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Prognostic Value of Platelet-Derived Growth Factor-A (PDGF-A) in Gastric Carcinoma

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Objective

Because our previous study indicated that PDGF-A mRNA expression in biopsy specimens might identify a subgroup of high-risk patients with gastric carcinoma, in this study we analyzed the prognostic value of platelet-derived growth factor-A (PDGF-A) gene expression in gastric carcinoma biopsy specimens.

Methods

Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to analyze the PDGF-A gene expression in 65 gastric carcinoma endoscopic biopsy specimens. The 65 patients were divided into a PDGF-A-positive group (29 patients) and a PDGF-A-negative group (36 patients).

Results

On the basis of 2-year follow-up data, the PDGF-A-positive group demonstrated a shorter overall survival rate compared with the PDGF-A-negative group ($p < 0.0001$). A similar correlation was found in 34 advanced-stage patients ($p = 0.003$) and in 24 advanced-stage patients who underwent a curative resection ($p = 0.003$). Multivariance analysis indicated that the transcription of PDGF-A gene is a potent prognostic factor that is independent of the traditional pathologic parameters.

Conclusions

Expression of PDGF-A mRNA in gastric biopsy specimens may be a new preoperative prognostic parameter in gastric carcinoma.

It is thought that autocrine and paracrine stimulation of tumor cells by several types of growth factors plays an important role in tumor growth and progression.[1–8] In our previous study, the mRNA expression of eight different tumor growth-related factors, which included cyclin D1, cyclin E, urokinase type plasminogen activator (uPA), 72 kD type IV collagenase (MMP2), vascular endothelial growth factor (VEGF), platelet-derived growth factor-A (PDGF-A), transforming growth factor-β (TGF-β), and interleukin-10 (IL-10) were examined in endoscopic biopsy gastric carcinoma specimens.[9] We also analyzed the statistical relationship between the mRNA expression of these genes and clinical pathologic parameters. The findings suggested that the transcription of the PDGF-A gene may be an independent prognostic indicator in gastric carcinoma.[9] Two years have passed since we started analyzing the relationship between the expression of tumor growth-related cytokine genes and traditional clinicopathologic parameters. This study was performed to confirm our hypothesis that the determination of PDGF-A gene expression in carcinoma specimens may be useful in the preoperative prognostic evaluation of patients with gastric carcinoma.

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PATIENTS AND METHODS

Patients and Biopsy Samples

The patients analyzed in this study are essentially the same as those used in our previous study.[9] Briefly, the tumor biopsy specimens were obtained during preoperative endoscopy of 78 patients with gastric carcinoma. All 78 primary gastric carcinoma surgically resected specimens were classified histologically using the General Rules for Gastric Cancer Study of the Japanese Research Society for Gastric Cancer.[10] In this study, only the 65 patients who underwent resection at the Department of Surgery, Saga Medical School from 1993 through 1995 were evaluated. The 13 patients who underwent surgery at other hospitals

were excluded from this study because of differing therapeutic schedules. Early gastric carcinomas are defined as those in which the invasion was limited to the gastric mucosa or submucosa, regardless of the nodal metastasis. Histologic staging demonstrated 31 early cases (t1) and 34 advanced cases (t2 or greater). According to the Japanese classification scheme, t1, t2, t3, and t4 correspond to tumor invasion of the mucosa or submucosa, muscularis propria or subserosa, the serosa without invasion of adjacent structures, and adjacent structures, respectively. The PDGF-A mRNA was expressed in 29 (PDGF-A-positive cases) of the 65 patients. The follow-up period ranged from 3 to 31 months (mean 15.1 months).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and Gel Electrophoresis

Total RNA from each biopsy specimen was isolated by a single step, acid guanidinium thiocyanate-phenol-chloroform extraction.[11] The biopsy specimens that were maintained on ice were minced and homogenized manually by a lysis buffer. The RNA fraction was resuspended in diethyl pyrocarbonate-treated water and quantitated by measuring the absorbance at 260 nm. The reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out according to the Perkin-Elmer/Cetus protocol for reverse transcription (RT) of RNA and amplification of cDNA. The RT reaction was carried out with 0.5 μg of RNA per sample. The cDNA amplification for PDGF-A was performed for 35 cycles using the following parameters: 94°C for 60 seconds, 60°C for 60 seconds, and 72°C for 120 seconds. The primer sequence was as follows: 5'-CCC CTG CCC ATT CGG AGG AAG AGA, 3'-TTG GCC ACC TTG ACG CTG CGG TG.[12] Aliquots of the PCR products (7.5 μL) were separated and visualized with ethidium bromide staining after electrophoresis on a 1.5% agarose gel in Tris acetate/ethylenediaminetetraacetic acid (EDTA) buffer at 100 V for 20 minutes. The specimens were scored as positive when the appropriately sized PCR band was visualized upon ethidium bromide staining. Therefore, a negative result does not necessarily indicate no mRNA expression. The RT-PCR was performed immediately after the sample collection by the same investigator from the previous study. The investigator had no knowledge of the corresponding clinical data. Determination of the PCR product specific for PDGF-A mRNA (positivity) was performed by two investigators. Consequently, none of the samples had different results.

PCR Product Verification by Southern Blotting

To verify that the PCR amplification was specific for PDGF-A, the PCR products were transferred to nylon membranes and probed with a radiolabeled oligonucleotide complementary to sequences within the region flanked by each pair of primers. The blots were hybridized at 50° with the probes labeled on their 5' end with τ−32P, (τ−32P-ATP; 7000 Ci/mM; ICN Pharmaceuticals, Costa Mesa, CA,) and T4 polynucleotidekinase (Pharmacia, Upssala, Sweden) for 18 hours. The membranes were then washed for 10 minutes with 2 × SSC and 0.1% SDS, followed by 0.2 × SSC and 0.1% SDS at room temperature and was subjected to autoradiography.

Immunohistochemistry

The resected specimens were fixed in formalin, embedded in paraffin, and sliced into 3-μm thick sections. These sections were stained with hematoxylin and eosin. An immunohistologic technique that is specific for PDGF-A was also used. A rabbit, antihuman PDGF polyclonal antibody (Cytokine Research Products, MA) specific for PDGF-A was used. This antibody specifically binds to human PDGF-AA and PDGF-AB and demonstrates less than 11% cross reactivity with human PDGF-BB.[13] The immunohistologic staining was performed using the avidin-biotin-peroxidase complex technique.

Statistics

Fisher's exact probability test was used for the statistical analyses that related the expression of PDGF-A mRNA and the traditional clinical pathologic parameters. The survival curves were calculated using the Kaplan-Meier method and analyzed by the log rank test. The influence of each variable on survival was assessed by the Cox's proportional hazard regression model. All calculations were carried out using Stat View (Abacus Concepts, Berkeley, CA,). A p level <0.05 was considered significant.

RESULTS

Of the 65 specimens evaluated, 36 (PDGF-A-negative cases) yielded no visible PCR product specific for PDGF-A mRNA and 29 (PDGF-A-positive case) displayed a distinct and clearly visible PCR product (Fig. 1). The correlation between the expression of PDGF-A mRNA and the clinicopathologic parameters is shown in Table 1. A significant correlation was found between PDGF-A mRNA expression and stage ($p = 0.002$), depth of invasion ($p = 0.022$), and nodal metastasis ($p = 0.010$). The prognosis of the PDGF-A-positive group was significantly worse than the PDGF-A-negative group ($p < 0.0001$ [Fig. 2]). Multivariate analysis indicated that serosal invasion ($p = 0.013$) and PDGF-A expression ($p = 0.009$) are independent prognostic factors (Table 2).

Because none of the 31 early-stage patients died during the follow-up period, similar analyses were performed in the 34 advanced-stage carcinomas. No correlation was found between the mRNA expression of PDGF-A and the pathologic parameters (Table 3). The prognosis of the 20 PDGF-

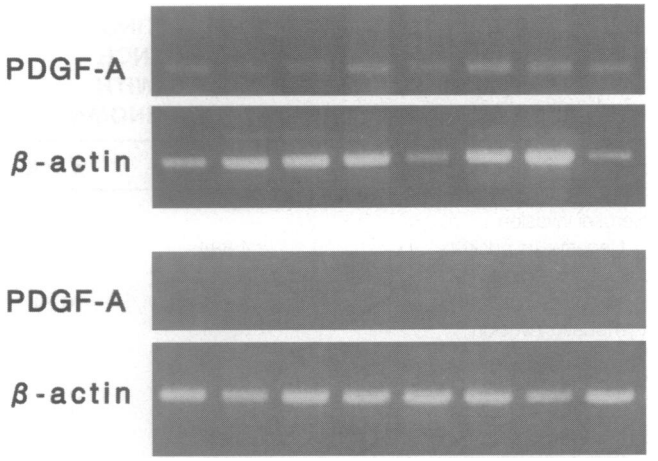

Figure 1. Expression of PDGF-A in 20 representative gastric carcinoma specimens. Upper: PDGF-A mRNA-positive carcinoma specimens. Lower: PDGF-A mRNA-negative carcinoma specimens.

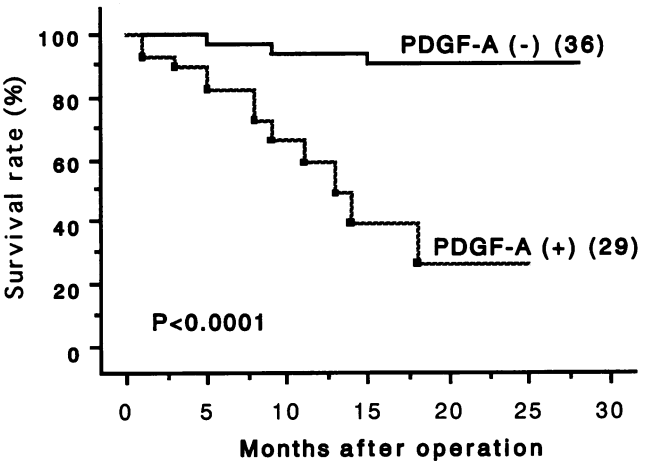

Figure 2. Survival curves of the 65 patients with all stages of gastric carcinoma, according to the expression of PDGF-A mRNA in the carcinoma specimens. The survival curves were determined by the method of Kaplan and Meier. The p value was determined using the log-rank test.

A-positive patients was significantly worse than that of the 14 PDGF-A-negative patients ($p = 0.003$ [Fig. 3]). Multivariate analysis demonstrated that serosal invasion ($p = 0.047$) and the mRNA expression of PDGF-A ($p = 0.006$) were significant independent prognostic factors (Table 4).

Finally, the prognoses of the 24 advanced carcinoma patients who underwent curative resection were evaluated.

Table 1. RELATIONSHIP BETWEEN THE EXPRESSION OF PDGF-A MRNA AND THE PATHOLOGIC PARAMETERS IN 65 PATIENTS WITH GASTRIC CARCINOMA

	PDGF-A (−)	PDGF-A (+)	p Value
Age	65.31 ± 12.76	63.05 ± 17.61	0.461
Sex			
Men	27	22	0.936
Women	9	7	
Stage			
I	25	10	
II	3	5	
III	7	6	0.002
IV	1	8	
Depth of Invasion			
t1	22	9	
t2	5	7	0.022
t3	8	11	
t4	1	2	
Nodal status			
node negative	27	12	
node positive	9	17	0.010
Histologic type			
Well differentiated	11	4	
Moderately differentiated	8	8	
Poorly differentiated	11	12	
Signet ring cell	3	3	0.609
Mucinous	2	2	
Papillary	1	0	

The prognoses of the 11 PDGF-A-positive patients also were worse than that of the 13 PDGF-A-negative patients ($p = 0.003$ [Fig. 4]). No correlation was found between the expression of PDGF-A mRNA and the pathologic parameters examined (Table 5). Multivariate analysis demonstrated that only the expression of PDGF-A mRNA was an independent prognostic factor in advanced patients with carcinoma who underwent a curative resection ($p = 0.011$ [Table 6]).

Ten patients underwent a noncurative resection because of peritoneal dissemination (4 cases), hepatic metastasis (5 cases), or ovarian metastasis (1 case) (Table 7). Of the 10 cases, 9 expressed detectable PDGF-A mRNA.

Immunohistochemical staining using polyclonal antibody specific for PDGF-A revealed that tumor cells that expressed PDGF-A mRNA as determined by RT-PCR also stained positive for PDGF-A protein (Fig. 5).

Table 2. RISK FACTORS AFFECTING SURVIVAL RATE BY MULTIVARIANCE ANALYSIS IN 65 PATIENTS WITH GASTRIC CARCINOMA

Parameter	Hazards Ratio	p Value
Serosal invasion		
Negative *vs* positive	15.652	0.013
Lymph node metastasis		
Negative *vs* positive	1.981	0.316
Lymphatic invasion		
Negative *vs* positive	1.344	0.751
Venous invasion		
Negative *vs* positive	1.037	0.954
Expression of PDGF-A mRNA		
Negative *vs* positive	5.964	0.009

Table 3. RELATIONSHIP BETWEEN THE EXPRESSION OF PDGF-A MRNA AND THE PATHOLOGIC PARAMETERS IN 34 PATIENTS WITH ADVANCED-STAGE GASTRIC CARCINOMA

	PDGF-A (−)	PDGF-A (+)	p Value
Age	60.86 ± 17.61	61.82 ± 13.17	0.857
Sex			
Men	8	14	0.440
Women	6	6	
Stage			
I	3	1	
II	3	5	
III	7	6	0.073
IV	1	8	
Depth of invasion			
t2	5	7	
t3	8	11	0.860
t4	1	2	
Nodal status			
node negative	5	6	>0.999
node positive	9	14	
Histologic type			
Well differentiated	2	2	
Moderately differentiated	4	6	
Poorly differentiated	6	9	0.991
Signet ring cell	1	1	
Mucinous	1	2	

Table 4. RISK FACTORS AFFECTING SURVIVAL RATE BY MULTIVARIANCE ANALYSIS IN THE 34 PATIENTS WITH ADVANCED-STAGE GASTRIC CARCINOMA

Parameter	Hazards Ratio	p Value
Serosal invasion		
Negative *vs* positive	4.884	0.047
Lymph node metastasis		
Negative *vs* positive	1.871	0.349
Lymphatic invasion		
Negative *vs* positive	1.498	0.645
Venous invasion		
Negative *vs* positive	0.862	0.801
Expression of PDGF-A mRNA		
Negative *vs* positive	6.461	0.006

DISCUSSION

Our previous study strongly indicated that the expression of PDGF-A in gastric carcinoma specimens may be an independent prognostic parameter.[9] In this study, we focused on the relationship between transcription of the PDGF-A gene and the patient's prognosis. As we postulated previously, PDGF-A mRNA expression is an important prognostic parameter, which is independent of the clinicopathologic parameters.

Because 2 years have passed since we started to collect gastric carcinoma specimens for the determination of the transcription of various tumor growth-factor related genes,[9] we felt that it was appropriate to analyze the correlation between PDGF-A mRNA expression and the survival time after surgery to see if our initial observation was confirmed. Recent studies on the malignant potential of various solid tumors have strongly suggested that tumors, including gastric carcinoma, can be divided genetically into two groups: a high malignancy group and a low malignancy group.[14] Although the follow-up period of this study was relatively short (mean 15.1 months), it is well known that gastric carcinoma frequently recurs within 2 years of surgery in patients with advanced gastric carcinoma.[15] Because of this observation, we postulated that a 2-year period of follow-up might be enough to identify the high-risk cases among all the patients with gastric carcinoma. Both sets of survival

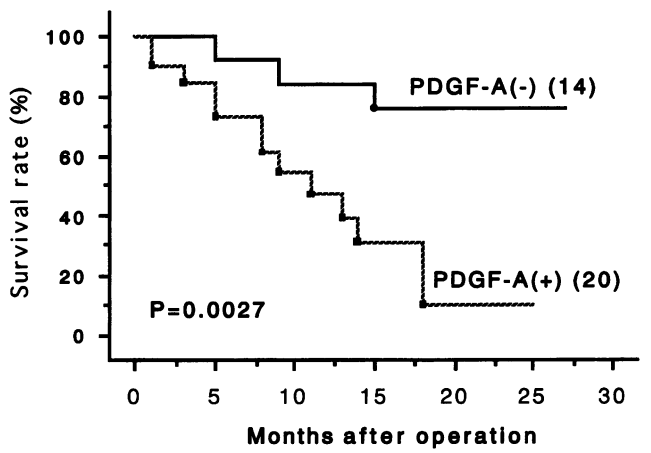

Figure 3. Survival curves of the 34 patients with advanced-stage gastric carcinoma, according to the expression of PDGF-A mRNA in the carcinoma specimens. The p value was determined using the log-rank test.

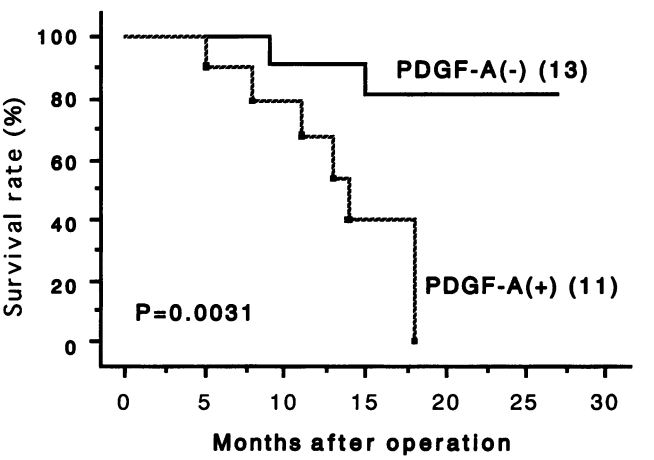

Figure 4. Survival curves of the 24 patients with advanced gastric carcinoma patients who underwent a curative resection. The p value was determined using the log-rank test.

Table 5. RELATIONSHIP BETWEEN THE EXPRESSION OF PDGF-A MRNA AND THE PATHOLOGIC PARAMETERS IN 24 PATIENTS WITH ADVANCED GASTRIC CARCINOMA WHO UNDERWENT CURATIVE RESECTION

	PDGF-A (−)	PDGF-A (+)	p Value
Age	59.83 ± 8.13	64.33 ± 16.45	0.393
Sex			
Men	8	8	0.562
Women	5	3	
Stage			
I	3	1	
II	3	4	
III	7	5	0.593
IV	0	1	
Depth of invasion			
t2	5	4	>0.999
t3	8	7	
Nodal status			
node negative	5	3	0.679
node positive	8	8	
Histologic type			
Well differentiated	2	1	
Moderately differentiated	4	3	
Poorly differentiated	5	4	0.944
Signet ring cell	1	1	
Mucinous	1	2	

curves were calculated using the Kaplan-Meier method (Figs. 2, 3, and 4) and multivariate analysis using the Cox's proportional hazard model (Tables 2, 4, and 6) was used in this study. Our data support the validity of using a 2-year follow-up period in gastric carcinoma, especially in those with advanced disease. Of the 31 early-stage patients (22 PDGF-A-negative patients and 9 PDGF-A-positive ones) who underwent a curative resection, 1 patient whose carcinoma biopsy specimen expressed PDGF-A mRNA developed multiple bone metastases 14 months after surgery.

Because all the carcinoma specimens were collected at our institute from 1993 to 1995 and all analyses were performed without knowledge of the corresponding clinical data, the patient population was rather homogeneous in terms of the surgical procedures performed, the postoperative therapeutic schedules, and the follow-up schedules. In addition, because this study was a prospective study, the determination of PDGF-A mRNA expression preceded the prognosis analysis.

The PDGF is a heterodimeric protein composed of two closely related A- and B-chain polypeptides encoded by separate genes.[16,17] The PDGF has three isoforms: PDGF-AA, PDGF-AB, and PDGF-BB. All three isoforms bind to the PDGF α, a receptor (PDGFR α). It has been reported that the expression of the PDGF genes was demonstrated in a few cell lines.[18–20] We also detected the expression of PDGF-A mRNA in four of the five human gastric carcinoma cell lines tested (data not shown). In addition, we confirmed that the PDGF-A protein is expressed in the tumor cells in all six PDGF-A-positive carcinoma specimens examined immunohistochemically by the avidin-biotin-peroxidase technique (Fig. 5). Thus, PDGF and PDGFR constitute a growth factor system that may participate in autocrine and paracrine loops in tumor tissues.[21] In patients with breast carcinoma, a poorer prognosis with a high plasma level of PDGF has been reported.[22,23] Takanami et al.[24] have demonstrated that the prognosis of patients with pulmonary adenocarcinoma tumors that exhibit positive immunohistochemical PDGF staining was significantly worse than that of those with negative PDGF staining. They used an antiPDGF polyclonal antibody that recognizes the three isoforms of PDGF.[25] Henriksen et al.[26] have demonstrated that PDGFRα expression is a prognostic parameter in epithelial ovarian neoplasms. In their report, they suggested that the presence of PDGF-A-chain may be related more strongly to the expression of PDGFRα. Smits et al.[27] have demonstrated that PDGFR α and PDGF-A were expressed in highly malignant fibroblast-derived tumors but not in benign tumors. It is well known that malignant tumors depend on neovascularization for their growth and metastasis, and PDGF is one of several angiogenic factors.[28]

Recently, it has been reported that vascular endothelial growth factor (VEGF), which is also an angiogenic factor, is an independent prognostic indicator for patients with gastric carcinoma that correlates with a worse prognosis.[29] The authors investigated the expression of VEGF immunohistochemically in resected specimens from 129 patients with gastric carcinomas. In our study, patients with VEGF-positive tumors also had a poorer prognosis than those with VEGF-negative tumors (data not shown). However, statistical analysis showed that the difference was not significant ($p = 0.09$). The different results in the two studies may be because of variations in methodology (immunostaining *vs.*

Table 6. RISK FACTORS AFFECTING SURVIVAL RATE BY MULTIVARIANCE ANALYSIS IN 24 PATIENTS WITH ADVANCED GASTRIC CARCINOMA WHO UNDERWENT RESECTION

Parameter	Hazards Ratio	p Value
Serosal invasion		
Negative *vs* positive	9.079	0.059
Lymph node metastasis		
Negative *vs* positive	2.769	0.357
Lymphatic invasion		
Negative *vs* positive	1.655	0.671
Venous invasion		
Negative *vs* positive	0.387	0.252
Expression of PDGF-A mRNA		
Negative *vs* positive	10.799	0.011

Table 7. 10 PATIENTS WITH ADVANCED GASTRIC CARCINOMA WHO UNDERWENT NONCURATIVE RESECTION

Patient No.	Reasons for Noncurative Resection	Expression of PDGF-A mRNA	Outcome
1	Peritoneal dissemination	Negative	5MDead
2	Peritoneal dissemination	Positive	8M Alive
3	Peritoneal dissemination	Positive	3MDead
4	Peritoneal dissemination	Positive	8MDead
5	Hepatic metastasis	Positive	5MDead
6	Hepatic metastasis	Positive	1MDead
7	Hepatic metastasis	Positive	1MDead
8	Hepatic metastasis	Positive	9MDead
9	Hepatic metastasis	Positive	25M Alive
10	Ovarial metastasis	Positive	3M Alive

RT-PCR) for detecting VEGF, the number of patients studied (129 *vs.* 65), and the different follow-up period (5 years *vs.* 2 years). Nonetheless, these studies strongly indicate that angiogenic factors play an important role in the prognosis of patients with gastric carcinoma.

Of the traditional clinicopathologic parameters, lymph node metastasis seems to be among the more important risk factors for predicting overall survival.[15,30] For this reason, that extended gastrectomy that includes D2 or D3 lymph node dissection has been recommended through research in Japan for better survival.[31] Even when extended gastrectomy is performed, approximately 30% of patients will have disease recurrence within a few years after surgery.[32] On the other hand, it has been demonstrated that approximately 50% of patients with advanced gastric carcinoma who undergo a curative resection survive without any additional postoperative therapy.[32] These clinical data are consistent with our results in which PDGF-A expression was found in 29 (44%) of 65 patients, which suggests that approximately 40% of these patients are at a high risk for recurrence. We now advocate that PDGF-A-positive advanced cases receive pre and postadjuvant chemotherapy because we believe that the PDGF-A-negative early stage cases do not need any adjuvant therapy. It is still too early to know whether endoscopic mucosal resection is sufficient for the early cases of PDGF-A-negative or whether the advanced cases of PDGF-A-positive need more radical therapies such as D4 lymph node dissection or intensive chemotherapy. Such questions can be addressed by future clinical trials.

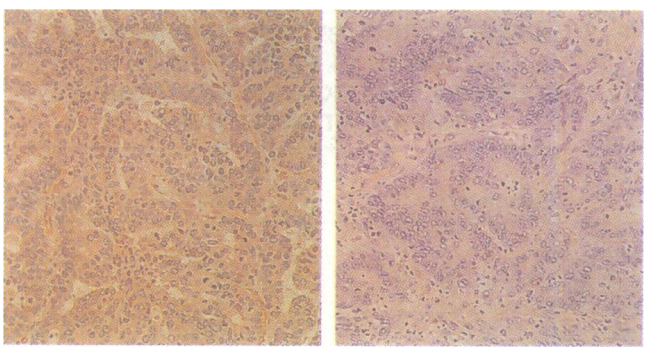

Figure 5. Immunohistochemical staining for PDGF-A in gastric carcinoma tissues. Left: Carcinoma tissue from a PDGF-A mRNA-positive patient. The positive staining is observed mainly in the cytoplasm of carcinoma cells. Right: Carcinoma tissue from a PDGF-A mRNA-negative patient. Positive staining is observed in some of the fibroblasts and endothelial cells but not in the carcinoma cells.

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