which are less sensitive to tyrosine kinase inhibitors. The *KIT/PDGFRA* WT GISTs are heterogeneous tumors and include succinate dehydrogenase (SDH)-deficient, neurofibromatosis 1-associated, *BRAF* mutant, and quadruple WT GISTs [3].

GISTs show a wide range of biological behaviors, which are predicted by tumor size and mitotic counts [2]. However, it is hard to predict the biological behavior based on histological findings alone.

The p16 gene is a tumor suppressor that inhibits cell cycling by arresting cells in the G1-S phase. This genetic alteration results in loss of p16 protein expression in many human cancers [4, 5]. In contrast, p16 overexpression was observed in breast cancer and premalignant lesions, breast ductal intraepithelial neoplasia, carcinoma in situ of the cervix, and prostatic intraepithelial neoplasia [4, 6-9]. The prognostic significance of p16 expression status has been reported in GISTs, but the results were quite inconsistent in studies [10-14]. The aim of this study was to investigate the expression status of p16 in GISTs and to assess its clinical and pathological significance.

Materials and methods

Patient characteristics

Between 1997 and 2016, a total of 226 GISTs from the stomach (154 cases), small intestine (67 cases), colon and rectum (three cases), and extra-gastrointestinal locations (pelvic cavity and abdominal cavity) were evaluated in this study. Medical records were reviewed to determine each patient's age, sex, most recent follow-up visit, survival status, and the presence or absence of GIST-related disease. The following clinicopathological characteristics were al-

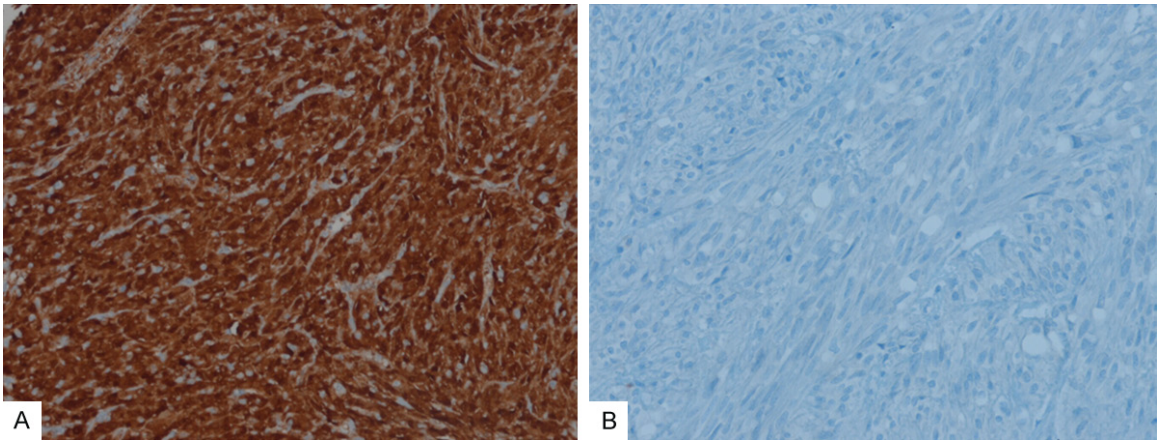

Figure 1. Immunohistochemical study for p16 in gastrointestinal stromal tumor. A. Expression, B. No expression.

Table 1. Correlation between clinicopathologic factors and p16 expression in gastrointestinal stromal tumors

Clinicopathologic factors	No.	p16 expression		*P*
		No	Expression	
Sex				0.646
Male	114	64 (56.1%)	50 (43.9%)	
Female	103	61 (59.2%)	42 (40.8%)	
Location				0.485
Stomach	146	89 (61.0%)	57 (39.0%)	
Small intestine	66	34 (51.5%)	32 (48.5%)	
Colorectum	3	1 (33.3%)	2 (66.7%)	
Extra-gastrointestinal	2	1 (50.0%)	1 (50.0%)	
Cell type				0.917
Spindle	199	114 (57.3%)	85 (42.7%)	
Epithelioid	7	4 (57.1%)	3 (42.9%)	
Mixed	11	7 (63.6%)	4 (36.4%)	
Mitosis				< 0.001
≤ 5/50 HPF	138	92 (66.7%)	46 (33.7%)	
> 5/50 HPF	79	33 (41.8%)	46 (58.2%)	
Risk				<0.001
Low	128	87 (68.0%)	41 (32.0%)	
Intermediate	40	21 (52.5%)	19 (47.5%)	
High	49	17 (34.7%)	32 (65.3%)	
Necrosis				0.002
No	182	113 (62.1%)	69 (37.9%)	
Yes	35	12 (34.3%)	23 (65.7%)	
Mucosal invasion				0.607
No	193	110 (57.0%)	83 (43.0%)	
Yes	24	15 (62.5%)	9 (37.5%)	
Recurrence or metastasis				< 0.001
No	192	121 (63.0%)	71 (37.0%)	
Yes	25	4 (16.0%)	21 (84.0 %)	

HPF, high-power field.

so assessed: tumor location, tumor size, mitotic count, tumor cell type, necrosis, mucosal ulceration, and recurrence or metastasis. The risk of malignant behavior was classified according to the system proposed by Miettinen and Lasota (the so-called AFIP criteria) [15] and further classified as low, moderate, or high risk. Overall survival (OS) was defined as the time from surgical resection to death or the last follow-up. The follow-up period ended in October 2016 (OS range: 0-215 months). This study was approved by our institutional Human Ethics Review Board.

Tissue microarray construction

Two to five 2-mm cores were obtained from the most representative tumor area of each block and arrayed in a new recipient block. Thus, 11 tissue microarray blocks were constructed. Four to five cores comprising breast carcinoma, thyroid papillary carcinoma, normal gastric mucosa, palatine tonsil, and uterine leiomyoma were used as control tissues.

Immunohistochemistry

Immunohistochemistry for p16 (clone E6H4, mouse monoclonal antibody, prediluted; Ventana, Tucson, AZ, USA) was performed after on-board heat-induced epitope retrieval in standard pH CC1 buffer (37°C, 32

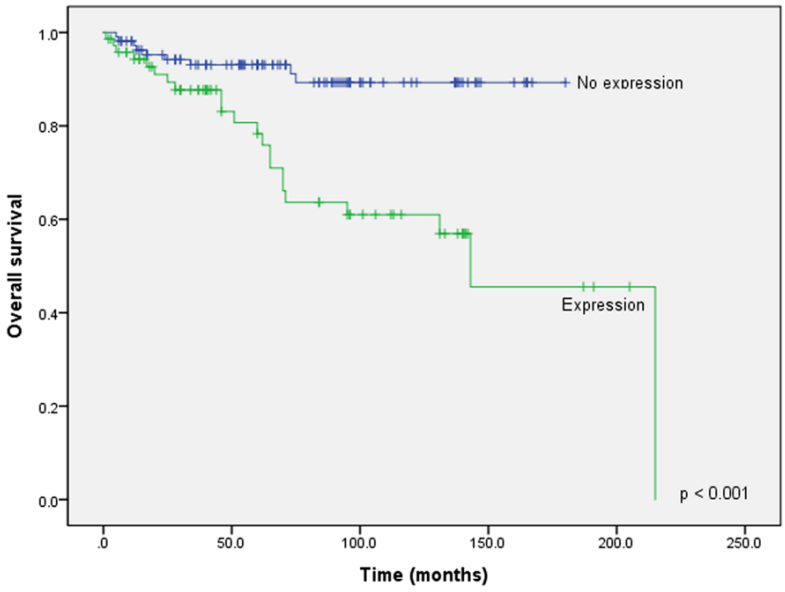

Figure 2. Survival curves of overall survival for p16 expression versus no p16 expression. The p16-expressing gastrointestinal tumors showed a shorter overall survival rate ($P < 0.001$).

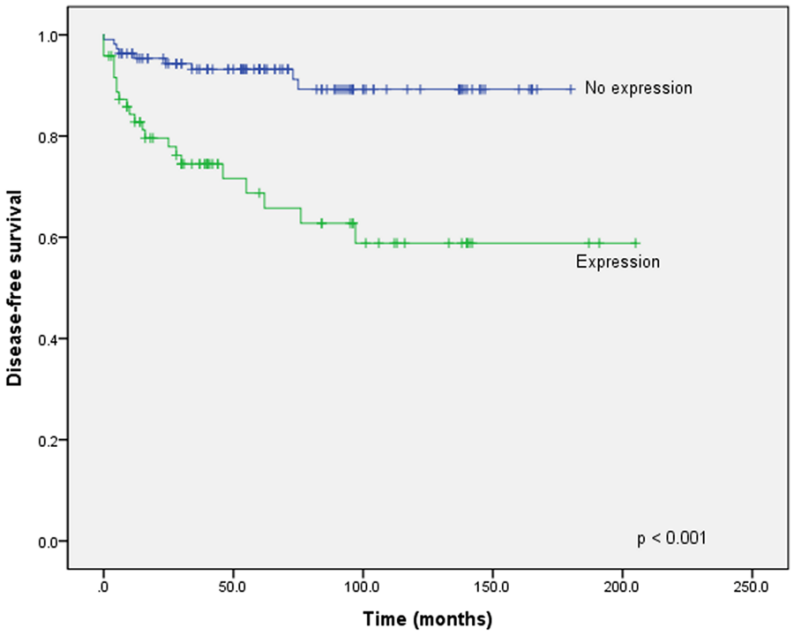

Figure 3. Survival curves of disease-free survival for p16 expression versus no p16 expression. The p16-expressing gastrointestinal tumors showed a shorter disease-free survival rate ($P < 0.001$).

min) on the automated Benchmark® platform (Ventana Medical Systems). The staining was visualized using the UltraView™ universal DAB detection kit (Automated BenchMark®, Ventana), which included a hydrogen peroxide substrate and a 3, 3'-diaminobenzidine chromogen solution. The slides were subsequently counterstained with hematoxylin.

Interpretation of immunohistochemistry

Slides were assessed by an investigator who was blinded to the patients' clinicopathological information. We defined p16 expression as more than 20% of total tumor cells showing nuclear staining with or without a cytoplasmic reaction. Lymphocytes and background stromal cells served as the positive controls.

Statistical analysis

Comparisons were performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). The χ^2 test and Fisher's exact test were used to examine associations between categorical variables. OS was defined as the time from surgical resection to death or the last follow-up examination. Disease-free survival (DFS) was the time that a patient lived without a known recurrence or metastasis. Survival rates were calculated using the Kaplan-Meier method. Associations between survival rates and various clinicopathological factors were evaluated using the log-rank test. Cox's proportional hazard regression model was used to evaluate the significance of the prognostic factors. The variables with significant results in univariate analysis were analyzed in multivariate analysis. Hazard ratios (HRs) and associated 95% confidence intervals (CIs) were calculated for each variable. Statistical significance was accepted for p values < 0.05.

Table 2. Univariate and multivariate analyses of clinicopathologic factors affecting the survival of patients with gastrointestinal stromal tumors

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Intermediate vs low risk	1.525 (0.976-2.383)	0.064	0.487 (0.204-1.160)	0.001
High vs low risk	0.759 (0,470-1.227)	0.260	0.924 (0.159-5.356)	< 0.001
Mitosis (> 5 vs ≤ 5)	2.413 (0.517-11.259)	0.262	14.953 (5.726-39.046)	< 0.001
Necrosis	4.768 (2.318-9.807)	< 0.001	1.010 (0.431-2.366)	0.981
Recurrence or metastasis	20.974 (7.187-61.209)	< 0.001	12,231 (3.692-40.525)	< 0.001
p16 (expression vs no)	4.039 (1.848-8.827)	< 0.001	1.215 (0.419-3.523)	0.720

HR, hazard ratio; CI, confidence interval.

Results

Clinicopathological characteristics

A total of 126 males and 120 female patients with median age of 58.5 years (range: 22-88 years) were included in this study. The median tumor size was 4.79 cm (range: 1-23 cm). Expression of CD117 and DOG1 was found in 222 (98.2%) cases. SDHB-negative GISTs were detected in only two gastric WT GISTs (one in a 56-year-old female and the other in a 15-year-old male patient). The SDHB-negative GISTs revealed diffuse strong positive staining on CD117 and DOG1.

Comparison between expression of p16 and clinicopathological factors

Expression of p16 was found in 42.4% (92/217) of GISTs (**Figure 1**). The p16 expression was significantly associated with GISTs with a higher mitotic count (> 5/50 high-power fields [HPF]), tumor necrosis, recurrence or metastasis, and a higher-risk group with respect to aggressive behavior (**Table 1**). Patients with p16-expressing GISTs showed shorter OS (P < 0.001) (**Figure 2**) and DFS (P < 0.001) (**Figure 3**) than those without p16 expression. On multivariate analysis, risk of malignant behavior and recurrence or metastasis were independent prognostic factors. The intermediate-risk group (P = 0.001, HR 10.370, CI 2.611-41.187) and high-risk group (P < 0.001, HR 13.459, CI 3.555-50.952) showed shorter survival than the low-risk group. Patients without recurrence or metastasis had better survival than those with recurrence or metastasis (P < 0.016, HR 0.369, CI 0.164-0.831) (**Table 2**).

Discussion

Most clinicopathological studies have demonstrated that tumor size and the mitotic index are the most important prognostic indicators of GISTs. However, they do not always reliably predict patient outcomes. The clinical behavior of GIST varies, and some small and mitotically inactive GISTs show aggressive behaviors [16]. A reliable method to predict the prognosis of GIST is necessary for clinical management.

Alteration of cell-cycle regulatory proteins has been implicated in the pathogenesis and tumor progression of various kinds of human cancers. Loss of p16 expression has been reported to be associated with progression to malignant disease [17]. However, p16 overexpression was found in some tumors, and it was associated with the aggressiveness of disease subtypes [6-8]. Although there have been extensive studies of p16 expression in GISTs, discrepancies still exist with respect to its prognostic value [18]. Loss of p16 expression has been previously reported as a negative prognostic factor in GISTs. Schneider-Stock et al. [19] did not find any correlation between p16 gene alteration and clinicopathologic variables, but p16 loss was associated with a poor prognosis and p16 expression was higher in the benign GISTs. Huang et al. [20] also demonstrated that complete loss of p16 expression preferentially affected intermediate- and high-risk groups, and they suggested that p16 deregulation might be involved in early tumorigenesis. Several other studies have confirmed this correlation and its implication for poor prognosis [21-23]. However, Haller et al. [24] demonstrated that loss of chromosomal region 9p21 led to reduced mRNA and p16 expression in GISTs. Steigen et al. [25] also showed that patients with p16-expressing GISTs had a significantly worse OS than those without p16 expression. In addition, p16-expressing GISTs tended to have a larger size and a higher mitotic count (> 5/50 HPF) compared with those not expressing

p16. Our study showed similar results, although p16 expression was not correlated with tumor size. These results were also confirmed in another study by Schmieder et al. [26], who revealed that p16-expressing GISTs tended to develop more recurrence or metastasis and showed a worse disease-specific survival and DFS compared with those not expressing p16. They also suggested that p16 expression might be an indicator for high-risk GIST. Our study showed nearly identical results with Schmieder's study in that p16-expressing GISTs were significantly associated with a higher-risk group and had a tendency of more recurrence or metastasis and worse OS and DFS. Regarding these contradictory results, although loss of p16 expression biologically contributes to malignancy, other oncogenic changes such as loss of RB or TP53 and aberrant activation of cyclin D1 may lead to increased proliferation and dysregulation of the cell cycle [14].

Prognostic factors in GISTs have been widely studied, and tumor size and mitotic count have been accepted as reliable factors. Other factors such as anatomic location, cellular atypia, and tumor necrosis have been shown to be independent prognostic factors in some studies [25]. However, it is still difficult to predict the risk of developing recurrence or metastasis, a higher mitotic count, and a higher risk, especially in small biopsied GISTs. Our study showed that p16 expression was a highly predictive factor for the presence of recurrence of metastasis and being in a higher-risk group for patients with GISTs.

In summary, p16 expression in GISTs was significantly associated with a higher mitotic count, tumor necrosis, recurrence or metastasis, and a higher-risk group with respect to aggressive behavior. Furthermore, p16-expressing GISTs revealed shorter OS and DFS compared with those without expression. The expression of p16 can be a highly predictive marker to predict recurrence or metastasis and aggressive behavior in GISTs.

Disclosure of conflict of interest

None.

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