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Prognostic relevance of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitors PAI-1 and PAI-2 in gastric cancer

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Abstract Expression of urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2) was evaluated in 125 surgically resected gastric cancers by immunohistochemical analysis. Tissue was stained immunohistochemically with a monoclonal antibody against human uPA and monoclonal antibodies against human PAI-1 and PAI-2. In addition, DNA ploidy patterns were determined by cytofluorometer after staining with propidium iodide. We found that 82 (66%) of the 125 gastric cancers expressed uPA as diffuse cytoplasmic staining, as intensely outlined luminal borders. PAI-1 expression was observed in 62 (50%) of 125 gastric cancer as a fine, diffuse and granular pattern in the cytoplasm. PAI-2 expression was observed in 65 (52%) of the 125 gastric cancers as a diffuse cytoplasmic staining. uPA-positive tumours showed a higher incidence of infiltration, lymph node metastasis and peritoneal dissemination than uPA-negative ones. Patients with uPA-positive tumours proved to have a significantly poorer prognosis than those with negative ones. PAI-1-negative tumours showed a higher incidence of liver metastasis and carried a poorer prognosis than PAI-1-positive ones. There was no significant correlation between uPA or PAI-1 expression and DNA ploidy patterns. Conversely, there was no significant relationship between PAI-2 expression and clinicopathological parameters and prognosis. According to the expression of uPA and PAI-1 status, groups of 19 uPA(–)/PAI-1(–), 44 uPA(+)/PAI-1(–), 23 uPA(–)/PAI-1(+) and 39 uPA(+)/PAI-1(+) were subdivided. Tumours with uPA(+)/PAI-1(–) had a significantly higher incidence of liver metastasis, lymph node metastasis and serosal invasion than the other groups of tumours. Patients with uPA(+)/PAI-1(–) tumours had a significantly poorer prognosis than those with uPA(–)/PAI-1(+) tumours. These results indicate that uPA expression is a useful biological prognostic indicator, and that uPA and PAI-1 may play an important part in the tumour progression and metastasis in gastric cancer.

Key words Urokinase-type plasminogen activator · Plasminogen activator inhibitor-1 · Plasminogen activator inhibitor-2 · Gastric cancer

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Introduction

The process of tumour cell infiltration and metastasis involves consecutive destruction and reconstitution of the extracellular matrix [11]. Plasminogen activation seems to be an important mechanism in the degradation of this matrix. Plasminogen activators (PAs) are serine proteases which catalyse the conversion of the inactive proenzyme plasminogen to plasmin [2, 8, 17, 21, 36, 49, 51, 62]. Plasmin plays a major part in the regulation of intravascular fibrinolysis, tissue remodelling, and the degradation of extracellular matrix, and active latent enzymes such as type-IV collagenase [26, 33–35, 52]. Two different types of plasminogen activator have been identified: tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) [6, 48]. tPA, localized predominantly in endothelial cells and detectable in all vascularized tissues, has been shown to play a major part in intravascular thrombolysis and in tumour growth [16, 29, 43]. uPA was initially identified in urine but is produced in many normal and malignant cells [28, 64]. It has been implicated in the destruction of extracellular matrix and basement membrane resulting in a metastatic spread of malignant cells. Previous studies have revealed remarkable changes in the plasminogen activator, particularly of uPA, profiles in several types of carcinomas, those from the colon, rectum, lung, breast, uterus, and stomach [9, 12, 14, 38, 41, 50, 53, 59, 61, 66].

Plasminogen activator inhibitors are a family of serine protease inhibitors (serpins) which control the activity of plasminogen activators and which have been divided into three genetically and immunologically distinct types, plasminogen activator inhibitor-1 (PAI-1), plasminogen activator inhibitor-2 (PAI-2), and protease nexin [31, 55]. PAI-1 is secreted by endothelial cells, vascular smooth muscle cells, fibroblasts and is also detectable in plasma and platelets. PAI-1 inhibits both uPA and tPA, forming a 1:1 covalent complex, and is thought to be a natural inhibitor of tPA in plasma [3, 7, 30, 32, 37, 46, 47, 60]. Accumulation of PAI-1 within the tissue environment may play a critical role in the control of stroma invasion and in the process of intravasation and extravasation by tumour cells [13, 19]. PAI-2 has been isolated from placenta, monocytes and several kinds of malignant cell lines. PAI-2 has been shown to be one of the primary physiological inhibitors of uPA and to inhibit two-chain tPA [1, 23, 24, 31, 46, 55]. The degree of tumour cell mediated proteolysis may therefore result from a regulation between uPA and inhibitors.

The aim of the present study was to determine the prognostic relevance of uPA, PAI-1 and PAI-2 in the overall survival of patients with gastric cancer. Therefore, we assessed the clinical outcome of 125 patients having operations for primary gastric cancer. The prognostic relevance of uPA, PAI-1 and PAI-2 was related to major clinicopathological variables.

Materials and methods

Patients and tissue samples

In all, 125 patients with primary gastric cancer, who were diagnosed and treated in the Second Department of Surgery, Kanazawa University, between 1988 and 1994, were enrolled in this study. There were 83 men and 42 women, with an age range from 27 to 96 years (mean, 63 years). The mean length of follow-up was 17 months (range, 1–63) at the time of this study. All these patients underwent total or subtotal gastrectomy combined with extensive lymph node dissection, and the resected lymph nodes were histologically examined for metastasis. Early gastric cancers in which the tumour invasion was confined to the mucosa or submucosa was diagnosed in 39 patients.

Immediately after surgical resection, a part of the primary tumour (approximately 1 cm thick) was fixed in acetone at 20°C overnight, dehydrated in acetone at 4°C for 15 min and in acetone at room temperature for 15 min, in sequence, cleared in methyl benzoate for 30 min and in xylene for 30 min, and then penetrated with paraffin at 60°C for 2 h in a vacuum evaporating embedder. This method is called the AMeX method [54].

Immunohistochemistry

uPA, PAI-1 and PAI-2 were identified immunohistochemically by a three-step indirect immunoperoxidase method (streptavidin–biotin–peroxidase complex). Briefly, three 5-μm sections were cut from each paraffin block prepared by the AMeX method, deparaffinized with graded xylene and alcohol and subsequently immersed in absolute methanol containing 0.3% H_2O_2 to block endogenous peroxidase activity. Following a phosphate-buffered saline (PBS) rinse, the sections were incubated with 10% normal goat serum for 30 min at room temperature to block non-specific binding, followed by incubation with avidin solution (Dakopatts) for 15 min and then with biotin solution (Dakopatts) for 15 min. The primary anti-uPA monoclonal antibody was obtained from American Diagnostica (Greenwich, Conn.). This antibody reacts against an epitope within the growth factor domain of A-chain of human uPA, amino acid sequence number 17–34, and reacts with the 54-kDa form of uPA [5]. The anti-PAI-1 and anti-PAI-2 monoclonal antibodies were obtained from Biopool (Umea Sweden). Then the slides were incubated with anti-uPA antibody (dilution 1:60), anti-PAI-1 antibody (dilution 1:60) and anti-PAI-2 antibody (dilution 1:60) at 4°C overnight. The sections were then treated with biotinylated goat anti-mouse IgG (Dakopatts) for 30 min. The peroxidase-labelled streptavidin (Dakopatts) was then added to sections for 30 min at room temperature. Reaction products were developed by immersing the sections in 3.3′-diaminobenzidine tetrahydrochloride solution containing 0.1% H_2O_2. Finally, the slides were counterstained with 0.3% methyl green, dehydrated, and mounted in a routine fashion. Nonimmune mouse serum was used for a negative staining study.

Human fibrosarcoma HT-1080, originally derived from a primary human acetabular bone tumour, was used as a positive control for uPA. This cell line produced uPA mRNA and PAI-1 [4]. HT-1080 was injected into chorioallantoic membrane veins of 10-day chick embryos, and the embryos were returned to a humidified, 37°C chick embryo incubator for 7 days. Embryo lungs were then dissected and fixed by the AMeX method. Metastases of HT-1080 to the embryonic lung were confirmed histologically 7 days after inoculation.

Immunohistochemical quantification of staining with uPA, PAI-1 and PAI-2

If tumour cells that stained positively for uPA, PAI-1 and PAI-2 were found in more than 10% of cancer tissues, the tumours were considered to be uPA, PAI-1 and PAI-2 positive [uPA(+), PAI-1(+), PAI-2(+)].

Immunoblot analyses

To confirm our evaluation of immunohistochemical staining, we performed immunoblot analyses on the HT-1080 conditioning medium for uPA, PAI-1 and PAI-2 [4]. In brief, samples were dissolved in a buffer containing 3% sodium dodecyl sulfate, and were electrophoresed in polyacrylamide gradient gels on sodium dodecyl sulfate polyacrylamide gel electrophoresis plates (Easy Gel II, Funakoshi, Tokyo Japan). After electrophoresis, the proteins were transferred to nitrocellulose sheets and incubated with anti-uPA MAb (×2000), anti-PAI-1 MAb (×2000) and anti-PAI-2 MAb (×2000). The immunoreactions were visualized using SMI LIGHT Western blot kit (Sumitomo Metal, Ibaraki, Japan).

Determination of DNA ploidy patterns

Carcinoma tissues from the same paraffin tissues as had been used in the immunohistochemical study were examined by cytofluorometry. Each paraffin-embedded tissue block was sliced into five 50-μm-thick sections, and cells were isolated by the method of Hedley et al. [15]. The specimen was first dewaxed with xylene, and then rehydrated in ethanol solutions. The tissue was then washed with distilled water and digested enzymatically with a 0.5% pepsin solution (pH 1.5) for 60 min at 37°C. After digestion, the solution was filtered through a 40-μm nylon monofilament mesh to remove debris or aggregates. The cell suspension was washed in 0.5% Tween 20 in PBS saline, and treated with 0.1% RNase and a fluorescent dye containing 20 μg/ml of propidium iodide. The fluorescence intensity was measured by a microfluorometry Olympus BH2-QRFL (Olympus, Tokyo, Japan). The DNA ploidy of a cell population was regarded as diploid when it exhibited a single G0/G1 peak and a DNA index of 1.0. Cell populations showing two discrete G0/G1 peaks were judged aneuploid,

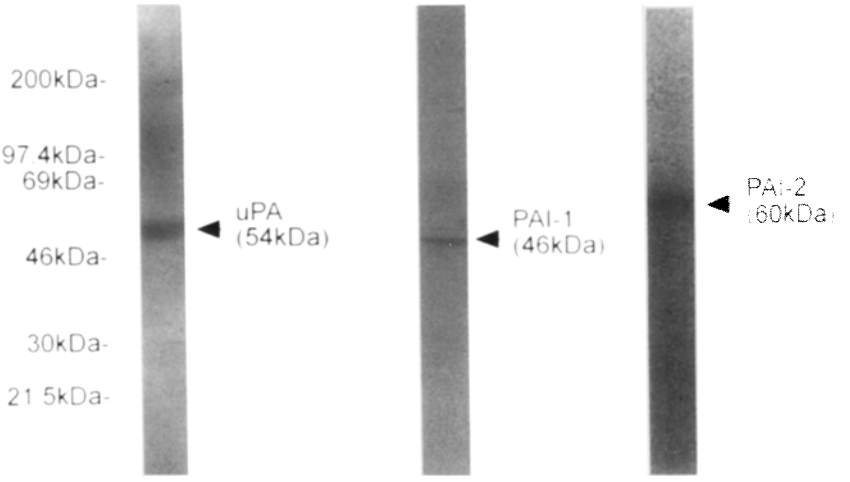


Fig. 1 Western blot analyses of the conditioning medium of HT-1080, using uPA MAb, PAI-1 MAb and PAI-2 MAb

as previously reported [27]. Human blood lymphocytes were used as an internal standard to identify the diploid population. The histogram was prepared using a minimum of 10,000 cells.

Statistics

The data are presented as mean±SD. Differences in the expression of uPA or PAI-1 or PAI-2 were evaluated by chi-square tests. Differences at $P<0.05$ were considered to be statistically significant.

Throughout this report, the general rules for gastric carcinoma study by the Japanese Society for Gastric Cancer [20] are used for the description and classification of variables.

Results

Specificity of the monoclonal antibodies

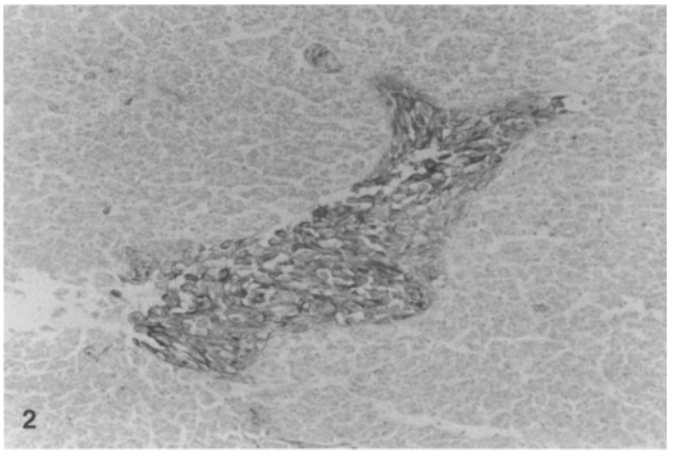
The results of immunoblotting analyses are shown in Fig. 1. Anti-uPA, anti-PAI-1 and anti-PAI-2 MAb recognized a single band of 54 kDa, 46 kDa and 60 kDa molecules, respectively. Control sections, stained with normal mouse IgG, (Coulter Immunology, Hialeah, Fla.) were prepared in slides stained positively for uPA, PAI-1 and PAI-2.

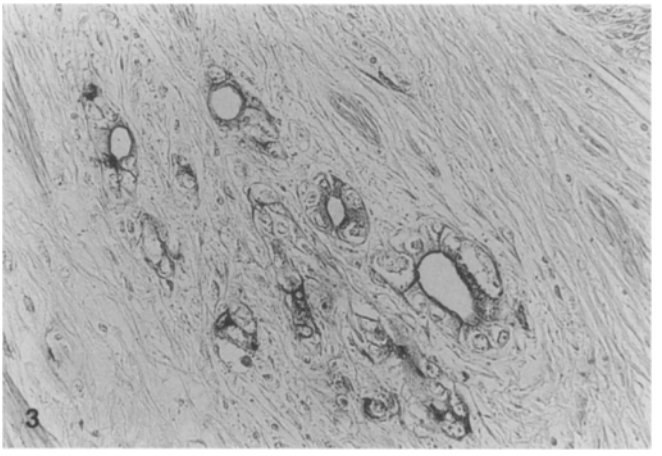
Immunohistochemistry

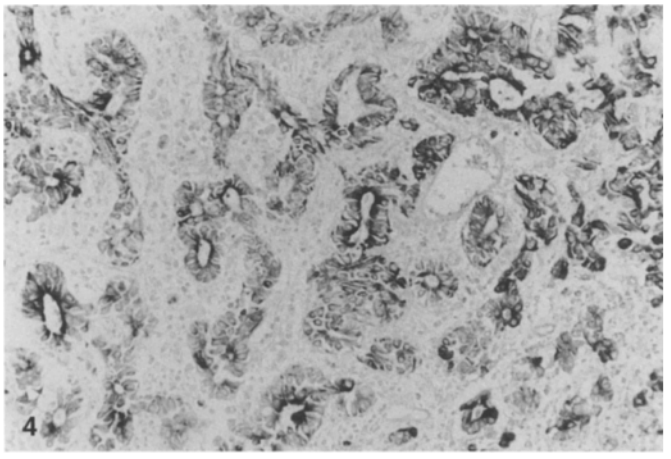
In HT-1080 cells, uPA can be observed as diffuse cytoplasmic staining (Fig. 2). In addition, PAI-1 can be observed as a diffuse cytoplasmic staining in HT-1080 cells, but PAI-2 was negative (data not shown). uPA immunoreactivities were seen in cytoplasm or luminal surface of normal gastric epithelium. In gastric cancers, uPA was observed as a diffuse cytoplasmic staining or in the luminal borders of cancer glands (Fig. 3). The staining of uPA was more prominent at the site of the growing edge of the tumour, whereas the adjacent stromal cells were not stained. uPA immunoreactivity was observed in 82 (66%) of the 125 tumours. PAI-1 expression was detected in 62 (50%) of the 125 primary tumours displaying a fine, diffuse and granular pattern in the cytoplasm of the carcinoma cells (Fig. 4). PAI-2 expression was observed in 65 (52%) of the 125 tumours as diffuse cytoplasmic staining (Fig. 5).

Fig. 2 uPA expression in HT-1080 cells, inoculated in the chick embryo; uPA can be observed as diffuse cytoplasmic staining. ×200

Fig. 3 uPA immunoreactivities are observed in the luminal borders of cancer glands of moderately differentiated adenocarcinoma. ×200







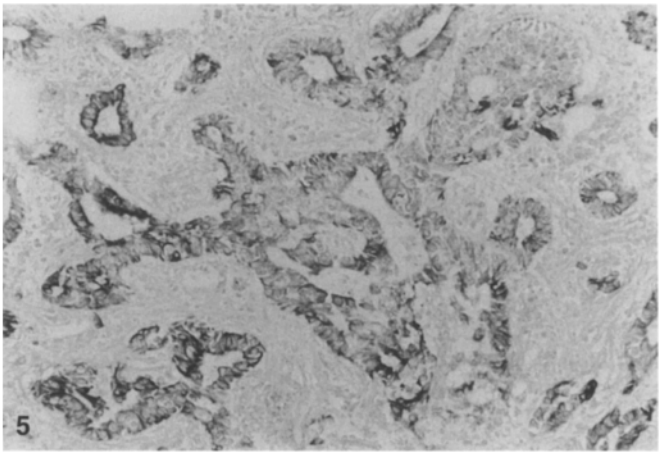


Fig. 4 The immunoreactivity pattern of PAI-1 is fine, diffuse and granular in the cytoplasm of carcinoma cells. ×200

Fig. 5 PAI-2 immunoreactivities are observed as a diffuse cytoplasmic staining in moderately differentiated adenocarcinoma. ×260

Table 1 Correlation between clinicopathological parameters and uPA expression in gastric cancer

Clinico-pathological parameters	No. of cases	uPA expression		p value
		Negative	Positive	
Depth of invasion				
m, sm	39	19 (49%)	20 (51%)	p<0.05
mp, ss, se, si	86	24 (28%)	62 (72%)	
Liver metastasis				
negative	114	42 (37%)	72 (63%)	NS
positive	11	1 (10%)	10 (90%)	
Peritoneal dissemination				
negative	93	37 (40%)	56 (60%)	p<0.05
positive	32	6 (19%)	26 (81%)	
Lymph node metastasis				
negative	55	28 (51%)	27 (49%)	p<0.01
positive	70	15 (21%)	55 (79%)	
Serosal invasion				
negative	64	32 (50%)	32 (50%)	p<0.01
positive	61	11 (18%)	50 (82%)	
Histological type				
differentiated	57	25 (44%)	32 (56%)	NS
undifferentiated	68	18 (26%)	50 (74%)	
Lymphatic invasion				
negative	43	18 (42%)	25 (58%)	NS
positive	82	25 (30%)	57 (70%)	
Venous invasion				
negative	70	26 (37%)	44 (63%)	NS
positive	55	17 (31%)	38 (69%)	

The correlation of uPA expression and clinicopathological variables is shown in Table 1. There was a significant correlation between uPA expression and depth of invasion, peritoneal dissemination, lymph node metastasis and serosal invasion. In contrast, no significant correlations were found between uPA expression and liver metastasis, histological type, lymphatic invasion and venous invasion. Tumours with uPA-positive expression had a higher incidence of infiltration, peritoneal dissemination and lymph node metastasis than did uPA-negative ones.

Table 2 shows the correlation between PAI-1 expression and clinicopathological variables. There was a significant correlation between PAI-1 expression and liver metastasis. Tumours that were negative for PAI-1 expression had a higher incidence of liver metastasis than positive ones. However other clinicopathological findings had no significant relationship with PAI-1 expression.

Then, according to the expression of uPA and PAI-1 status, groups of 19 uPA(–)/PAI-1(–), 44 uPA(+)/PAI-1(–), 23 uPA(–)/PAI-1(+), 39 uPA(+)/PAI-1(+) were subdivided. The correlation of combination analysis of uPA

Table 2 Correlation between clinicopathological parameters and PAI-1 expression in gastric cancer

Clinico-pathological parameters	No. of cases	PAI-1 expression		p value
		Negative	Positive	
Depth of invasion				
m, sm	39	21 (54%)	18 (46%)	NS
mp, ss, se, si	86	42 (49%)	44 (51%)	
Liver metastasis				
negative	114	54 (47%)	60 (53%)	p<0.05
positive	11	9 (82%)	2 (18%)	
Peritoneal dissemination				
negative	93	47 (51%)	46 (49%)	NS
positive	32	16 (50%)	16 (50%)	
Lymph node metastasis				
negative	55	28 (51%)	27 (49%)	NS
positive	70	35 (50%)	35 (50%)	
Serosal invasion				
negative	64	34 (53%)	30 (47%)	NS
positive	61	29 (48%)	32 (52%)	
Histological type				
differentiated	57	36 (63%)	21 (37%)	NS
undifferentiated	68	27 (40%)	41 (60%)	
Lymphatic invasion				
negative	43	21 (49%)	22 (51%)	NS
positive	82	42 (51%)	40 (49%)	
Venous invasion				
negative	70	37 (53%)	33 (47%)	NS
positive	55	26 (47%)	29 (53%)	

Table 3 Correlation between clinicopathological parameters and uPA/PAI-1 expression in gastric cancer

Clinico-pathological parameters	No. of cases	uPA and PAI-1 tissue status				p value
		uPA (–)/ PAI-1 (–)	uPA (+)/ PAI-1 (–)	uPA (–)/ PAI-1 (+)	uPA (+)/ PAI-1 (+)	
Depth of invasion						
m, sm	39	11 (28%)	10 (26%)	8 (20%)	10 (26%)	NS
mp, ss, se, si	86	8 (9 %)	34 (40%)	15 (17%)	29 (34%)	
Liver metastasis						
negative	114	18 (16%)	36 (32%)	23 (20%)	37 (32%)	p<0.05
positive	11	1 (9%)	8 (73%)	0 (0%)	2 (18%)	
Peritoneal dissemination						
negative	93	17 (18%)	30 (32%)	19 (20%)	27 (30%)	NS
positive	32	2 (6%)	14 (44%)	4 (13%)	12 (37%)	
Lymph node metastasis						
negative	55	13 (24%)	15 (27%)	15 (27%)	12 (22%)	p<0.05
positive	70	6 (9%)	29 (41%)	8 (11%)	27 (39%)	
Serosal invasion						
negative	64	15 (23%)	19 (30%)	17 (27%)	13 (20%)	p<0.05
positive	61	4 (7%)	25 (41%)	6 (10%)	26 (42%)	
Histological type						
differentiated	57	15 (26%)	21 (37%)	10 (18%)	11 (19%)	NS
undifferentiated	68	4 (6%)	23 (34%)	13 (19%)	28 (41%)	
Lymphatic invasion						
negative	43	10 (23%)	11 (26%)	8 (19%)	14 (32%)	NS
positive	82	9 (11%)	33 (40%)	15 (18%)	25 (31%)	
Venous invasion						
negative	70	14 (20%)	23 (33%)	12 (17%)	21 (30%)	NS
positive	55	5 (9%)	21 (38%)	11 (20%)	18 (33%)	

and PAI-1 expression and clinicopathological findings is shown in Table 3. There was a significant correlation between uPA(+)/PAI-1(–) status and liver metastasis, lymph node metastasis and serosal invasion. Tumours with uPA(+)/PAI-1(–) had a significantly higher incidence of liver metastasis, lymph node metastasis and serosal invasion than did the other groups of tumours.

There was no significant relationship between PAI-2 expression and clinicopathological variables.

uPA and PAI-1 tissue status and DNA ploidy patterns

Among the 115 tumours, a diploid DNA pattern was observed in 32 patients (28%) and an aneuploid pattern in 83 patients (72%). Ten patients were excluded because of a lack of tissue. There was no significant association between uPA tissue status and DNA ploidy patterns. In addition, no correlation between PAI-1 tissue status and DNA ploidy patterns could be found.

uPA, PAI-1 and PAI-2 tissue status and prognosis

The analysis of uPA tissue status and survival is shown in Fig. 6. The 5-year survival rates of the patients with uPA-positive tumours and with negative tumours were 40.1% and 82.4%, respectively. There was a significant survival advantage for patients with uPA-negative tumours. Patients with PAI-1-positive tumours survived a better time than those with PAI-1-negative ones. The 5-year survival rates of the patients with PAI-1-positive tumours and with negative tumours were 59.3% and 53%. Patients with PAI-2-negative tumours survived longer than those with PAI-2-positive ones. The 5-year survival rates of the patients with PAI-2-negative tumours and with positive tumours were 61.6 and 47.7%, respectively. Figure 7 shows the survival curves of four groups subdivided according to the uPA and PAI-1 expression. The 5-year survival rate of the patients with uPA(–)/PAI-1(+) tumours was 85.2%, and there was a significant difference in survival advantage between these two groups. The 5-year survival rates of patients with uPA(–)/PAI-1(+), uPA(–)/PAI-1(–), uPA(+)/PAI-1(–) and uPA(+)/PAI-1(+) tumours were 79.0%, 40.9% and 37.8%, respectively. Patients with uPA(+)/PAI-1(–) tumours survived a significantly shorter time than those with uPA(–)/PAI-1(+) ones. On the other hand, the 5-year survival rates of patients with uPA(+)/PAI-2(–) tumours and with uPA(+)/PAI-2(+) tumours were 42.9 and 34.2%, respectively. Patients with uPA(+)/PAI-2(+) tumours had shorter survival times than those with uPA(+)/PAI-2(–) tumours.

The patterns of recurrent sites after operation from the aspects of uPA and PAI-1 tissue status are shown in Table 4. Three (13%) of 23 patients with uPA(–)/PAI-1(+) tumours had recurrence in the peritoneum, while, in contrast, 20 (45%) of 44 patients with uPA(+)/PAI-1(–) tumours had recurrence. There was a significant correlation between uPA/PAI-1 tissue status and recurrence.

Several prognostic variables were analysed by a generalized Wilcoxon test (Table 5). uPA, liver metastasis,

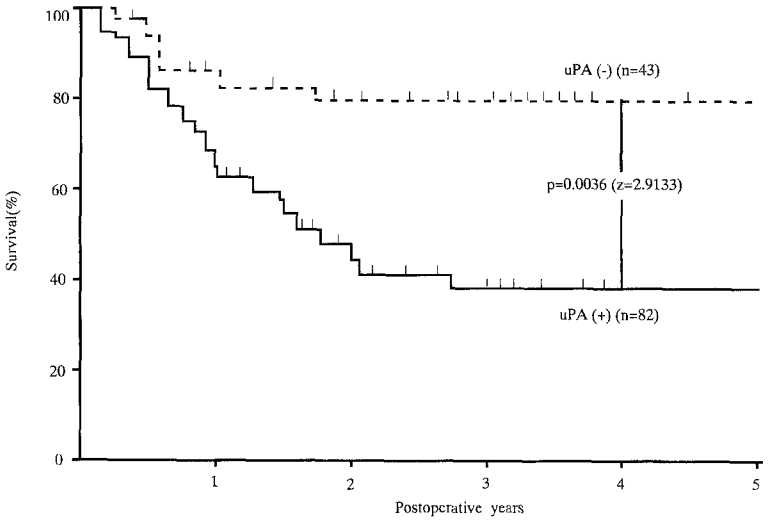


Fig. 6 Survival curves of patients with gastric cancer, according to the uPA expression; patients with uPA-positive tumours survived a significantly shorter time than those with uPA-negative tumours

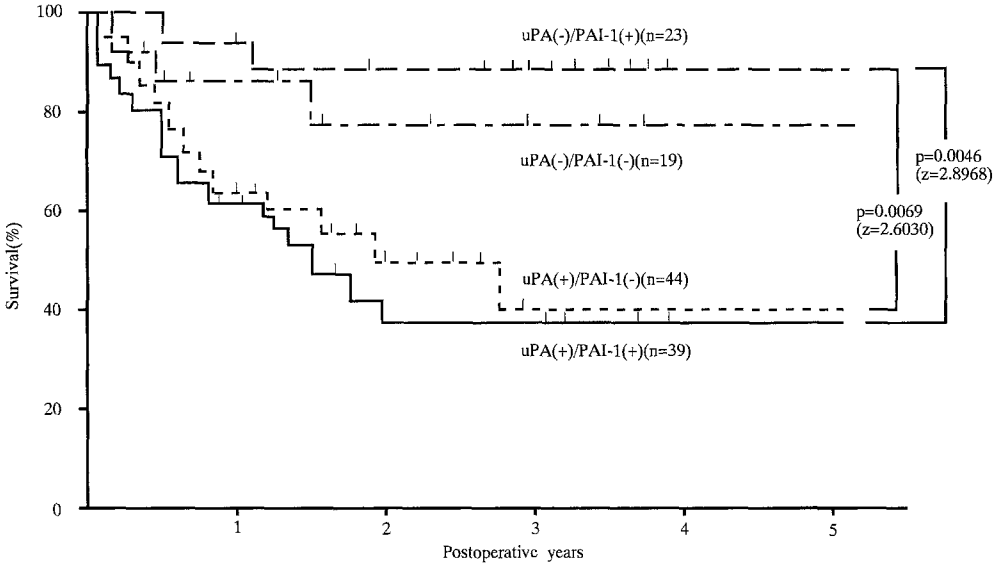


Fig. 7 Survival curves of four groups subdivided according to the uPA and PAI-1 expression; the 5-year survival rate of patients with uPA(–)/PAI-1(+) tumours was 85.2%, and it was the highest among these four groups

Table 4 Recurrent sites according to the uPA/PAI-1 tissue status

	No. of cases	Recurrent sites			
		Liver	Peritoneum	Lymph node	
uPA (–)/PAI-1 (–)	19	1 (5%)	1 (5%)	1 (5%)	
uPA (+)/PAI-1 (–)	44	7 (16%)	9 (20%)	4 (9%)	p<0.01
uPA (–)/PAI-1 (+)	23	0 (0%)	3 (13%)	0 (0%)	
uPA (+)/PAI-1 (+)	39	1 (3%)	12 (31%)	2 (5%)	

peritoneal dissemination, lymph node metastasis, venous invasion, serosal invasion, depth of invasion and lymphatic invasion were the significant variables for survival. However, PAI-1, PAI-2, DNA ploidy, histological type were not prognostic factors.

Discussion

Tumour cell invasion and metastasis formation is a multifactorial process divided into steps, such as detachment of malignant cells from the primary tumour, invasion into surrounding tissues, penetration into lymphatic and blood vessels, circulation, and development of a tumour mass at a distant secondary locus. The remodelling it involves requires the coordinated action of cell-secreted proteolytic enzymes and their inhibitors. Several groups have reported on the possible role of plasminogen activators in tumorigenesis, tumour invasion and metastasis [8, 11, 26, 33–35, 52]. The presence of these proteolytic enzymes in tumours results in the conversion of plasminogen to plasmin, which not only directly destroys components of the extracellular matrix, such as laminin and fibrin, but also activates procollagenases to collagenases, which degrade collagen in the matrix [2, 8, 11, 36, 51]. In recent studies in breast cancer, Duffy et al. reported the positive correlation of uPA tissue status and tumour size and metastasis [10], and uPA seem to be a better prognostic parameter than the known clinical and histological variables. In gastric cancer, uPA levels increased with the degree of nodal involvement and with increasing tumour stage [63]. However, the precise role of the inhibitors, PAI-1 and PAI-2, in gastric cancer is not

Table 5 Clinicopathological parameters, DNA ploidy and uPA, PAI-1 and PAI-2 tissue status as prognostic parameters in 125 patients with gastric cancer

Variables	Generalized Wilcoxon test	
	z value	p value
uPA expression		
Negative	3.266	<0.001
Positive		
Liver metastasis		
Negative	4.835	<0.001
Positive		
Peritoneal dissemination		
Negative	5.815	<0.001
Positive		
Lymph node metastasis		
Negative	5.675	<0.001
Positive		
Venous invasion		
Negative	5.729	<0.001
Positive		
Serosal invasion		
Negative	6.294	<0.001
Positive		
Depth of invasion		
m, sm	4.487	<0.001
mp, ss, se, si		
Lymphatic invasion		
Negative	3.833	<0.001
Positive		
Histological type		
Differentiated	1.873	0.061
Undifferentiated		
DNA ploidy		
Diploid	1.117	0.264
Aneuploid		
PAI-2 expression		
Negative	0.875	0.382
Positive		
PAI-1 expression		
Negative	0.477	0.633
Positive		

known. In the current study, we investigated the uPA, PAI-1 and PAI-2 expression immunohistochemically and evaluated whether these protease and its inhibitor were related to the extent of gastric cancer progression.

Our immunohistochemical staining against anti-uPA, anti-PAI-1, and anti-PAI-2 antibodies revealed intensive staining for these proteins in the cytoplasm of cancer cells, which was more prominent at the growing front. Previous studies support these findings [39, 41, 47, 66]. We investigated the interrelationship between tumour infiltration and metastatic potentials of gastric cancer and the coexpression of uPA and PAI-1, or PAI-2. The current study revealed that the expression of uPA was significantly correlated with depth of invasion, peritoneal dissemination and lymph node metastasis. Wang et al. [65] also reported the same results, i.e. that the positive expression of uPA correlated with lymph node metastasis in gastric cancer. PAI-1 expression was significantly associated with liver metastasis. Conversely, no relationship between PAI-2 expression and clinicopathological findings could be found.

In gastric cancer, Nekarda et al. [40] reported that elevated uPA or PAI-1 levels measured by ELISA were found to be associated with poor prognosis. The present study showed that uPA and PAI-1 expression were closely associated with patient prognosis. Patients with uPA-positive tumours survived a significantly shorter time than those with uPA-negative ones. Patients with PAI-1-positive tumours survived better than those with PAI-1-negative ones. In addition, patients with uPA(+)/PAI-1(–) tumours survived a significantly shorter time than those with uPA(–)/PAI-1(+) ones. We suggest that uPA and PAI-1 tissue status are good prognostic indicators [10, 13, 19, 47]. The present study revealed that positive expression of uPA was associated with a poor prognosis [13, 19, 47, 58, 66], while in contrast, negative expression of PAI-1 was associated with poor prognosis. The correlation between uPA and PAI-1 levels raised two possibilities [47]: either PAI-1 has a role in the defence mechanism against tumour invasion, or it is involved in the activation of plasminogen. In this study, we did not measure the content of uPA and PAI-1 in cancer tissue, but our results, showing a link between negative expression of PAI-1 and liver metastasis and a poor outcome, tend to support the first hypothesis. But the inhibitory effect of PAI-1 on uPA-mediated extracellular matrix degradation was not completely clear in this study. Other mechanisms for overexpression of PAI-1 in tumour tissues, such as autocrine or paracrine oversynthesis under the control of growth factors (tumour necrosis factor-α, β-epidermal growth factor, tumour growth factor-β, cytokines and hormones) are suggested [3, 25, 26, 33]. Further study is needed to clarify the role of PAI-1 in tumour growth.

These results suggest that, when a tumour shows uPA overexpression and negative PAI-1 expression concurrently, it may have powerful invasive and metastatic potential. In the present study, patients with uPA(+)/PAI-1(–) tumours had a significantly higher incidence of liver metastasis, lymph node metastasis and serosal invasion than those with the other groups of tumours. Regarding the relationship between prognosis and uPA and PAI-1 expression, the 5-year survival rate in patients with uPA(+)/PAI-1(–) tumours was 40.9%, in those with uPA(–)/PAI-1(+) tumours it was 85.2%, and there was a significant survival advantage. In addition, with regard to the relationship between recurrence and uPA and PAI-1 expression, recurrence was most frequently observed in patients with uPA(+)/PAI-1(–) tumours. These results may suggest that tumours with overexpression of uPA and negative PAI-1 expression have a powerful potential for invasiveness and metastasis.

Several reports have shown that only uPA receptor (uPAR) is produced in cancer cells and that both uPA and PAI-1 are produced in fibroblasts or endothelial cells around tumour tissue through a paracrine mechanism stimulated by tumour cells [44, 45]. The positive staining

of uPA and PAI-1 was explained as being due to internalization and accumulation of these complexes in tumour cells after binding of uPA to uPAR on the cell surface, followed by complex formation with PAI-1 [42]. However, the presence of mRNA for uPA, PAI-1, and PAI-2 in cancer cells [46, 53] and the rather strong staining for these proteins in cancer cells indicate that the proteins are synthesized mainly in the cells. These proteins seem to be released from cancer cells after production, followed by the binding to cell surface uPAR. Schwartz et al. [55] have also reported that gastric cancer cell lines expressed uPA and the 72-kDa form of collagenase type IV, mRNA expression of the 72-kDa type IV collagenase may be more useful in distinguishing invasive from non-invasive cells than uPA. In our current study, uPA and PAI-1 immunoreactivities were seen in the cytoplasm of cancer cells, whereas the adjacent stromal cells were not stained. Further study is needed to localize and elucidate the role of uPAR, as well as uPA, PAI-1 and PAI-2 in gastric cancer.

De Bruin et al. [9] reported that the reduction of tPA activity in cancer tissue might be due to a lesser degree of vascularization accompanied by invasion and rapid clearance due to the formation of tPA-PAI-1 complex, because PAI-1 antigen levels were elevated in cancer tissue. In the current study, tumours with PAI-1 overexpression had a higher incidence of inhibition for liver metastasis and survival times were better. Our results support the conclusions that PAI-1 is synthesized mainly in cancer cells and form a covalent complex with tPA, which has a major role in intravascular thrombolysis and in tumour growth, and that PAI-1 plays a critical part in the control of stromal invasion. Sier et al. [56] have reported that the heterogeneous increase of uPA antigen concentration (uPA/tPA antigen ratio of the carcinoma was higher than normal mucosa) might be of value in determining prognosis in oesophageal and gastric cancer. Elucidation of the role of tPA in tumour growth also appears to be important for understanding the mechanism of tumour infiltration and metastasis.

The biological characteristics of highly malignant tumours have been studied by DNA analyses [18, 22], and estimation of DNA abnormalities by means of flow cytometry or cytofluorometry may substantially contribute to knowledge of the biological characteristics of gastric cancer. Takai et al. [57] reported that aneuploid tumours had significantly higher levels of uPA content than diploid ones. In this analysis, however, no relationship between uPA tissue status and DNA ploidy patterns could be found. In addition, no correlation between PAI-1 tissue status and DNA ploidy patterns was evident. In the current study, uPA tissue status was a better prognostic variable than DNA ploidy patterns, but uPA tissue status was not the strongest one of the prognostic findings; liver metastasis, peritoneal dissemination, lymph node metastasis, venous invasion, serosal invasion, depth of invasion and lymphatic invasion were all stronger. PAI-1 tissue status was not a better prognostic parameter than DNA ploidy patterns.

In conclusion, our results indicate that uPA expression is a useful biological prognostic indicator, and that uPA and PAI-1 may have an important role in tumour progression and metastasis in gastric cancer.

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