Urokinase-type plasminogen activator expression correlates with tumor angiogenesis and poor outcome in gastric cancer

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Urokinase plasminogen activating system (PA system) and vascular endothelial growth factor (VEGF) were recently suggested to contribute synergistically to tumor progression. To evaluate the roles of the PA system and VEGF in gastric cancer, the effects of the PA system and VEGF on tumor angiogenesis and the survival of patients with gastric cancer were investigated. Cancer tissues from 101 gastric cancer patients were assayed immunohistochemically for expression of urokinase-type plasminogen activator (uPA), uPA receptor (uPAR), PA inhibitor-1 (PAI-1) and VEGF protein. The positive rates of uPA, uPAR, PAI-1, VEGF expression were 22.8%, 32.7%, 36.6% and 26.7%, respectively. Positive staining was observed in tumor cells (uPA, uPAR, VEGF), or in both tumor cells and stromal cells (PAI-1). The expressions of uPA, uPAR, PAI-1 and VEGF were significantly correlated with the clinicopathological factors: uPA, depth of tumor invasion, differentiation, lymphatic and vascular invasion; uPAR, tumor size, depth, lymph node involvement, differentiation, vascular invasion; PAI-1, tumor size, depth, lymph node involvement, differentiation, vascular invasion; VEGF, differentiation, vascular invasion. The microvessel density (MVD) assessed immunohistochemically was significantly higher in the patients with expression of uPA, uPAR or VEGF, and stepwise analysis identified uPA as an independent correlated factor with MVD. Furthermore, multivariate analysis demonstrated that depth of tumor invasion, lymph node involvement and uPA expression were independent prognostic factors. uPA is a key factor in the PA system, being associated with a poor outcome of gastric cancer, and contributing not only to invasive activity, but also to angiogenesis. (Cancer Sci 2003; 94: 43–49)

Destruction of the extracellular matrix and basement membrane is essential for tumor invasion and metastasis. The extracellular matrix is degraded by extracellular proteolytic enzymes such as metalloproteases and serine proteases.[1, 2] Plasminogen activators (PA) catalyze the conversion of the inactive pro-enzyme plasminogen to plasmin.[3] Plasmin acts to degrade the extracellular matrix and activates latent enzymes such as type-IV collagenase.[3, 4] Among the plasminogen activators, urokinase-type plasminogen activator (uPA) and uPA receptor (uPAR) have been reported to play an important role in tumor progression.[5, 6] uPA, produced in both normal and malignant cells, has roles in tissue remodelling of normal cells, degradation of extracellular matrix and destruction of the basement membrane in malignant cell proliferation and metastasis. uPA activation occurs on the cell surface after binding to its specific receptor and is regulated by the number of uPAR.[7] The relevance of uPA or uPAR in tumor progression has been demonstrated by the poor prognoses of patients with a high content of uPA or uPAR in tumor tissue.[8–10] The activities of PA are controlled not only through its synthesis and secretion, but also by its two physiologic PA inhibitors, type 1 (PAI-1) and type 2 (PAI-2), both of which belong to the serine protease inhibitor superfamily.[11] Elevated PAI-1 levels were found to be associated with poor prognosis.[9, 10, 12] A possible promoting function of PAI-1 in tumor growth is suggested by its potential to modify cell adhesion capacity, which is independent of uPA inhibitory activity.[13, 14]

Angiogenesis, the formation of new capillaries from existing blood vessels, is essential for the growth of a solid tumor.[15] Many studies have shown that malignant tumors depend on angiogenesis for their growth and metastasis.[3, 16] It is generally assumed that microvessel formation around a tumor is stimulated by various angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and IL-1 and angiopoietin-2, secreted by the tumor cells.[16] Among them, VEGF, a selective mitogen for endothelial cells, is regarded as the most important factor in angiogenesis of gastrointestinal tumors.[17] The overexpression of VEGF has been reported in various tumors,[18, 19] and a significant correlation has been demonstrated between microvessel density (MVD) and VEGF expression in tumor cells.[20] Recently a role of the PA system in tumor angiogenesis has also been demonstrated.[21, 22] In this context, we examined here the roles of the PA system and VEGF in tumor invasion and angiogenesis, and whether the two factors are independently prognostic for gastric cancer.

Materials and Methods

Patients. Tumors were obtained from 101 patients (72 men and 29 women; mean age 60.8; range 18–87) who had undergone surgery for gastric cancer at our department from November 1995 through November 1999. Tumor sizes ranged from 1–14 cm in greater diameter (median, 4.6 cm). No patients had received prior chemotherapy or radiation. The Japanese Classification of Gastric Carcinoma (The 13th Edition) was used for pathologic diagnosis and for the classification of variables.

Immunohistochemistry of uPA, uPAR, PAI-1 and VEGF. Immunohistochemical stainings for VEGF, uPA, uPAR and PAI-1 in formalin-fixed, paraffin-embedded cancer tissues were performed using the indirect immunoperoxidase technique. Samples were fixed with 10% formaldehyde in phosphate-buffered saline (PBS), embedded in paraffin, and cut into 4 μm thick sections, which then were deparaffinized with xylene and dehydrated with ethanol. Endogenous peroxidase activity was blocked by incubation with 0.3% H_2O_2 for 15 min. The sections were washed with PBS, and treated with 10% normal goat serum (Histofine SAB-PO kit, Nichirei, Tokyo) for 10 min to block nonspecific protein binding. The sections were then incubated with anti-human VEGF monoclonal antibody (MoAb) (IBL Co., Ltd., Gunma) at 1:500 dilution, anti-uPA MoAb (3785, American Diagnostica, Inc. [ADI], Greenwich, CT) at 1:200 dilution, anti-uPAR MoAb (3936, ADI) at 1:100 dilution, and anti-PAI-1 MoAb (3989, ADI) at 1:100 dilution, for 60 min at 4°C. Sections were then washed and treated with biotinylated rabbit antimouse antibody for 30 min, and streptavidin horseradish peroxidase (HRP)-conjugated reagent for 10 min at room

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temperature (uPA and uPAR) or Envision+ (K4000, Dako Japan, Tokyo) in the cases of PAI-1 and VEGF. Diaminobenzidine was used as the chromogen. The sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted.

All slides were evaluated by two clinicians without knowledge of the patients' identity or clinical status. Specimens taken adjacent to the invasive front were used for the staining examination. Analysis of staining was exclusively restricted to tumor cell reactions. Staining of stromal cells was not considered. uPA, uPAR, PAI-1 and VEGF expressions were classified into three groups. When strong immunostaining was seen in more than 50% of cancer cells, the tumors were determined to be positive (+). When weak immunostaining was seen in less than 50% of cancer cells, the tumors were determined to be negative (−). Weak immunostaining in more than 50% of cells, or strong immunostaining in less than 50% of cells was designated +/−.

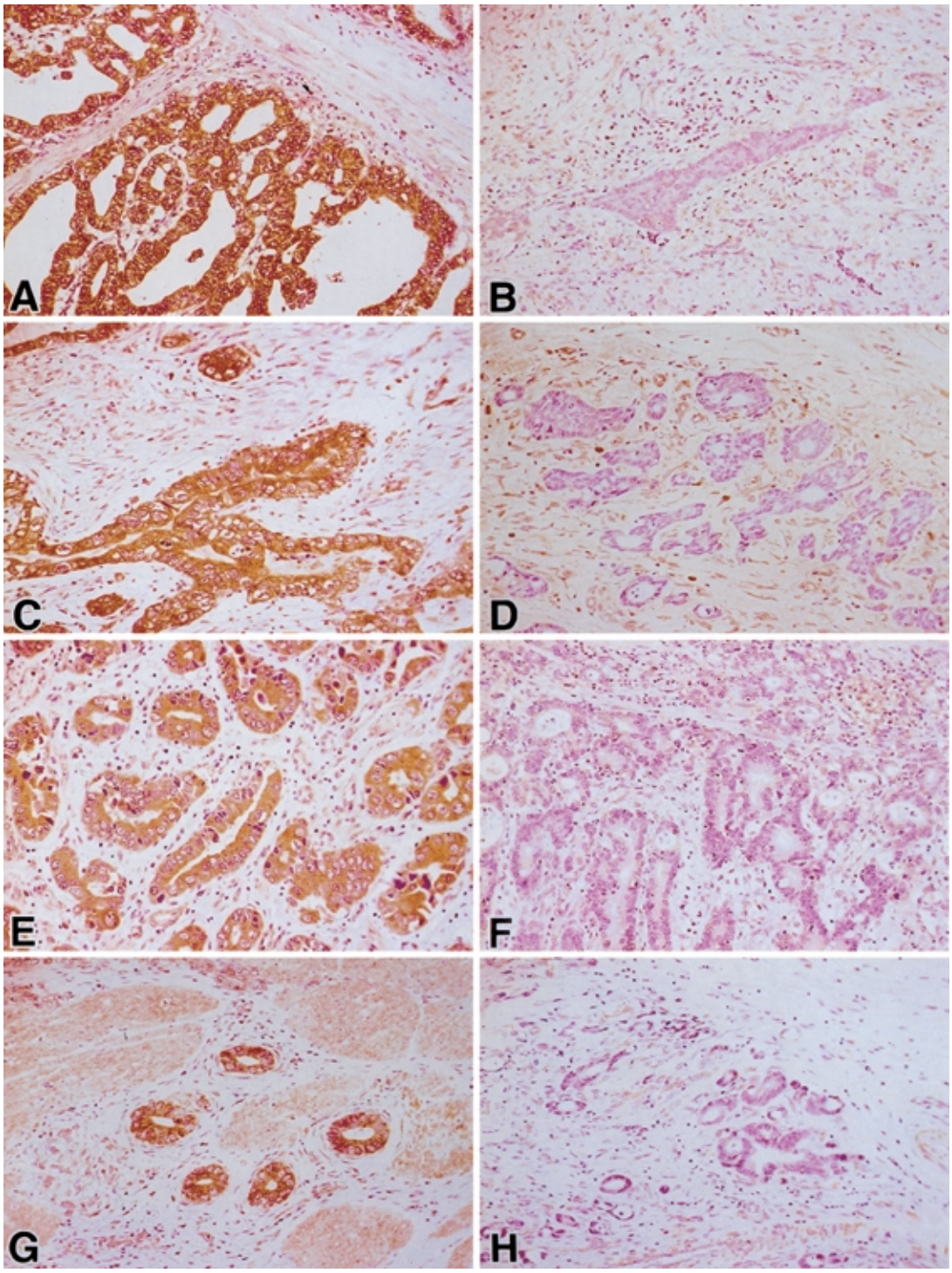

Fig. 1. Immunohistochemical analysis of uPA, uPAR, PAI-1, and VEGF expression in gastric cancer tissue. Positive immunoreactivity for uPA (A), uPAR (C), PAI-1 (E) and VEGF (G). Negative immunoreactivity for uPA (B), uPAR (D), PAI-1 (F) and VEGF (H). uPA, uPAR and VEGF were mainly expressed in cancer cells.

MVD. MVD was determined by immunohistochemical staining with an antihuman factor VIII-related antigen monoclonal antibody (M0616, Dako Japan), as described elsewhere. Microwave pretreatment (500 W, 15 min, 0.1 *M* citrate buffer) of dewaxed tissue sections and the treatment of tissue with trypsin (0.1% trypsin, 37°C, 30 min) were performed in the case of factor VIII-related antigen. Endogenous peroxidase activity was blocked by incubation with 0.3% H_2O_2 in methanol and nonspecific protein binding was blocked by treatment with 10% normal goat serum for 10 min. This was followed by incubation with anti-factor VIII-related antigen at a dilution of 1:20 overnight at 4°C. Sections were then washed and treated with Envision+ (K4000, Dako). Positive staining was observed in vascular endothelial cells. MVD was evaluated by counting the number of endothelial deposits/field by light microscopy at 200× magnification without knowledge of patients' status. The mean of four counts for each specimen was calculated and statistically analyzed.

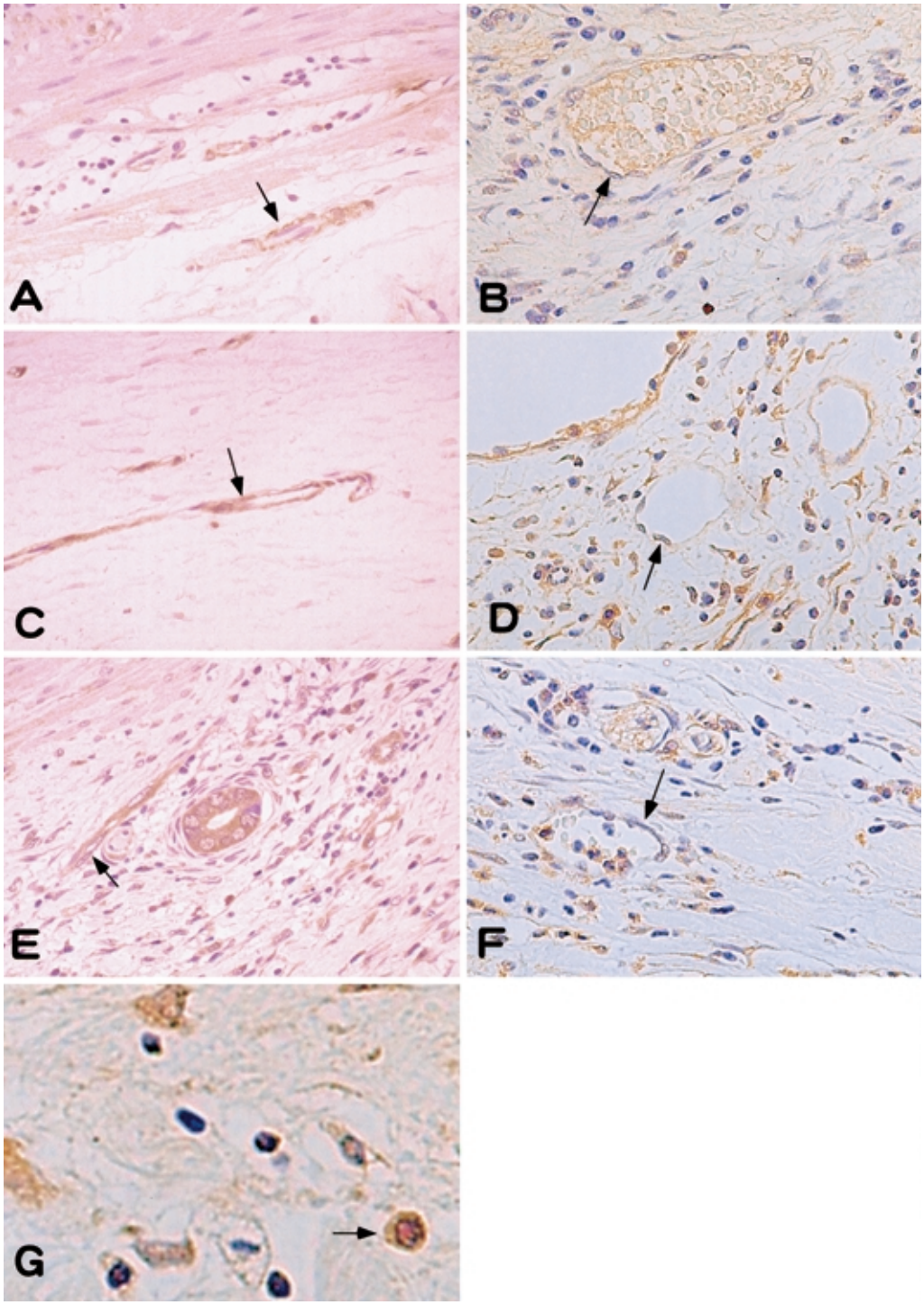

Fig. 2. Immunohistochemical staining of uPA (A), uPAR (C) and PAI-1 (E) in endothelial cells, and VEGF (G) in macrophages. Negative immunoreactivity for uPA (B), uPAR (D) and PAI-1 (F) in endothelial cells. There were a few stromal cells with positive expression of these factors, but the number of those cells was too small to evaluate quantitatively.

Statistical analysis. Statistical analysis was performed using a statistical software package (Statview 4.5, Abacus Concepts, Berkeley, CA). VEGF, uPA, uPAR and PAI-1 expression and MVD in relation to various clinicopathologic factors were assessed using the χ^2 test. Survival was calculated from the date of surgery to the date of death or of the last follow-up. The factors related to MVD were identified by stepwise analysis. The survival rate was estimated by the Kaplan-Meier method and analyzed by means of the log-rank test. To define independent risk factors for prognosis, multivariate analysis was performed with a Cox proportional hazards model. Differences were considered significant when P values were less than 0.05.

Results

Relationships between immunoreactivity of uPA, uPAR, PAI-1, VEGF and clinicopathological factors. Positive staining for uPA, uPAR and VEGF was observed mainly in cancer cells, but also in a number of stromal cells including endothelial cells. Among stromal cells, positive staining of uPA and uPAR was observed mainly in endothelial cells and that of VEGF was mainly in macrophages (Figs. 1, 2). However, the numbers of these cells were too small to allow quantitative evaluation of the correlation with clinicopathlogical factors.

The rates of positive expression in cancer cells of uPA, uPAR, PAI-1 and VEGF were 22.8%, 32.7%, 36.6%, and 26.7%, respectively. The positive relationships of the expression of each factor to clinicopathological factors are shown in Table 1. It was shown that PA system is associated with local growth and invasion of the cancer and lymph node involvement. Among the components of the PA system, there was a significant relationship between uPA and uPAR, and between uPAR and PAI-1 (data not shown).

Relationships of uPA, uPAR, PAI-1 and VEGF expressions to the MVD. By univariate analysis, a significant association of MVD with uPA, uPAR and VEGF was shown (Fig. 3). Stepwise analysis revealed that only uPA was significantly correlated with MVD (Table 2).

Impact of expression of uPA, uPAR, PAI-1 and VEGF proteins on survival. The survival rate of patients with positive uPA or uPAR expression was significantly lower than that of patients without it (Fig. 4). In univariate analysis using the Cox proportional hazards model for survival rate, uPA, uPAR, MVD, lymphatic invasion, vascular invasion, lymph node involvement, depth of tumor invasion, tumor differentiation and tumor size were significant prognostic factors. In multivariate analysis, depth of tu-

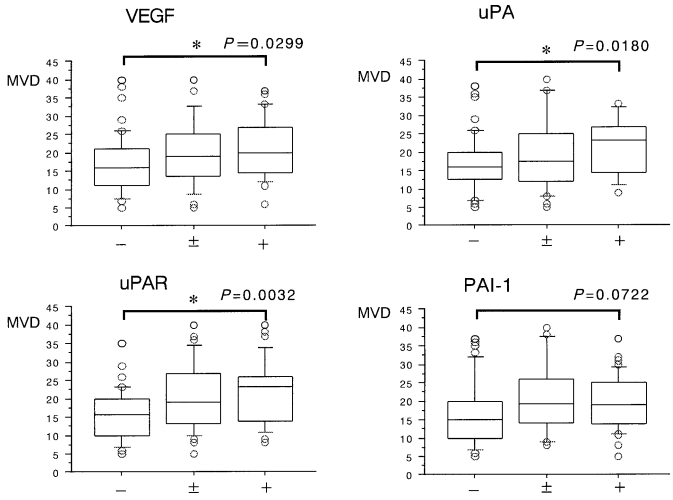

Fig. 3. Relations between the expression of VEGF, uPA, uPAR, and PAI-1 and the MVD. There were significant correlations between the expressions of VEGF, uPA and uPAR and the MVD (P=0.0299, P=0.018, P=0.0032), but there was no correlation between the expression of PAI-1 and the MVD. *: Significant difference between two groups (P<0.05).

Table 1. Relation of immunoreactivity of uPA, uPAR, PAI-1 and VEGF to clinicopathological factors

Factor	Case	uPA expression			P value	uPAR expression			P value	PAI-1 expression			P value	VEGF expression			P value
		−	±	+		−	±	+		−	±	+		−	±	+	
Age (years)																	
<65	53	31	10	12		19	16	18		18	13	22		27	11	15	
≥65	48	21	16	11	0.211	19	14	15	0.924	20	13	15	0.553	23	13	12	0.751
Tumor size																	
<40	53	31	11	11		26	16	11		26	11	16		31	16	18	
≥40	48	21	15	12	0.310	12	14	22	0.013	12	15	21	0.045	19	8	9	0.887
Depth of tumor invasion																	
<mp[1)]	47	33	10	4		26	11	10		27	11	9		28	10	9	
≥mp	54	19	16	19	<0.001	12	19	23	0.003	11	15	28	<0.001	22	14	18	0.141
Lymph node involvement																	
Negative	64	37	14	13		31	16	17		31	17	16		34	16	14	
Positive	37	15	12	10	0.242	7	14	16	0.013	7	9	21	0.003	16	8	13	0.347
Tumor differentiation																	
Differentiated	49	17	18	14		12	18	19		12	13	24		16	16	17	
Undifferentiated	52	35	8	9	0.004	26	12	14	0.030	26	13	13	0.015	34	8	10	0.004
Lymphatic invasion																	
Negative	48	32	11	5		23	13	12		23	11	14		27	12	9	
Positive	53	20	15	18	0.005	15	17	21	0.109	15	15	23	0.119	23	12	18	0.214
Vascular invasion																	
Negative	55	39	10	6		30	12	13		28	13	14		32	16	7	
Positive	46	13	16	17	<0.001	8	18	20	<0.001	10	13	23	0.007	18	8	20	0.002
Peritoneal dissemination																	
Negative	92	48	24	20		36	28	28		36	23	33		45	23	24	
Positive	9	4	2	3	0.731	2	2	5	0.302	2	3	4	0.604	5	1	3	0.638

1) mp, muscularis propria.

mor invasion, lymph node involvement and uPA expression emerged as independent prognostic factors (Table 3).

Discussion

The components of the PA system promote tumor invasion by cleavage of the extracellular matrix in various cancer cells, like matrix metalloproteinases (MMPs), which can collectively degrade almost all extracellular matrix and basement membrane proteins.[23] The PA system also plays a vital role in the early phase of tumor angiogenesis.

Table 2. Correlations between MVD and uPA, uPAR, PAI-1, and VEGF (stepwise analysis)

Variable	*F* value	*P* value
uPA	9.660	0.0025
uPAR	6.154	
PAI-1	3.050	
VEGF	3.325	

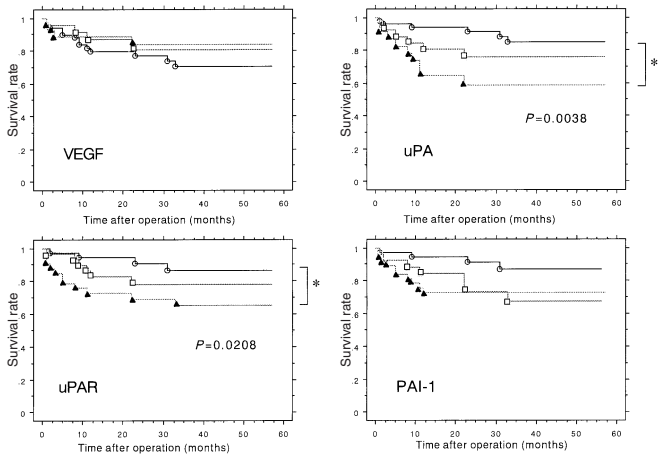

Fig. 4. Survival curves of gastric cancer patients showing VEGF, uPA, uPAR or PAI-1 expression. The survival rate of patients with positive expression of uPA was significantly worse than that of patients with negative expression of uPA (P=0.0038). The survival rate of patients with positive expression of uPAR was significantly worse than that of patients with negative expression of uPAR (P=0.0208). ▲ +, □ ±, ○ −.

We reported previously that the factors in the PA system, especially uPAR and PAI-1, were involved in tumor progression, and that the uPAR level in tumor tissue was a useful indicator of tumor progression in patients with colorectal cancer.[24] We also reported the synergistic effect of the PA system and VEGF in promoting liver metastasis of colorectal cancer.[25] However, it is still controversial which factor among those of the PA system is most important in the progression of gastric cancer. We thus assessed the relevance of the PA system to tumor invasion, angiogenesis and survival in gastric cancer. The relevance of the PA system in gastric cancer has been investigated by using qualitative and quantitative methods. Migita *et al.*[26] reported that uPA expression in cancer cells was qualitatively more pronounced in patients with liver metastasis. Allgayer *et al.*[27] reported that uPAR was a potential antigen for positive selection of disseminated tumor cells during the postoperative time course. Quantitative analysis was also performed in some studies, confirming an important role of uPA or uPAR.[28–30] Okusa *et al.*[29] reported that uPA promoted the invasive capacity of uPAR-positive cancer cells, and suggested that uPA expression in stromal cells is significantly correlated with tumor histology and peritoneal metastasis.[28]

Since there was a significant relationship among the factors of PA system, uPA, uPAR and PAI-1 may synergistically contribute to the progression of gastric cancer. It is likely that uPA promotes the invasive ability of uPAR-positive gastric cancer cells, and that stromal cells play an important role in cancer cell invasion by supplying uPA and promoting uPA production. Since both uPAR-bound uPA and nonbound uPA are identified immunohistochemically, the isolation of both types of uPA may contribute to clarifying the role of uPA.[29] PAI-1 has also been shown to promote tumor growth and invasion by potentiating tumor cell detachment from the matrix.[13, 14] The binding of uPA to uPAR enhances binding to vitronectin.[31] Because PAI-1 binds to the same somatomedin B-like domain of vitronectin, it can compete with the binding of the uPA/uPAR complex.[13, 32] Inhibition of adhesion to vitronectin by PAI-1 may also promote cell migration on vitronectin-rich matrices, and in this regard PAI-1 may have a function unrelated to its antiprotease activity and may promote rather than inhibit cell invasion.[33–35] Such a modification of cell adhesion to the matrix by uPAR and PAI-1 appears to be intimately involved in tumor growth, invasion and metastasis.

Both the PA system and VEGF are key factors in tumor angiogenesis. The PA system degrades the basement membrane and stimulates the migration and progression of endothelial cells in the early phase of angiogenesis.[36] Aside from the in-

Table 3. Results of univariate and multivariate analysis of factors influencing the survival of the patients

Variables	Comparison	Univariate analysis			Multivariate analysis		
		Odds ratio	*P* value	95%CI[1)]	Odds ratio	*P* value	95%CI
uPA	−, ±, +	2.015	0.0070	1.211–3.352	2.442	0.0081	1.261–4.731
uPAR	−, ±, +	1.892	0.0221	1.096–3.265	—	NS	
PAI-1	−, ±, +	—	NS				
VEGF	−, ±, +	—	NS				
MVD		1.061	0.0092	1.015–1.109	—	NS	
Lymphatic invasion	0, 1, 2, 3	3.170	<0.0001	2.092–4.801	—	NS	
Vascular invasion	0, 1, 2, 3	2.464	<0.0001	1.651–3.676	—	NS	
Lymph node involvement	0, 1, 2, 3, 4	2.268	<0.0001	1.717–2.995	1.883	0.0067	1.192–2.974
Depth of tumor invasion	m, sm, mp, ss, se, si[2)]	2.687	<0.0001	1.781–4.054	1.965	0.0201	1.111–3.474
Histology	diff., undiff.[3)]	2.661	0.0430	1.031–6.866	—	NS	
Size		1.023	<0.0001	1.012–1.033	—	NS	
Sex	male, female	—	NS				

1) CI, confidence interval.
2) m, mucosa; sm, submucosa; mp, muscularis propria; ss, subserosa; se, serosa exposed; si, serosa infiltrating.
3) diff., differentiated; undiff., undifferentiated.

duction of tumor angiogenesis, VEGF has several additional functions that serve to enhance tumor progression, including enhancing the permeability of tumor vessels[37] and inhibiting either apoptosis of endothelial cells[38, 39] or the maturation of dendritic cells.[40] An association between the PA system and angiogenesis has been reported.[36, 41, 42] VEGF has been shown to cause up-regulation of uPA and uPAR in endothelial cells, and we demonstrated in the previous study that the PA system and VEGF synergistically contributed to liver metastasis of colorectal cancer.[25] A significant relation of VEGF to both uPA and uPAR was also observed in the present study (data not shown). VEGF promotes proliferation of endothelial cells and tube formation after degradation of the extracellular matrix by the PA system and/or MMPs.[36, 41] In the present study, we observed positive immunogenicity of uPA, uPAR or VEGF in the stromal cells, suggesting that stromal cells with positive expression may play an important role in tumor angiogenesis. However, the number of these stromal cells was small. Our results revealed that cancer cells with positive expression may be mainly correlated with tumor angiogenesis. Interestingly, stepwise analysis demonstrated that only uPA was significantly correlated with MVD among uPA, uPAR, PAI-1 and VEGF. The uPA produced from gastric cancer cells destroys the extracellular matrix, which may promote migration of both cancer cells and endothelial cells. On the other hand, cancer cells with high invasive ability may have various malignant potentials, including VEGF production. Since a positive correlation between VEGF expression and both uPA and uPAR expression was found in the present study, it is possible that the PA system enhances VEGF-induced tumor angiogenesis. Although additional studies are needed to clarify the difference of tumor angiogenesis among various tumors, this is the first report to suggest that uPA is a key factor in the angiogenesis of gastric cancer. Recently, it was reported that endostatin inhibited angiogenesis through the down-regulation of the PA system.[43] Thus, the inhibition of uPA activity may inhibit not only tumor invasion, but also angiogenesis in gastric cancer. Ganesh *et al.*[30] reported that t-PA and PAI-1 levels are independently associated with survival. Yonemura *et al.*[8] reported that overexpression of uPA protein was associated with several clinicopathologic factors and poor prognosis. Nekarda *et al.*[9] found elevated uPA levels to be associated with poor prognosis. However, there have been few studies in which the survival impact of all three factors was assessed in gastric cancer. Allgayer *et al.*[44] immunohistochemically studied the uPA system and demonstrated by multivariate Cox analysis that PAI-1 was an independent parameter. Heiss *et al.*[45] reported that univariate analysis revealed highly significant inverse correlations between uPA, uPAR and PAI-1 expression and survival time, while in multivariate analysis PAI-1 was an independent prognostic factor.

In the present study, survival analysis with the Kaplan-Meier method demonstrated that patients with expression of uPA or uPAR protein had a significantly lower survival rate than those without it. However, multivariate analysis revealed only uPA expression as an independent prognostic factor, in addition to depth and lymph node involvement. Our results suggest that the PA system contributes synergistically to tumor invasion, but uPA is especially important in angiogenesis of gastric cancer. uPA might thus be identified as an independent prognostic factor.

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1. Dano K, Andreasen PA, Grondahl HJ, Kristensen P, Nielsen LS, Skriver L. Plasminogen activators, tissue degradation, and cancer. *Adv Cancer Res* 1985; **44**: 139–266.
2. Andreasen PA, Kjoller L, Christensen L, Duffy MJ. The urokinase-type plasminogen activator system in cancer metastasis: a review. *Int J Cancer* 1997; **72**: 1–22.
3. Duffy MJ, Reilly D, O'Sullivan C, O'Higgins N, Fennelly JJ, Andreasen P. Urokinase-plasminogen activator, a new and independent prognostic marker in breast cancer. *Cancer Res* 1990; **50**: 6827–9.
4. Sumiyoshi K, Serizawa K, Urano T, Takada Y, Takada A, Baba S. Plasminogen activator system in human breast cancer. *Int J Cancer* 1992; **50**: 345–8.
5. Duffy MJ. The role of proteolytic enzymes in cancer invasion and metastasis. *Clin Exp Metastasis* 1992; **10**: 145–55.
6. Dano K, Behrendt N, Brunner N, Ellis V, Ploug M, Pyke C. The urokinase receptor. Protein structure and role in plasminogen activation and cancer invasion. *Fibrinolysis* 1994; **8** Suppl 1: 189–203.
7. Ellis V, Behrendt N, Dano K. Plasminogen activation by receptor-bound urokinase. A kinetic study with both cell-associated and isolated receptor. *J Biol Chem* 1991; **266**: 12752–8.
8. Yonemura Y, Nojima N, Kaji M, Fujimura T, Itoh H, Ninomiya I, Miyazaki I, Endo Y, Sasaki T. E-Cadherin and urokinase-type plasminogen activator tissue status in gastric carcinoma. *Cancer* 1995; **76**: 941–53.
9. Nekarda H, Schmitt M, Ulm K, Wenninger A, Vogelsang H, Becker K, Roder JD, Fink U, Siewert JR. Prognostic impact of urokinase-type plasminogen activator and its inhibitor PAI-1 in completely resected gastric cancer. *Cancer Res* 1994; **54**: 2900–7.
10. Costantini V, Sidoni A, Deveglia R, Cazzato OA, Bellezza G, Ferri I, Bucciarelli E, Nenci GG. Combined overexpression of urokinase, urokinase receptor, and plasminogen activator inhibitor-1 is associated with breast cancer progression: an immunohistochemical comparison of normal, benign, and malignant breast tissues. *Cancer* 1996; **77**: 1079–88.
11. Sprengers ED, Kluft C. Plasminogen activator inhibitors. *Blood* 1987; **69**: 381–7.
12. Chambers SK, Ivins CM, Carcangiu ML. Plasminogen activator inhibitor-1 is an independent poor prognostic factor for survival in advanced stage epithelial ovarian cancer patients. *Int J Cancer* 1998; **79**: 449–54.
13. Deng G, Curriden SA, Wang S, Rosenberg S, Loskutoff DJ. Is plasminogen activator inhibitor-1 the molecular switch that governs urokinase receptor-mediated cell adhesion and release? *J Cell Biol* 1996; **134**: 1563–71.
14. Stefansson S, Lawrence DA. The serpin PAI-1 inhibits cell migration by blocking integrinαVβ3 binding to vitronectin. *Nature* 1996; **383**: 441–3.
15. Folkman J. Tumor angiogenesis. *Adv Cancer Res* 1985; **43**: 175–203.
16. Nakata S, Ito K, Fujimori M, Shingu K, Kajikawa S, Adachi W, Matsuyama I, Tsuchiya S, Kuwano M, Amano J. Involvement of vascular endothelial growth factor and urokinase-type plasminogen activator receptor in microvessel invasion in human colorectal cancers. *Int J Cancer* 1998; **79**: 179–86.
17. Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* 1989; **246**: 1309–12.
18. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Senger DR, Dvorak HF. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. *Cancer Res* 1993; **53**: 4727–35.
19. Maeda K, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T, Sowa M. Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. *Cancer* 1996; **77**: 858–63.
20. Ferrara N. Vascular endothelial growth factor: molecular and biological aspects. *Curr Top Microbiol Immunol* 1999; **237**: 1–30.
21. Brodsky S, Chen J, Lee A, Akassoglou K, Norman J, Goligorsky MS. Plasmin-dependent and -independent effects of plasminogen activators and inhibitor-1 on *ex vivo* angiogenesis. *Am J Physiol Heart Circ Physiol* 2001; **281**: H1784–92.
22. Bajou K, Masson V, Gerard RD, Schmitt PM, Albert V, Praus M, Lund LR, Frandsen TL, Brunner N, Dano K, Fusenig NE, Weidle U, Carmeliet G, Loskutoff D, Collen D, Carmeliet P, Foidart JM, Noel A. The plasminogen activator inhibitor PAI-1 controls *in vivo* tumor vascularization by interaction with proteases, not vitronectin. Implications for antiangiogenic strategies. *J Cell Biol* 2001; **152**: 777–84.
23. Johnson LL, Dyer R, Hupe DJ. Matrix metalloproteinases. *Curr Opin Chem Biol* 1998; **2**: 466–71.
24. Abe J, Urano T, Konno H, Erhan Y, Tanaka T, Nishino N, Takada A, Nakamura S. Larger and more invasive colorectal carcinoma contains larger amounts of plasminogen activator inhibitor type 1 and its relative ratio over urokinase receptor correlates well with tumor size. *Cancer* 1999; **86**: 2602–11.
25. Konno H, Abe J, Kaneko T, Baba M, Shoji A, Sunayama K, Kamiya K, Tanaka T, Suzuki S, Nakamura S, Urano T. Urokinase receptor and vascular endothelial growth factor are synergistically associated with the liver metastasis of colorectal cancer. *Jpn J Cancer Res* 2001; **92**: 516–23.

26. Migita T, Sato E, Saito K, Mizoi T, Shiiba K, Matsuno S, Nagura H, Ohtani H. Differing expression of MMPs-1 and -9 and urokinase receptor between diffuse- and intestinal-type gastric carcinoma. *Int J Cancer* 1999; **84**: 74–9.
27. Allgayer H, Heiss MM, Schildberg FW. Prognostic factors in gastric cancer. *Br J Surg* 1997; **84**: 1651–64.
28. Okusa Y, Ichikura T, Mochizuki H. Prognostic impact of stromal cell-derived urokinase-type plasminogen activator in gastric carcinoma. *Cancer* 1999; **85**: 1033–8.
29. Okusa Y, Ichikura T, Mochizuki H, Shinomiya N. Urokinase type plasminogen activator and its receptor regulate the invasive potential of gastric cancer cell lines. *Int J Oncol* 2000; **17**: 1001–5.
30. Ganesh S, Sier CF, Heerding MM, van Krieken JH, Griffioen G, Welvaart K, van de Velde CJ, Verheijen JH, Lamers CB, Verspaget HW. Prognostic value of the plasminogen activation system in patients with gastric carcinoma. *Cancer* 1996; **77**: 1035–43.
31. Wei Y, Waltz DA, Rao N, Drummond RJ, Rosenberg S, Chapman HA. Identification of the urokinase receptor as an adhesion receptor for vitronectin. *J Biol Chem* 1994; **269**: 32380–8.
32. Kjoller L, Kanse SM, Kirkegaard T, Rodenburg KW, Ronne E, Goodman SL, Preissner KT, Ossowski L, Andreasen PA. Plasminogen activator inhibitor-1 represses integrin- and vitronectin-mediated cell migration independently of its function as an inhibitor of plasminogen activation. *Exp Cell Res* 1997; **232**: 420–9.
33. Chapman HA. Plasminogen activators, integrins, and the coordinated regulation of cell adhesion and migration. *Curr Opin Cell Biol* 1997; **9**: 714–24.
34. Waltz DA, Natkin LR, Fujita RM, Wei Y, Chapman HA. Plasmin and plasminogen activator inhibitor type 1 promote cellular motility by regulating the interaction between the urokinase receptor and vitronectin. *J Clin Invest* 1997; **100**: 58–67.
35. Sugiura Y, Ma L, Sun B, Shimada H, Laug WE, Seeger RC, DeClerck YA. The plasminogen-plasminogen activator (PA) system in neuroblastoma: role of PA inhibitor-1 in metastasis. *Cancer Res* 1999; **59**: 1327–36.
36. Min HY, Doyle LV, Vitt CR, Zandonella CL, Stratton-Thomas JR, Shuman MA, Rosenberg S. Urokinase receptor antagonists inhibit angiogenesis and primary tumor growth in syngeneic mice. *Cancer Res* 1996; **56**: 2428–33.
37. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science* 1983; **219**: 983–5.
38. Gerber HP, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. *J Biol Chem* 1998; **273**: 13313–6.
39. Gupta K, Kshirsagar S, Li W, Gui L, Ramakrishnan S, Gupta P, Law PY, Hebbel RP. VEGF prevents apoptosis of human microvascular endothelial cells via opposing effects on MAPK/ERK and SAPK/JNK signaling. *Exp Cell Res* 1999; **247**: 495–504.
40. Gabrilovich BD, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, Carbone DP. Vascular endothelial growth factor inhibits the development of dentic cells and dramatically affects the differentiation of multiple hematopoietic lineages *in vivo*. *Blood* 1998; **92**: 4150–66.
41. Pepper MS, Ferrara N, Orci L, Montesano R. Vascular endothelial growth factor (VEGF) induces plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells. *Biochem Biophys Res Commun* 1991; **181**: 902–6.
42. Mandriota SJ, Seghezzi G, Vassalli JD, Ferrara N, Wasi S, Mazzieri R, Mignatti P, Pepper MS. Vascular endothelial growth factor increases urokinase receptor expression in vascular endothelial cells. *J Biol Chem* 1995; **270**: 9709–16.
43. Wickstrom SA, Veikkola T, Rehn M, Pihlajaniemi T, Alitalo K, Keski-Oja J. Endostatin-induced modulation of plasminogen activation with concomitant loss of focal adhesions and actin stress fibers in cultured human endothelial cells. *Cancer Res* 2001; **61**: 6511–6.
44. Allgayer H, Babic R, Grutzner KU, Beyer BC, Tarabichi A, Schildberg FW, Heiss MM. Tumor-associated proteases and inhibitors in gastric cancer: analysis of prognostic impact and individual risk protease patterns. *Clin Exp Metastasis* 1998; **16**: 62–73.
45. Heiss MM, Allgayer H, Gruetzner KU, Funke I, Babic R, Jauch KW, Schildberg FW. Individual development and uPA-receptor expression of disseminated tumour cells in bone marrow: a reference to early systemic disease in solid cancer. *Nat Med* 1995; **1**: 1035–9.