Aust N Z J Obstet Gynaecol 2002; 42: 4: 385

Clinical relevance of urokinase-type plasminogen activator and its inhibitor type 1 (PAI-1) in squamous cell carcinoma of the uterine cervix

LC Horn,[1] S Pippig,[2] G Raptis,[1] U Fischer,[2] U Köhler,[3] B Hentschel[4] and R Martin[2]

Institute of Pathology, Gynecopathology;[1] Institute of Clinical Chemistry, Laboratory of Medicine and Molecular Diagnostics;[2] Department of Obstetrics and Gynecology;[3] Department of Medical Statistics and Epidemiology,[4] University of Leipzig, Leipzig, Germany

ABSTRACT

Objective

The expression of uPA and PAI-1 as parameters of tumour-associated proteolysis has been implicated in the process of tumour cell invasion and the metastatic process. However, there is limited information on the impact of these parameters in cervical carcinoma.

Methods

Quantitative levels for uPA (n = 114) and PAI-1 (n = 103) were researched in operatively treated, surgically staged squamous cell cancer of the uterine cervix, using an ELISA-technique. Results were assessed regarding their impact in predicting pelvic lymph nodes metastases, tumour recurrence rate and recurrence free survival (RFS) using uni- and multivariate analysis.

Results

Median levels of both parameters were significantly higher in tumour tissue than in normal cervical tissue ($p < 0.001$). Detection of uPA gave no useful prognostic information. PAI-1 concentration showed a positive correlation with advanced tumour stage ($p = 0.008$), but no significant correlation with nodal status (pN0: 2.6 vs. pN1: 4.0 ng/mg protein; $p = 0.092$). Using a cut-off level of 2.4 ng/mg protein, patients with elevated PAI-1 levels demonstrated reduced RFS (45.9 versus 52.9 months; $p = 0.1$). Multivariate analysis, including nodal status, tumour stage, lymphovascular space involvement and grading failed to demonstrate any prognostic impact of uPA and PAI-1.

Conclusions

The results indicate, that PAI-1 expression is of some prognostic impact in cervical cancer, indicating an association of elevated PAI levels with local tumour progression and reduced recurrence-free survival.

INTRODUCTION

Cell recruitment and invasion are multi-step processes regulated by cytokines, growth factors and chemokines through multiple signalling pathways and coordinated among different cells.[1,2] Infiltration and destruction of extracellular matrix (ECM) by degradative enzymes are required to facilitate the spread and invasion of tumour cells into the host tissue. At least four different types of tumour-associated proteases may be responsible for ECM-degradation: matrix metalloproteases, cystine, aspartate and serine proteases.

The serine proteases uPA is one of the most important enzymes produced by cancer cells. It is associated with pleiotropic functions, including involvement in proteolysis, cell adhesion, motogenesis and angiogenesis of malignant tumors.[1–3] Recent studies have reported that the presence of uPA and PAI-1 was associated with lymphatic spread and poor prognostic outcome in several tumour sites, including gynaecologic malignancies.[4–7] The aim of this study was to investigate the role of uPA and its specific inhibitor PAI-1 in cervical cancer patients.

MATERIALS AND METHODS

One hundred and fourteen consecutive patients with squamous cell carcinoma of the uterine cervix,

Address for correspondence

Lars-Christian Horn MD
Institute of Pathology
Leipzig University
Liebigstrasse 26
Leipzig, D-04103
Germany

LC Horn MD PhD, S Pippig MD, G Raptis Bc, U Fischer MD, U Köhler MD PhD, B Hentschel PhD, R Martin PhD

treated by radical hysterectomy according to Wertheim-Meigs (radicality Piver III) between January 1993 and December 1995 were included in the study. Immediately after radical surgery, tumour tissue samples were collected by the gynaecological pathologist, shock frozen and stored until use in liquid nitrogen. The assessment of histomorphological parameters and staging were carried out as described,[8] using WHO classification for tumour typing and pTNM-classification for staging. The tumour characteristics are summarised in Table 1.

Table 1 Tumor characteristics

	n (%)
pT category	
pT1b1	41 (36.0)
pT1b2	19 (16.7)
pT2a	7 (6.1)
pT2b	47 (41.2)
pelvic lymph node metastases	
pN0	68 (59.6)
pN1	46 (40.4)
Depth of invasion	
< 33%	10 (8.8)
33–66%	22 (19.3)
> 66%	71 (62.3)
Missing	11 (9.6)
Recurrent disease	
No	94 (82.5)
Yes	20 (17.5)

Frozen tissue (about 150–400 mg) was pulverised by the use of the microdismembrator (Braun-Melsungen, Melsungen, Germany) set to maximum power for 30 seconds. The powder was suspended in Triton-X-100-TBS (20 mM Tris-HCl, 25 mM NaCl, 1% Triton-X-100, pH 8.5), then shaken for 12 hours at 4°C and centrifuged (100,000 g, 4°C for 1 hour). The supernatant was kept and frozen in liquid nitrogen until use.

Commercially available ELISA kits[3,9] determined uPA and PAI-1 as follows: Immubind uPA ELISA kit (American Diagnostics Inc, Greenwich, Connecticut): The intra-assay variations were 3.8% and 6.3%, respectively. This assay recognises sc-uPA, pro-uPA and HMW forms of uPA, as is receptor bound uPA and uPA complex with PAI-1 and PAI-2. Innotest PAI-1 ELISA kit (BYC-Sangtec Diagnostica, Dreieich, Germany) the intra-assay and the inter-assay varaitions were 1.9% and 3.0%, respectively. The lower detection limits of the uPA and PAI-1 kits are 10 ng uPA/mL and 2.5 ng PAI-1/mL. All results were normalised to mg of protein, determined in each sample. Pooled human breast tumour cytosols were used as internal control samples.

The statistical evaluation was carried out with the statistic package SPSS for Windows, version 9.0, using chi-squared cross tabulation for univariate and Cox stepwise regression model for multivariate analysis. Two-sided p values below 0.05 were considered statistically significant (CI 95%).

RESULTS

The measurement of uPA-concentration was possible in all 114 tested tumour samples. Because of a small sample size in 11 cases, PAI-1 concentration was available only in 103 cases.

In tissue extracts of cervical cancer the uPA-antigen concentration was significantly higher (median 0.22 ng/mg protein; range 0.0–0.23) than in tumour-free cervical tissue (median 0.02, range 0–0.2 ng/mg protein; $p < 0.001$). The same was seen for PAI-1 (tumour tissue: median 3.15; range 0.03–18.82 ng/mg protein versus normal cervical tissue: median 0.21; range 0.0–3.08 ng/mg protein; $p < 0.001$). The uPA and PAI-1 results did not show any significant association regarding the presence of lymphovascular space involvement within the tumour (data not shown).

Contrary to uPA, PAI-1 concentrations exhibited a distinct rise when they were compared with tumour stage (Table 2). The presence of lymph node metastases correlated with elevated PAI-1 concentration, but without statistical significance (Table 2). An optimum of sensitivity (66.7%) and specificity (41.3%) of PAI-1, calculated with the use of a receiver operator curve (ROC), was seen at a PAI-concentration of 2.4 ng/mg; therefore, an empirical cut-off value of 2.4 ng/mg protein was established for further analysis. Patients with lower PAI-1 values represented a prolonged mean disease-free survival (≤ 2.4 ng/mg: 52.9 ± 2.17 versus > 2.4 ng/mg: 45.9 ± 2.8 months; $p = 0.1$), but the difference was not statistically significant. The frequency of recurrent disease was up to 2.1 times higher when PAI-1 values were high compared with lower levels (≤ 2.4 ng/mg: 5/48 = 10.4% versus > 2.4 ng/mg: 10/46 = 21.7%; $p = 0.009$; Figure 1). Tumours with parametrial involvement (pT2b) exhibited higher PAI-1 values (2.45 in pT1b versus 4.4 ng/mg in pT2b; $p = 0.000$). There were no differences in uPA and PAI-1 concentrations

Figure 1 Recurrence free survival in patients with elevated PAI-1 levels, using a cut off-value of 2.4 ng/mg protein

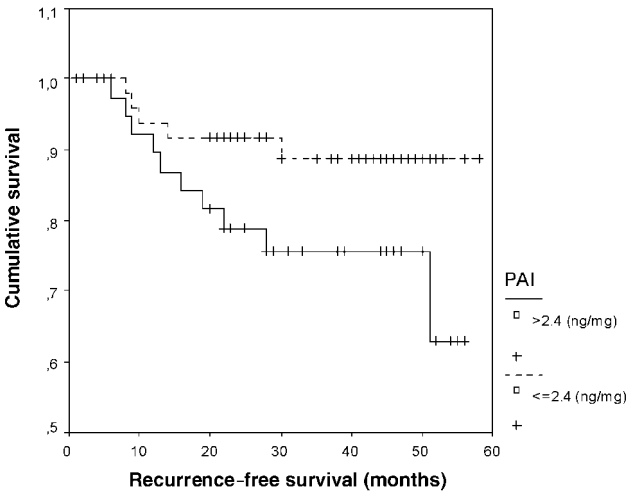

Table 2 uPA- and PAI-1 concentration in cervical squamous cell carcinoma

	n	uPA concentration (ng/mg protein)	n	PAI-1 concentration (ng/mg protein)
pT-catogory				
pT1b1	41	0.21	30	1.0
pT1b2	19	0.19	19	3.9
pT2a	7	0.34	7	2.7
pT2b	47	0.23	47	4.4
p value		0.6		0.008*
Pelvic lymph node status				
pN0	68	0.21	59	2.6
pN1	46	0.23	44	4.0
p value		0.72		0.092
Depth of invasion*				
< 33%	10	0.32	7	1.65
33–66%	22	0.2	17	2.41
> 66%	71	0.23	63	3.92
p value		0.52		0.2
Recurrent disease				
No	95	0.38	90	3.2
Yes	20	0.29	13	4.4
p value		0.68		0.55

*In the missing cases in the uPA (n = 11) and PAI-1-group (n = 16) the depth of invasion was not available

to the depth of invasion of the tumour into the cervical stroma (Table 2).

In multivariate analysis (n = 78), using the stepwise Cox-regression model, including pT-category, histologically proven pelvic lymph node status, lymphovascular space involvement and tumour grading, uPA and PAI-1 failed to give additional prognostic information regarding disease-free and overall survival, even when cases with and without pelvic lymph node involvement were considered separately.

DISCUSSION

The results regarding the impact of plasma levels of uPA and PAI-1 are conflicting. Koelbl et al[10] reported significantly increased plasma uPA-concentrations in cervical cancer patients, when compared with age-matched controls. Others failed to confirm these results using tPA and PAI-1 as study parameters. In the present study we preferred the use of ELISA technique for determining uPA and PAI-1 levels of the tumour and stromal cells.

Intra-tumour levels of uPA and PAI-1, determined by ELISA technique are quite different, comparing different tumour sites and histological tumour types. Cervical carcinomas and tumour-free cervical tissue exhibited the lowest concentrations, compared with vulvar squamous cell cancers and endometrial adenocarcinomas,[5,6] indicating tumour-specific expression.

Tumours with immunohistochemical detection of uPA or increased uPA levels represented poor prognosis. In lung cancer, uPA-expression in cancer cells was correlated only with the tumour size,[4] but the detection in stromal cells showed a positive correlation with the presence of lymph node metastases and patient's outcome. Similar results were reported for ovarian, cervical and breast cancer.[9,11] In gastric cancer uPA expression showed a correlation with increased values of other proteases, like MMP-2 and 72 kD-collagenase IV.[12]

These results and the findings of Jarrad et al[3] who demonstrated that in prostatic cancer cell lines anti-uPA antibodies may reduce but not completely block the tumour cell invasion, indicate a complex network of proteases establishing the malignant potential of tumours. This is in concordance with the findings of Tsuchiya et al,[13] who have demonstrated an insignificant reduction of metastatic potential of fibrosarcoma cell lines after application of anti-uPA antibodies.

There is some agreement that uPA and PAI-1 levels are elevated in cervical cancer tissue, compared with tumour-free tissue[9,14] or CIN-lesions.[15] As indicated in Table 2, our results failed to show any correlation between uPA levels and tumour stage in pathologically staged cervical carcinoma. In contrast, Daneri-Navarro et al[15] reported a 1.4–4.2-fold increase for mean uPA and PAI levels in FIGO II–IV cervical cancers, compared to stage IB disease. However, their study population was rather small, the data were pooled and clinical (FIGO) staging was used. In an earlier study, including 20 cervical carcinoma FIGO-IB and -IIA, Sugimara et al[14] suspected that the detection of uPA may be used as a marker for predicting pelvic lymph node metastases (PNM). In stage FIGO II cervical carcinoma uPA activity was approximately three times higher in patients with PNM than in those without nodal spread.[11] The present study was unable to confirm these results (Table 2). The causes of these conflicting results are unclear.

In accordance with an earlier study with short-term follow-up, we were unable to demonstrate any prognostic significance of uPA regarding recurrent disease or disease free survival (DFS).[16] This is in conflict with the study of Kobayashi et al[9] which demonstrated a poorer recurrence free and overall survival in cases of elevated uPA levels. This diversity may be caused by methodological problems (different ELISA-kits), with the detected uPA values of this Japanese group being ten times higher, even in normal cervical tissue which served as controls, compared with our study and the results presented by Daneri-Navarro et al.[15]

Increased PAI-1 concentrations correlated with advanced stage of the disease. PAI-1 was also elevated in tumours from patients with pelvic LNM, not reaching statistical significance (Table 2). As indicated by others, an increase of PAI-1 is well correlated with metastatic potential in several tumours[7,13] as well as with poor prognostic outcome.[7,11] In the present study tumours with high PAI-1 levels demonstrated a shorter disease-free survival of about nine months (44.9 versus 52.4 months), so that PAI-1 may represent a prognosticator in patients with malignant tumours.

PAI-1 seems to play a role in the generation and functional stabilisation of tumour stroma against proteolytic self-destruction.[11] Hildebrand et al[17] have been shown an increased uPA concentration at the front of invasion, compared with non-invasive areas. In cervical cancer specimens strong immuno-staining for uPA was found in areas with invasive growth, whereas marked PAI-1 staining was observed in the centre of the tumour nests.[9] It is probable that some of the cleavage products of plasminogen may have anti-angiogenetic activity,[2] indicating that any down-regulation of uPA in malignant tumours may result in a shift from anti-angiogenesis to neo-angiogenesis in malignant neoplasms.[2] In that context PAI-1 concentration may represent an indirect indicator for angiogenetic potential in tumours.

In multivariate analysis, measurement of uPA and PAI-1 conveyed no additional prognostic impact. Further, the interpretation of the results of uPA and PAI-1 levels must include tumour type and tumour site diversities. Probably, the use of combination of immuno-histochemistry and ELISA-technique may highlight several problems.

In conclusion, PAI-1 is of more prognostic impact than uPA in surgically treated squamous cell cancer of the uterine cervix. Elevated PAI-1 levels, using a cut-off of 2.4 ng/mg protein, are associated with local tumour progression (pT category) and reduced disease-free survival.

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