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Prognostic Relevance of Plasminogen Activators and Their Inhibitors in Colorectal Cancer[1]

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ABSTRACT

Human colorectal carcinogenesis has been shown previously to be associated with impressive changes in the tissue levels of plasminogen activators and their inhibitors, exemplified by an increase in the urokinase-type plasminogen activator (u-PA) and the inhibitors PAI-1 and PAI-2, and a decrease in tissue-type plasminogen activator (t-PA). In the present study we evaluated the prognostic significance of these parameters to the overall survival of patients with colorectal cancer, in conjunction with several major clinicopathological parameters like age, gender, differentiation grade, and Dukes' stage. Univariate analyses revealed that a low t-PA antigen level, low t-PA activity, and high u-PA/t-PA antigen ratio in normal mucosa and a high u-PA and PAI-2 antigen level in carcinomas are prognostic for a poor overall survival of patients with colorectal cancer. The prognostic value of t-PA antigen and activity in normal mucosa, the antigen ratio of u-PA in carcinoma (C) and t-PA in corresponding normal (N) mucosa [u-PA(C)/t-PA(N) antigen ratio], and PAI-2 antigen in carcinomas was found to be independent from clinicopathological parameters by multivariate analyses. These observations illustrate the clinical importance of the plasminogen activation cascade at the tissue level in colorectal cancer invasion, metastasis, and survival.

INTRODUCTION

PAs[3] are serine-proteases which play an important role in neoplastic growth (1–3). t-PA, predominantly localized in endothelial cells and detectable in all vascularized tissues, has been shown to play a major role in intravascular thrombolysis and probably a minor role in tumor growth (4). u-PA activates some proteinases, especially plasminogen and indirectly collagenases, which results in breakdown of extracellular matrix and basal membranes, surrounding for example (pre)malignant tissue (1–6). Furthermore, it has also been shown that proliferation, invasive growth, and metastasis of tumors is correlated with the PA level, particularly of u-PA, and that tumor cell metastasis can be inhibited in an animal model with specific antibodies against u-PA (7).

Previous studies revealed remarkable changes in the plasminogen activator profiles in several types of carcinomas, particularly those from the colon and breast (8–21). We showed that colorectal adenocarcinomas have an approximately 10-fold increase in u-PA antigen level and a 5-fold increase of u-PA activity compared to normal mucosa. In contrast, both t-PA activity and t-PA antigen levels were found to be significantly decreased in colorectal carcinomas compared to normal colorectal mucosa (10, 19). Recent studies demonstrated that an increased u-PA content in breast cancer tissue is an independent prognostic factor in predicting early relapse, through which high and low risk patients could be selected (15–18). Interestingly, a high t-PA level in breast cancer seemed to be associated with a longer disease-free interval and survival (22). With respect to PAIs, we found increased levels of PAI-1 and PAI-2 antigen in colorectal cancer (19), while breast cancer has been found to contain significantly higher antigen levels of only PAI-1 compared with benign breast tissue (15, 20). Moreover, in breast cancer these elevated PAI-1 levels were found to be associated with a higher risk for relapse and a shorter overall survival (15, 18). t-PA, u-PA, and PAIs in colorectal cancer have not been found to be clearly associated with malignancy parameters such as tumor grade, stage, etc. (8–13, 19, 21, 23–25). The observation that t-PA, u-PA, and PAI-1 levels are prognostic factors in breast cancer raised the suggestion that plasminogen activators might also be relevant prognostic markers in colorectal cancer.

The aim of the present study was to determine the prognostic relevance of plasminogen activators and inhibitors in the overall survival of patients with colorectal cancer. Therefore, we assessed the clinical outcome of 92 patients having operations for colorectal cancer, with a follow-up of at least 5 years, and performed uni- and multivariate survival analyses. The prognostic relevance of u-PA, t-PA, PAI-1, and PAI-2 determined in normal mucosa and carcinomas of resection specimens was compared to major clinicopathological parameters.

MATERIALS AND METHODS

Patients and Study Design. During the period from November 1983 to December 1985, 92 fresh surgical resection specimens of histologically confirmed colorectal cancer were obtained. Part of the carcinoma and normal mucosa, taken approximately 10 cm from the tumor, were immediately frozen and stored at −70°C. Clinical data for the patients and histological data for the tumors were registered. The colorectal carcinomas were histologically classified according to the Dukes' stage modified by Astler and Coller (26–28). Two patients had a tumor extending through the full thickness of the intestinal wall, without lymph node involvement. These could not be classified according to the method of Astler and Coller and were included as Dukes' stage B2. All the patients entered the study at the operation date and had a clinical follow-up for local recurrence and/or metastasis and survival for at least 5 years. Patient time experience ended in the event of death or at the common closing date (August 1991) of the study, when the patients were still alive.

Tissue Preparation. After homogenization of the tissue in 0.1% (v/v) Tween 80–0.1 M Tris-HCl buffer (pH 7.5; 60 mg wet tissue/ml) as described before (9), the homogenates were centrifuged twice at 8000 × *g* for 2.5 min at 4°C. Protein concentration of the supernatant was determined by the method of Lowry *et al.* (29).

u-PA and t-PA Determination. u-PA antigen was determined by a sandwich ELISA according to the method of Binnema *et al.* (30), with rabbit anti-u-PA as catching antibody. The samples were incubated overnight followed by an incubation with affinopurified goat anti-u-PA IgG as second antibody. After a washing, the samples were incubated with donkey anti-goat IgG-alkaline phosphatase conjugate. The amount of u-PA antigen in the samples was calculated from a standard curve of u-PA (0–3.3 ng/ml).

t-PA antigen was measured by an ELISA using goat anti-t-PA as catching antibody and anti-t-PA horseradish peroxidase conjugate as second antibody,

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[3] The abbreviations used are: PA, plasminogen activator; u-PA, urokinase-type plasminogen activator; t-PA, tissue-type plasminogen activator; PAI, plasminogen activator inhibitor; ELISA, enzyme-linked immunosorbent assay; u-PA(C)/t-PA(N)antigen ratio, calculated antigen ratio of u-PA in carcinoma and t-PA in corresponding normal mucosa.

according to the method of Rijken *et al.* (31), while 3,3′;5,5′-tetramethylbenzidine was used as substrate. Quantities of t-PA antigen were calculated using an 8-point standard curve of t-PA (Biopool, Umeå, Sweden; 0–4 ng/ml).

u-PA and t-PA activities were enzymatically determined in a spectrophotometric assay, consisting of tissue extracts incubated with plasminogen, fragments of fibrinogen, and a chromogenic plasmin substrate (32). PA activities were distinguished by adding specific inhibitory antibodies against t-PA and u-PA, respectively rabbit anti-human t-PA IgG and goat anti-human u-PA IgM/IgD, to parallel incubations. The activator activities were calculated by the amount of inhibition. The percentage of u-PA activity was calculated as 100 times the u-PA activity divided by the sum of the u-PA and t-PA activity. u-PA and t-PA standard preparations (National Institute of Biological Standards and Control, London, United Kingdom; batch nos. 66/46 and 83/517, respectively) were included to express activities in IU. The inhibiting antibodies used were monospecific, showed no cross-reactivity, and blocked maximum standard u-PA and t-PA completely.

PAI-1 and PAI-2 Determination. Total PAI-1 antigen, *i.e.*, latent, active, and complexed PAI-1, was determined using the Tintelize PAI-1 ELISA (Biopool) without prior denaturation of the samples as described before (19). In brief, mouse monoclonal anti-human PAI-1 was used as catching antibody. After incubation with the tissue homogenates a goat polyclonal anti-human PAI-1, conjugated to peroxidase, was used to form a "sandwich" ELISA and *o*-phenylenediamine was added as substrate. The assay included the use of quenching and nonspecific antibodies to exclude falsely elevated results. In order to increase the sensitivity of the assay sample volumes of up to 40 μl were used, instead of the recommended 20 μl, resulting in a detection limit of 0.3 ng/ml.

The determination of PAI-2 antigen was performed using the Tintelize PAI-2 ELISA (Biopool) as described previously (19). The first antibody used was mouse monoclonal anti-human PAI-2 and the second was goat polyclonal anti-PAI-2 IgG conjugated to peroxidase. *o*-Phenylenediamine was added as substrate. Unspecific response was excluded using quenching antibodies. The detection limit was decreased to 0.5 ng/ml by using 50 μl homogenate instead of 20 μl and by increasing sample incubation, conjugate incubation, and substrate incubation times.

Statistical Analyses. For the statistical analyses, the clinicopathological parameters were dichotomized, with tumor localization in the colon divided into right sided (from cecum to splenic flexure) and left sided (from splenic flexure to end of rectum).

The cutoff points of the age and PA parameters were determined by slowly increasing the level until the point of best discrimination was found, *i.e.*, the optimal dichotomization. Significance of differences in the PA results between normal tissue and carcinomas and between survivors and nonsurvivors were tested statistically (2-sided) using the paired and unpaired Student *t* test, with separate variance estimation if the standard deviations were significantly different according to the *F* test. Besides dichotomization, as mentioned before, parameters were also categorized into 3 to 4 subgroups based on integer cutoff points with sufficient numbers in each group.

Univariate survival analysis was performed with both the χ^2 test and Cox's proportional hazards model (33), the latter using the EGRET statistical package (SERC Corp., Seattle, WA), resulting in identification of covariates which were significantly correlated with the overall survival. Multivariate survival analyses were performed using the Cox proportional hazards method by separately adding the significant plasminogen activator variables to the clinicopathological parameters to estimate the independent prognostic value in the overall survival. Overall survival curves were constructed by the method of Kaplan and Meier (34), and the Mantel-Haenszel test for linear association was performed using the SPSS/PC+ statistical package (SPSS, Inc., Chicago, IL). Statistical values of $P < 0.05$ were considered significant.

RESULTS

The 92 patients included in the study had all had operations for colorectal carcinoma and had had clinical follow-up for at least 5 years. Forty-eight (52.2%) of the patients had died [15 females and 33 males; mean age, 71.9 ± 1.5 (SE) years, and mean survival time, 1.7 ± 0.2 years] whereas 44 (47.8%) patients were still alive (20 females and 24 males; mean age, 64.3 ± 1.5 years; and mean follow-up, 5.5 ± 0.1 years). As expected the overall survival of the patients was gradually decreasing from those with lesions classified as Dukes' A (100%), Dukes' B (57%), Dukes' C (38%) to those with Dukes' D (0%), indicating a representative population of colorectal cancer patients.

Similar to our previous studies we found a significantly increased level of u-PA antigen and activity and a decreased level of t-PA antigen and activity in colorectal carcinomas compared to normal mucosa ($P < 0.001$), as presented in Table 1. Also the percentage u-PA activity, which is that part of the total PA activity contributed by u-PA, and the calculated u-PA/t-PA antigen ratio were significantly higher in carcinomas compared to normal mucosa ($P < 0.001$).

Comparing the PA parameters of the tissues from the patients who had died to those who were still alive (Table 2), we found that the normal mucosa of nonsurvivors had a significantly lower t-PA activity and antigen level, whereas the u-PA/t-PA antigen ratio was increased in this group. Moreover, the u-PA(C)/t-PA(N) antigen ratio was significantly higher in the patients who had died ($P = 0.008$), while the PAI-2 antigen level of the carcinomas tended to be higher ($P < 0.08$). All the other plasminogen activator parameters showed no differences between the patients who had died and those who were alive.

After determining the best discrimination point as cutoff level for each parameter between survivors and nonsurvivors, *i.e.*, optimal dichotomization, several plasminogen activator parameters seemed to be associated with the overall survival. Univariate survival analysis

Table 1 *Plasminogen activator parameters in normal mucosa and colorectal carcinomas*

	Normal mucosa (*n*)	Carcinomas (*n*)	*P*
t-PA activity[a]	1670 ± 91[b] (88)	575 ± 54 (92)	<0.001
t-PA antigen[c]	5.3 ± 0.5 (89)	2.5 ± 0.3 (92)	<0.001
u-PA activity[a]	37 ± 3 (88)	112 ± 9 (92)	<0.001
u-PA antigen[c]	2.2 ± 0.1 (89)	14.2 ± 0.9 (92)	<0.001
% of u-PA activity[d]	7.8 ± 0.6 (88)	45.4 ± 2.6 (92)	<0.001
u-PA/t-PA antigen ratio	0.7 ± 0.1 (89)	11.8 ± 1.2 (92)	<0.001
PAI-1 antigen[c]	ND[e]	6.2 ± 1.3 (89)	
PAI-2 antigen[c]	ND[e]	2.8 ± 0.4 (86)	

[a] mIU/mg protein.
[b] Mean ± SE.
[c] ng/mg protein.
[d] $$\%\text{ of u-PA activity} = \frac{100 \times \text{u-PA activity}}{\text{u-PA activity} + \text{t-PA activity}}$$
[e] ND, not done.

Table 2 *Plasminogen activator parameters in normal mucosa and colorectal carcinomas of patients divided in survivors and nonsurvivors*

	Alive	(*n*)	Dead	(*n*)	*P*
Normal mucosa					
t-PA activity[a]	1905 ± 151[b]	(40)	1473 ± 104	(48)	<0.02
t-PA antigen[c]	6.3 ± 0.7	(41)	4.4 ± 0.5	(48)	<0.04
u-PA activity[a]	40 ± 5	(40)	34 ± 3	(48)	NS[d]
u-PA antigen[c]	2.2 ± 0.2	(41)	2.1 ± 0.2	(48)	NS
% of u-PA activity[e]	7.2 ± 0.9	(40)	8.4 ± 0.9	(48)	NS
u-PA/t-PA antigen ratio	0.6 ± 0.1	(41)	0.8 ± 0.1	(48)	<0.05
Carcinomas					
t-PA activity[a]	548 ± 69	(44)	600 ± 83	(48)	NS
t-PA antigen[c]	2.6 ± 0.4	(44)	2.4 ± 0.4	(48)	NS
u-PA activity[a]	103 ± 11	(44)	121 ± 14	(48)	NS
u-PA antigen[c]	13.4 ± 1.1	(44)	15.1 ± 1.5	(48)	NS
% of u-PA activity	42.7 ± 3.4	(44)	47.8 ± 3.8	(48)	NS
u-PA/t-PA antigen ratio	11.1 ± 1.6	(44)	12.5 ± 1.8	(48)	NS
PAI-1 antigen[c]	6.7 ± 2.2	(43)	5.8 ± 1.4	(46)	NS
PAI-2 antigen[c]	2.1 ± 0.4	(43)	3.4 ± 0.6	(43)	<0.08
u-PA(C)/t-PA(N) antigen ratio	3.8 ± 0.6	(41)	6.6 ± 0.8	(48)	0.008

[a] mIU/mg protein.
[b] Mean ± SE.
[c] ng/mg protein.
[d] NS, not significant.
[e] $$\%\text{ u-PA activity} = \frac{100 \times \text{u-PA activity}}{\text{u-PA activity} + \text{t-PA activity}}$$

Table 3 *Univariate analysis of dichotomized clinicopathological parameters in relation to overall survival of patients with colorectal cancer*

Parameter dichotomized	No. of survivors/total	Survival (%)	χ^2 (P)	Cox univariate hazard ratio (95% CI,[a] P)
Gender				
Female	20/35	57.1	1.4 (NS)	
Male	24/57	42.1		1.6 (0.9–2.9, NS)
Age (yr)				
≤72.2	35/57	61.4	9.7 (0.002)	
>72.2	9/35	25.7		2.3 (1.3–4.1, 0.003)
Localization				
Right colon	15/31	48.4	0.0 (NS)	
Left colon	29/61	47.5		1.1 (0.6–1.9, NS)
Differentiation grade				
Well	12/24	50.0	0.0 (NS)	
Moderate-poor	32/68	47.1		1.1 (0.6–2.1, NS)
Dukes' stage				
AB	32/53	60.4	6.8 (0.009)	
CD	12/39	30.8		2.3 (1.3–4.1, 0.004)

[a] CI, confidence interval; NS, not significant.

showed that of all the dichotomized clinicopathological parameters only age (>72.2 years *versus* ≤72.2 years) and Dukes' stage were associated with the overall survival (Table 3; Fig. 1). With regard to the PA parameters, Table 4 shows that both the χ^2 and Cox univariate analysis gave similar results in relation to survival. In normal mucosa, low t-PA antigen [≤3.15 ng/mg protein (Fig. 2)], low t-PA activity [≤1600 mIU/mg protein (Fig. 3)], and a high u-PA/t-PA antigen ratio (>0.62) were prognostic for poor overall survival, whereas in the carcinomas this was found with a high level of u-PA antigen (>10.45 ng/mg protein), a high percentage of u-PA activity (>33%), and a high PAI-2 antigen level [>4.95 ng/mg protein (Fig. 4)]. Also a high u-PA(C)/t-PA(N) antigen ratio [>7.0 (Fig. 5)] was associated with a poor overall survival. In fact, the u-PA(C)/t-PA(N) antigen ratio was found to be the best univariate prognostic parameter (hazard ratio, 2.9) followed by t-PA activity and antigen in normal mucosa (both hazard ratios, 0.4), and age and Dukes' stage (both hazard ratios, 2.3). Performing the multivariate Cox analysis by adjusting the plasminogen activator parameters separately to the clinicopathological parameters revealed that in normal mucosa all the PA parameters, *i.e.*, t-PA antigen and activity and the u-PA/t-PA antigen ratio, remained prognostically significant. In the carcinomas, the PAI-2 antigen and u-PA(C)/t-PA(N) antigen ratio also remained significant prognostic parameters, while u-PA antigen and the percentage of u-PA activity lost their prognostic significance. These observations indicated that in this patient population, the u-PA antigen level in carcinoma is a prognostic variable which is not independent from clinicopathological parameters, while the other PA parameters are independent prognostic variables. This was confirmed by the finding that patients with Dukes' stage C/D lesions had a higher mean u-PA antigen level of the carcinomas compared to those with Dukes' stage A/B lesions (16.6 ± 1.8 *versus* 12.5 ± 0.9 ng/mg protein, $P = 0.03$). The other plasminogen activator parameters did not show any difference between these two histological groups (in normal mucosa, t-PA activity 1544 ± 104 *versus* 1843 ± 161 mIU/mg protein; t-PA antigen 5.6 ± 0.5 *versus* 4.8 ± 0.8 ng/mg protein; and u-PA/t-PA antigen ratio 0.7 ± 0.1 *versus* 0.7 ± 0.1; in carcinomas, PAI-2 antigen 3.4 ± 0.7 *versus* 2.3 ± 0.4 ng/mg protein, percentage of u-PA activity 46.5% ± 4.3 *versus* 44.5% ± 3.2; and u-PA(C)/t-PA(N) antigen ratio 6.2 ± 0.8 *versus* 4.7 ± 0.7, all not significant).

Besides survival analysis with the best discrimination point, the parameters were also categorized and the same uni- and multivariate survival analyses were performed. The results (Table 5) show that higher age remained associated with a poor overall survival. Regarding the PA parameters the univariate analysis showed that there was a decreased overall survival in patients with lower t-PA antigen level (≥9 ng/mg protein, 61.5%, to <3 ng/mg protein, 31.4%), lower t-PA activity (≥2200 mIU/mg protein, 60%, to <1100 mIU/mg protein, 32.0%), and higher u-PA/t-PA antigen ratio (<0.4, 54.5%; to ≥1.2, 31.3%) in normal mucosa, with higher PAI-2 antigen level (<1, 68.0%; to ≥3, 31.8%) in carcinomas, and with higher u-PA(C)/t-PA(N) antigen ratio (<3, 57.5%; to ≥9, 25.0%). A good continuity in the association of the categorized parameters with the overall survival was observed with age, t-PA antigen in normal mucosa, and the u-PA(C)/t-PA(N) ratio as can be seen from the percentages of survival and the corresponding hazard ratios of the univariate Cox analysis, which was confirmed by the Mantel-Haenszel test for linear association (Table 5). The multivariate Cox analysis of the categorized parameters revealed that particularly the t-PA activity, and to a lesser extent t-PA antigen, in normal mucosa and the u-PA(C)/t-PA(N) antigen ratio remained independent prognostic variables for the overall survival of patients with colorectal cancer.

DISCUSSION

Many studies have been performed investigating clinical, histological, genetic, and biological variables expressed in colorectal cancer and their possible impact on the overall survival of these patients (35–42). Our present and previous studies have shown that colorectal carcinomas are characterized by an impressive increase of u-PA and a decrease of t-PA, compared with the corresponding normal mucosa (9, 10, 19, 23). Several studies reported on the possible role of plasminogen activators in tumorigenesis, tumor invasion, and metastases (1–6). The presence of these proteolytic enzymes in tumors results in the conversion of plasminogen to plasmin, which not only directly breaks down components of the extracellular matrix, such as laminin and fibrin, but also activates procollagenases to collagenases which degrade collagen in the matrix (1–4). Recent studies in breast cancer have shown that plasminogen activators, particularly u-PA, seem to be better prognostic parameters than the known clinical and histological parameters (15–18, 43–45). The survival of colorectal

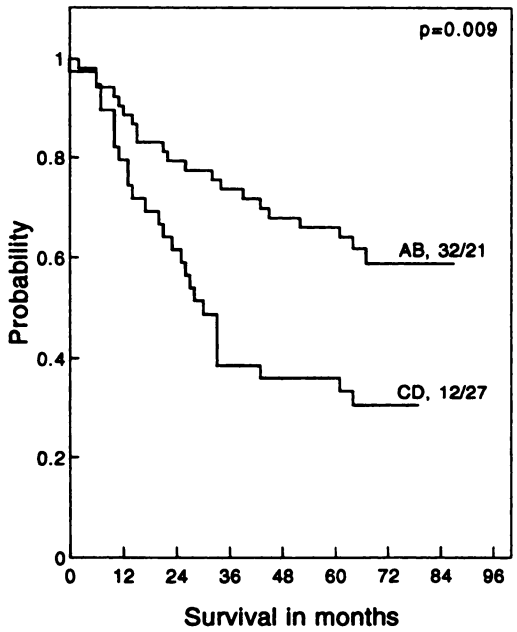

Fig. 1. Overall survival curves according to Dukes' stage A + B and C + D of the patients with colorectal cancer. Values are the number of patients alive/dead at the end of follow-up.

Table 4 *Univariate and multivariate analysis of dichotomized plasminogen activator parameters in relation to overall survival of patients with colorectal cancer*

Adjustment was performed by adding the significant plasminogen activator parameters separately to the clinicopathological parameters (gender, age, tumor localization, tumor differentiation grade, and Dukes' stage).

Parameter dichotomized	No. of survivors/total	Survival (%)	χ^2 (*P*)	Cox proportional hazards analyses (95% CI,[a] (*P*) Univariate hazard ratio	Adjusted hazard ratio
Normal mucosa					
t-PA antigen[b]					
≤3.15	11/37	29.7	5.7 (0.02)		
>3.15	30/52	57.7		0.4 (0.3–0.8, 0.005)	0.4 (0.2–0.8, 0.007)
t-PA activity[c]					
≤1600	14/46	30.4	7.6 (0.006)		
>1600	26/42	61.9		0.4 (0.2–0.8, 0.009)	0.3 (0.2–0.7, 0.002)
u-PA/t-PA antigen ratio					
≤0.62	27/45	60.0	6.0 (0.01)		
>0.62	14/44	31.8		2.1 (1.2–3.8, 0.01)	2.0 (1.0–3.9, 0.05)
Carcinomas					
u-PA antigen[b]					
≤10.45	20/30	66.7	5.3 (0.02)		
>10.45	24/62	38.7		2.2 (1.1–4.4, 0.03)	1.7 (0.8–3.6, NS)
% of u-PA activity[d]					
≤33	20/31	64.5	4.3 (0.04)		
>33	24/61	39.3		2.0 (1.0–3.9, 0.04)	1.9 (0.9–3.8, 0.07)
PAI-2 antigen[b]					
≤4.95	41/74	55.4	4.8 (0.03)		
>4.95	2/12	16.7		2.2 (1.1–4.4, 0.03)	2.2 (1.0–4.8, 0.04)
u-PA(C)/t-PA(N) antigen ratio					
≤7.0	37/65	56.9	9.9 (0.002)		
>7.0	4/24	16.7		2.9 (1.6–5.2, <0.001)	2.5 (1.3–4.7, 0.006)

[a] CI = confidence interval; NS, not significant.
[b] ng/mg protein.
[c] mIU/mg protein.
[d] $$\%\text{ of u-PA activity} = \frac{100 \times \text{u-PA activity}}{\text{u-PA activity} + \text{t-PA activity}}$$

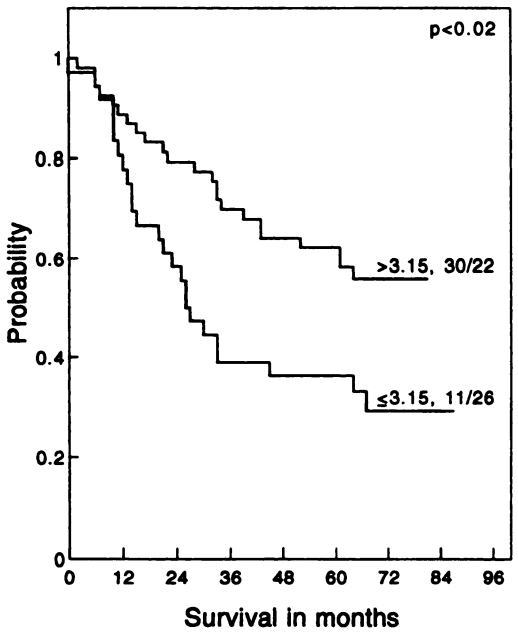

Fig. 2. Overall survival curves according to high (>3.15 ng/mg protein) and low (≤3.15 ng/mg protein) t-PA antigen level in normal mucosa of patients with colorectal carcinoma. Values are the number of patients alive/dead at the end of follow-up.

cancer patients is correlated with the rate of invasiveness and metastases classified by histological (Dukes) stage, which is used to identify subgroups of patients with different prognosis (26, 27, 46, 47). In this study we evaluated the prognostic relevance of the plasminogen activation cascade to the overall survival of these patients because this has not been studied before in colorectal cancer.

Regarding the clinicopathological parameters, age and Dukes' stage were the only significant prognostic parameters found, irrespective whether the analyses were performed by the χ^2 test or the Cox model. The fact that age was a prognostic factor in our study could be expected since patients who had died were older than those who were still alive, an observation which is in agreement with the findings of others (39, 48, 49). The association between survival and the Dukes' stage of the tumors, which is the most generally used histological prognostic parameter in patients with colorectal carcinoma, was also confirmed in our population since the overall 5-year survival was gradually decreasing from 100% in patients with Dukes' A to 0% in patients with Dukes' D lesions. Although some studies indicate that a poor differentiation of the tumor is associated with a poor survival (40, 41, 48) this was not found in our population. With respect to the localization of the tumor, *i.e.*, right sided *versus* left sided, we did not find it to be related to survival of the patients, which is similar to most of the other reports (36–42).

Comparing survivors and nonsurvivors in our patient population (Table 2) major differences were found in the t-PA levels and the u-PA/t-PA antigen ratio in normal mucosa, the PAI-2 antigen level in carcinomas, and the u-PA(C)/t-PA(N) antigen ratio. Optimal dichotomization of the parameters and subsequent univariate analysis revealed that low t-PA levels, antigen, and activity and a high u-PA/t-PA antigen ratio in normal mucosa and high levels of u-PA, antigen and percentage activity and PAI-2 antigen in carcinomas, and u-PA(C)/t-PA(N) antigen ratio were associated with a poor overall survival of the patients with colorectal cancer. Multivariate analysis, however, showed that particularly t-PA in normal mucosa, PAI-2 in

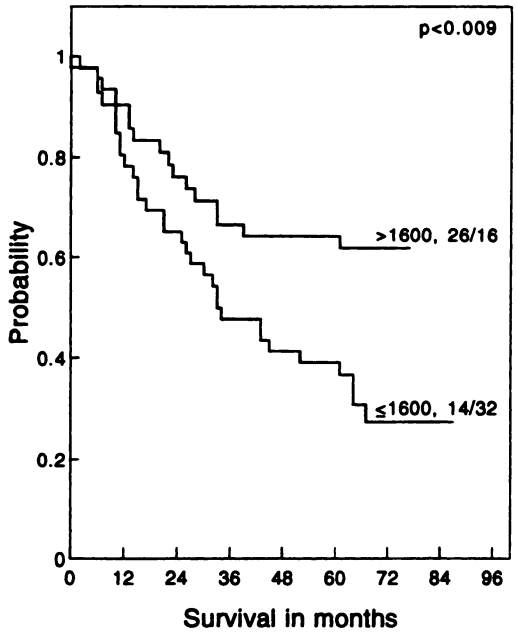

Fig. 3. Overall survival curves according to high (>1600 mIU/mg protein) and low (≤1600 mIU/mg protein) t-PA activity in normal mucosa of patients with colorectal carcinoma. Values are the number of patients alive/dead at the end of follow-up.

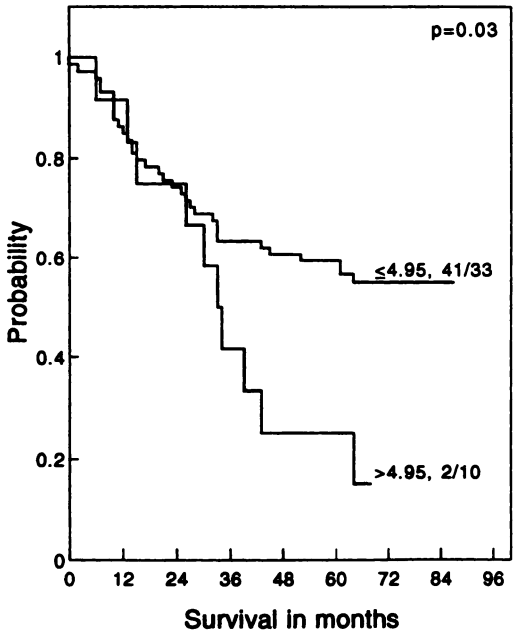

Fig. 4. Overall survival curves according to high (>4.95 ng/mg protein) and low (≤4.95 ng/mg protein) PAI-2 antigen level of the carcinomas of the patients with colorectal cancer. Values are the number of patients alive/dead at the end of follow-up.

carcinoma, and the u-PA(C)/t-PA(N) antigen ratio remained significant prognostic parameters, independent from the clinicopathological parameters. Categorization of the parameters and performing similar analyses (Table 5) revealed that the t-PA antigen and activity levels in normal mucosa and the u-PA(C)/t-PA(N) antigen ratio remained independent prognostic parameters, with gradual and continuous changes in survival of patients.

With respect to u-PA, several studies in breast cancer have demonstrated previously that a high u-PA content, especially a high u-PA antigen level, is associated with a poor survival (15–18, 43–45). In carcinomas of the colorectum, breast, and stomach high levels of PAI antigen, especially PAI-1, were detected (15, 19–21). Recently several studies (15, 18) showed that the PAI-1 antigen level was an independent prognostic factor for relapse-free and overall survival in breast cancer. In contrast, we did not find PAI-1 antigen to be of prognostic relevance for the overall survival in colorectal cancer. However, a high level of PAI-2 antigen was clearly associated with a poor overall survival, which appeared to be independent from the clinicopathological parameters. This finding indicates that the inhibitor PAI-2 might be more important for colorectal tumor growth and metastasis than PAI-1.

Remarkable in our study was the observation that a low t-PA content in normal mucosa of patients with colorectal cancer is associated with a poor overall survival. Moreover, the prognostic value of t-PA was independent from the evaluated clinical parameters of the patients and histopathological parameters of the tumor, a phenomenon that has never been evaluated before either in normal mucosa of patients with colorectal cancer or in corresponding normal tissues of other human carcinomas. This observation suggests that t-PA probably has a more important role in carcinogenesis than assumed, which is also supported by the study of Duffy *et al.* (22) who found a high t-PA antigen level in breast cancer to be associated with longer disease-free interval and better survival. Since it is clearly shown that histologically normal mucosa of patients with colorectal carcinoma has an increased proliferation (50–53) through which patients at high risk could be identified, it is reasonable to believe that the early development of colorectal carcinoma is associated with changes in the mucosa, which probably cannot be detected by routine histological examination. Taking this into account, our findings suggest that the changes in normal mucosa may result in changes in the plasminogen activator profile, reflected by changes in the t-PA level, through which the prognosis of the patient might be predicted. In relation to our previous findings, that there is a gradual decrease of t-PA level in adenomatous polyps and colorectal carcinoma compared to histologically normal mucosa (9, 10, 19), our present findings are very interesting because also in normal mucosa of colorectal cancer patients lower t-PA levels are associated with a poor overall survival.

Because of the diametrical observation, low t-PA in normal mucosa

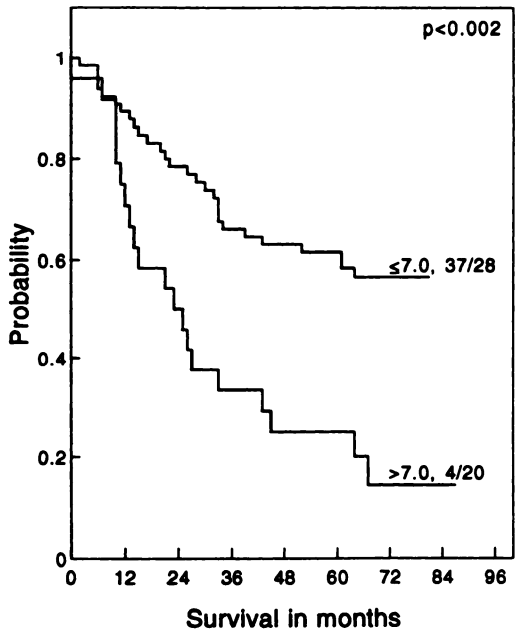

Fig. 5. Overall survival curves according to high (>7.0) and low (≤7.0) antigen ratio of u-PA in carcinomas and t-PA in normal mucosa of the patients with colorectal cancer. Values are the number of patients alive/dead at the end of follow-up.

Table 5 *Univariate and multivariate analysis of categorized age and plasminogen activator parameters in relation to overall survival of patients with colorectal cancer*

Adjustment was performed by adding the significant plasminogen activator parameters separately to the clinicopathological parameters (gender, age, tumor localization, tumor differentiation grade, and Dukes' stage).

Parameter categorized	No. of survivors/total	Survival (%)	χ^2 (*P*) *Mantel-Haenszel* (*P*)	Cox proportional hazards analyses (95% CI,[a] *P*) Univariate hazard ratio	Adjusted hazard ratio
Age (yr)					
<60	13/22	59.1	10.1 (0.02)		
60–70	17/29	58.6	*7.4 (0.007)*	1.0 (0.4–2.4, NS)	
70–80	13/29	44.8		1.4 (0.6–3.1, NS)	
≥80	1/12	8.3		3.3 (1.3–7.9, 0.009)	
Normal mucosa					
t-PA antigen[b]					
<3	11/35	31.4	5.9 (NS)		
3–6	10/21	47.6	*5.4 (0.02)*	0.6 (0.3–1.3, NS)	0.5 (0.2–1.1, 0.08)
6–9	12/20	60.0		0.4 (0.2–1.0, 0.05)	0.5 (0.2–1.3, NS)
≥9	8/13	61.5		0.4 (0.2–1.1, 0.09)	0.3 (0.1–0.9, 0.04)
t-PA activity[c]					
<1100	8/25	32.0	3.6 (NS)		
1100–2200	20/43	46.5	*3.5 (0.06)*	0.6 (0.3–1.2, NS)	0.5 (0.3–1.1, 0.09)
≥2200	12/20	60.0		0.5 (0.2–1.1, 0.08)	0.3 (0.1–0.8, 0.01)
u-PA/t-PA antigen ratio					
<0.4	18/33	54.5	2.4 (NS)		
0.4–0.8	10/23	43.5	*1.9 (NS)*	1.6 (0.7–3.3, NS)	1.2 (0.6–2.7, NS)
0.8–1.2	8/17	47.1		1.2 (0.5–2.7, NS)	1.1 (0.4–2.5, NS)
≥1.2	5/16	31.3		2.0 (0.9–4.3, 0.09)	1.5 (0.6–3.9, NS)
Carcinomas					
u-PA antigen[b]					
<11	20/36	55.6	1.5 (NS)		
11–22	17/41	41.5	*0.7 (NS)*	1.4 (0.7–2.6, NS)	1.3 (0.7–2.6, NS)
≥22	7/15	46.7		1.3 (0.6–3.1, NS)	0.9 (0.3–2.3, NS)
% of u-PA activity[d]					
<25	13/21	61.9	3.5 (NS)		
25–50	15/38	39.5	*0.7 (NS)*	1.8 (0.8–4.1, NS)	2.1 (0.9–4.8, 0.08)
50–75	10/18	55.6		1.1 (0.4–3.0, NS)	1.4 (0.5–4.0, NS)
≥75	6/15	40.0		1.6 (0.6–4.2, NS)	1.3 (0.5–3.5, NS)
PAI-2 antigen[b]					
<1	17/25	68.0	7.5 (0.06)		
1–2	11/26	42.3	*4.2 (0.04)*	2.6 (1.1–6.2, 0.03)	3.6 (1.5–8.9, 0.006)
2–3	8/13	61.5		1.4 (0.4–4.2, NS)	1.3 (0.4–4.2, NS)
≥3	7/22	31.8		2.8 (1.2–6.5, 0.02)	2.2 (0.9–5.5, 0.08)
u-PA(C)/t-PA(N) antigen ratio					
<3	23/40	57.5	6.1 (NS)		
3–6	9/18	50.0	*5.9 (0.02)*	1.3 (0.6–2.8, NS)	0.9 (0.3–2.1, NS)
6–9	5/15	33.3		1.9 (0.9–4.1, NS)	1.3 (0.5–3.1, NS)
≥9	4/16	25.0		2.6 (1.2–5.4, 0.01)	2.3 (1.0–5.4, 0.05)

[a] CI = confidence interval; NS, not significant.
[b] ng/mg protein.
[c] mIU/mg protein.
[d] $$\%\ \text{u-PA activity} = \frac{100 \times \text{u-PA activity}}{\text{u-PA activity} + \text{t-PA activity}}$$

and high u-PA in carcinoma associated with a poor survival, we evaluated the prognostic relevance of the u-PA(C)/t-PA(N) antigen ratio. As expected, a high level of the u-PA(C)/t-PA(N) antigen ratio was associated with a poor overall survival. More importantly, however, this ratio was not only found to be independent from the clinicopathological parameters but also seemed to have a stronger prognostic value than the separate PA parameters.

Similar to studies in breast cancer our findings show that plasminogen activators are prognostic parameters independent from the histological stage of the tumor. In the literature, several multivariate studies reported the prognostic relevance of routine clinicopathological and new parameters, like tumor DNA index and ploidy, secretory component, mucins, serotonin, perioperative blood transfusion, and large bowel obstruction, to the overall survival of colorectal cancer (35–42, 54, 55). Taking into account the role of u-PA in tumor invasion and metastasis, the u-PA antigen level was expected to lose its significance in the multivariate analysis, because it was found to be related to the Dukes' stage. However, the fact that the other plasminogen activator parameters [t-PA and PAI-2, and u-PA(C)/t-PA(N) antigen ratio] remained independent prognostic parameters indicates that these plasminogen activator-related parameters are of major importance with respect to colorectal cancer survival. Thus, our data support and extend the relationship between the PA expression in tumors and their impact on survival of the patients, as reported previously for breast cancer (15–18, 22, 43–45).

In conclusion, a low t-PA antigen level, t-PA activity, and u-PA/t-PA antigen ratio in normal colorectal mucosa; a high u-PA antigen level, percentage of u-PA activity, and PAI-2 antigen level in carcinomas; and a high u-PA(C)/t-PA(N) antigen ratio are associated with a poorer overall survival of patients with colorectal cancer. Multivariate analysis revealed that in both the dichotomized and categorized groups of patients t-PA antigen and activity levels, PAI-2 antigen

level, and the u-PA(C)/t-PA(N) antigen ratio are independent prognostic variables for the overall survival of patients with colorectal cancer. These observations illustrate the clinical relevance of the plasminogen activators at the tissue level with respect to colorectal cancer development and survival of the patients.

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Prognostic Relevance of Plasminogen Activators and Their Inhibitors in Colorectal Cancer

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