ONCOLOGY

Urokinase and Tissue Plasminogen Activators and Their Inhibitor PAI-1 in Human Tumors

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The content of urokinase and tissue plasminogen activators and their inhibitor PAI-1 in tumors and histologically intact tissues from patients with breast, ovarian, and non-small-cell lung cancer was measured by enzyme immunoassay. The content of urokinase plasminogen activator and PAI-1 was considerably increased in all malignant tumors, however the correlation between the expression of components of the plasminogen activation system and clinical morphological features and prognosis of the disease depends on the type of tumor.

Key Words: *urokinase plasminogen activator; tissue plasminogen activator; breast cancer; ovarian cancer; lung cancer*

Destruction of the basal membrane and extracellular matrix by tumor-associated proteases is the main mechanism of tumor invasion [12]. Proteases participate in tumor metastasizing and neoangiogenesis [7,10,13]. Plasminogen activation and plasmin formation system play a central role in proteolytic processes associated with progress of malignant tumors [1,6,12]. Plasmin destroys the components of tumor stroma and activates metalloproteases, e.g. collagenase IV which cleaves collagen and other components of the basal membrane and promotes metastasizing and invasion of tumors and the formation of new vessels.

Plasmin is formed as a result of cyclic amplification regulated by the feedback mechanism. The key component of this proteolytic cascade is urokinase plasminogen activator (uPA), whose activity is regulated by several factors. First, uPA binding to cell receptors stimulates plasminogen activation and plasmin formation, second, the receptor is inactivated via the feedback mechanism as a result of its cleavage by plasmin or uPA [11]. uPA activity is suppressed by two protein inhibitors belonging to the serpin family, PAI-1 and PAI-2 [3]. Tissue type activator (tPA) also participates in plasminogen activation, but plays an opposite role in tumor development: it destroys tumor cells and protects adjacent tissues from tumor dissemination [4,5]. The receptors for uPA and tPA are different, but PAI-1 and PAI-2 effectively inhibit both plasminogen activators [3].

The level and ratio of expression of different components of the plasminogen activation system in the tumor can serve as indicators of its metastatic and invasive activity, and are therefore biologically significant prognostic factors in various tumors [1,6,8]. Moreover, suppression of urokinase type plasminogen activation at different levels can become one of approaches to the development of new therapies [14]: the use of monoclonal antibodies to uPA or its receptor for preventing their interactions; the use of soluble uPA receptor for binding of active uPA; competitive blockers of uPA interactions with the receptor (inactive enzyme, fragments of uPA molecule); natural or synthetic inhibitors of uPA enzymatic activity or antisense oligonucleotides to uPA, PAI-1, or uPA receptor.

For effective clinical use of antimetastatic drugs it is necessary to determine the types of tumors and the

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groups of patients in whom the best effect can be expected. An important factor is the ratio between the content of target protein in the tumor and adjacent intact tissue.

The purpose of this study was to evaluate the role of uPA, tPA, and PAI-1 in tumors of different location and their potential significance as the targets for new antitumor antimetastatic drugs. This report presents the data on breast, ovarian, and non-small-cell lung cancer (BC, OC, NSLC, respectively).

MATERIALS AND METHODS

The study was carried out in 102 patients with breast cancer (stages I-IV) aged 32-81 years; 69 with ovarian tumors aged 23-76 years (40 malignant, 19 benign, 3 with borderline tumors) and 3 healthy women with normal ovaries; contralateral ovaries were examined in 47 patients and tissue specimens of the greater omentum in 43; and 58 patients (88% men) with NSLC. The predominant clinical anatomic form of NSLC was central cancer (60%); the majority of patients had stage III (50%) or IV (41%). Tumors and histologically normal lung tissue were examined in all patients with lung cancer.

The concentration of uPA in the cytosol of tumors and histologically intact tissues was measured using the routine steroid hormone receptor binding assay [2]. The preparations were diluted 10-fold with K,Na-phosphate buffer (14 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4) containing 0.1% Tween-20 and 1% BSA. Standard reagents for enzyme immunoassay [9] were used as described previously [2]. The measurements were carried out on an EL_X800 automated universal microplates reader (Bio-Tek Instruments, Inc.) at 490/630 nm. The results were processed using the formula:

TABLE 1. Concentrations of uPA, PAI-1, and tPA (ng/mg Protein) in Tumor Cytosol of Patients with Breast Cancer of Different Stages and Dissemination (*M*±*m*)

Parameters of tumor		uPA	PAI-1	tPA
Stage	I (*n*=15)	1.19±0.49	0.32±0.11*	4.82±1.22
		(0.05-7.73)	(0-1.26)	(0-15.44)
	Ia (*n*=26)	1.58±0.41	0.75±0.29	3.67±0.92
		(0.02-8.93)	(0-6.14)	(0-16.70)
	Ib (*n*=32)	1.74±0.48	1.47±0.35*	2.03±0.62
		(0.04-13.24)	(0.0-6.00)	(0-16.16)
	IIIa (*n*=13)	0.70±0.15^{o}	0.54±0.24	1.25±0.43^{*+}
		(0.04-1.66)	(0.0-2.78)	(0-5.3)
	IIIb (*n*=9)	0.49±0.10^{+}	0.57±0.23	3.20±2.12
		(0.12-0.96)	(0.0-1.79)	(0-19.31)
	IV (*n*=7)	0.69±0.17^{o}	0.91±0.57	4.62±2.46
		(0.23-1.41)	(0.0-4.19)	(0.0-15.67)
Tumor size	T1 (*n*=24)	1.7±0.5	0.32±0.09	4.57±0.99
		(0.05-8.93)	(0-1.26)	(0-15.4)
	T2 (*n*=50)	1.47±0.32	1.10±0.26*	2.46±0.54
		(0.02-13.24)	(0-6.14)	(0-16.7)
	T3 (*n*=12)	0.72±0.12	1.43±0.46**	2.31±1.02
		(0.04-1.31)	(0-4.19)	(0-12.1)
	T4 (*n*=15)	0.62±0.12^{**+}	0.48±0.16^{o}	3.26±1.56
		(0.12-1.66)	(0-1.79)	(0-19.3)
Lymph node involvement	N0 (*n*=40)	1.04±0.22	0.79±0.22	3.78±0.71
		(0.02-7.73)	(0-6.14)	(0-16.7)
	N1 (*n*=45)	1.81±0.40	1.11±0.24	2.26±0.58
		(0.04-13.24)	(0-6)	(0-16.16)
	N2 (*n*=17)	0.58±0.11	0.33±0.14*	3.35±1.36
		(0.04-1.66)	(0-1.75)	(0-19.31)

Note. Significant differences between stages: $p<0.05$: **vs.* stage I, $^{+}$*vs.* stage IIa, ^{o}*vs.* stage IIb; between tumors of different size: $^{*}p<0.01$, $^{**}p<0.05$ *vs.* T1; $^{+}p<0.05$ *vs.* T2; $^{o}p<0.01$ *vs.* T3; depending on lymph node involvement: $^{*}p<0.05$ *vs.* N1.

$$Y=a+bX+cX^2,$$

where X is the concentration of analyzed protein (ng/ml), Y optical density at 492 nm, and a, b, c known coefficients. The concentrations of analyzed proteins were expressed in ng/mg cytosol protein. Protein was measured by the method of Lowry.

The parameters were compared using Student's t test, Cruscal—Wallis' median test, and Spearman rank correlation test. Kaplan—Meyer test was used for the analysis of relapse-free and total survival. Cox proportionate risk model was used for multifactorial regression analysis of survival. The results were statistically processed using Statistica for Windows 5.0 software.

RESULTS

In breast cancer uPA was present in all studied samples (0.02-13.24 ng/mg protein), PAI-1 was found in tumors of 64 (63%) patients (0.02-6.14 ng/mg protein), and tPA was found in tumors of 76 (75%) patients (0.01-19.31 ng/mg protein).

The mean concentrations of uPA, PAI-1, and tPA in tumors and histologically intact mammary tissue were compared in 35 patients. The mean concentration of uPA in tumors was 1.26±0.31 (0.02-0.31) ng/mg *vs.* 0.32±0.05 (0-1.31 ng/mg; $p<0.01$) in intact mammary tissue; the concentration of PAI-1 in tumors was 5.2 times higher than in intact tissue (0.89±0.15 and 0.12± 0.02 ng/mg, respectively, $p<0.05$; ranges 0.04-2.78 and 0.01-0.34 ng/mg, respectively). On the other hand, the concentration of tPA was virtually the same in tumors and histologically intact tissue. Only in 4 of 35 patients (11%) the concentration of uPA in normal tissue was slightly higher than in the tumor. For PAI-1 this ratio was observed in only 1 of 20 examines (5%). For tPA different ratios of concentrations in tumors and intact mammary tissue were detected.

Hence, tissue concentrations of uPA and PAI-1 were increased in the majority of malignant breast tumors in comparison with intact tissue. No regularity of this kind was observed for tPA.

The highest concentration of uPA was detected in patients with stage IIb BC; this parameter was significantly higher than in patients with stages III-IV or I and IIa (Table 1). Two trends were seen: at early stages (I-IIb) the concentration of uPA increased in parallel with tumor expansion, while then this parameter decreased with further tumor growth. Similar trends were observed for PAI-1. Opposite trends were seen for

TABLE 2. Cytosol Concentrations of uPA (ng/mg Protein) in Tumors, Intact Ovarian Tissue, and Greater Omentum

Tissue	*M*±*m*	Median	Range
Ovarian cancer (*n*=40)	0.87±0.19	0.45	0.01-6.52
Borderline ovarian tumors (*n*=3)	0.33±0.13	0.25	0.15-0.58
Benign ovarian tumors (*n*=19)	0.65±0.36	0.16	0-6.79
Intact ovaries (control group) (*n*=3)	0.05±0.02	0.07	0.01-0.07
Intact ovaries of patients with cancer or benign tumors (*n*=11)	0.12±0.06*	0.09	0.01-0.28
All intact ovaries (*n*=14)	0.10±0.02	0.07	0.01-0.28
Tumor-involved ovary of patients with cancer (*n*=33)	0.41±0.08	0.21	0-2.32
Intact omentum (*n*=23)	0.38±0.17	0.18	0-3.95
Metastases in the omentum (*n*=20)	0.78±0.22	0.34	0-3.95

TABLE 3. Cytosol Concentration of uPA (ng/mg Protein) in Ovarian Cancer Depending on Stage and Size of Primary Tumor

Tumor dissemination		*M*±*m*	Median	Range
Stage	I (*n*=6)	1.30±1.05	0.23	0.03-6.52
	II (*n*=6)	0.77±0.33	0.43	0.04-2.00
	III (*n*=19)	0.62±0.11	0.42	(0.01-1.64)
	IV (*n*=8)	1.30±0.48	0.80	0.25-4.35
Tumor size, cm	up to 5 (*n*=4)	0.43±0.13	0.34	0.23-0.79
	5-10 (*n*=14)	1.31±0.43	0.88	0.19-6.52
	10-20 (*n*=11)	0.86±0.40	0.42	0.03-4.35
	more than 20 (*n*=11)	0.48±0.14	0.30	0.01-1.28

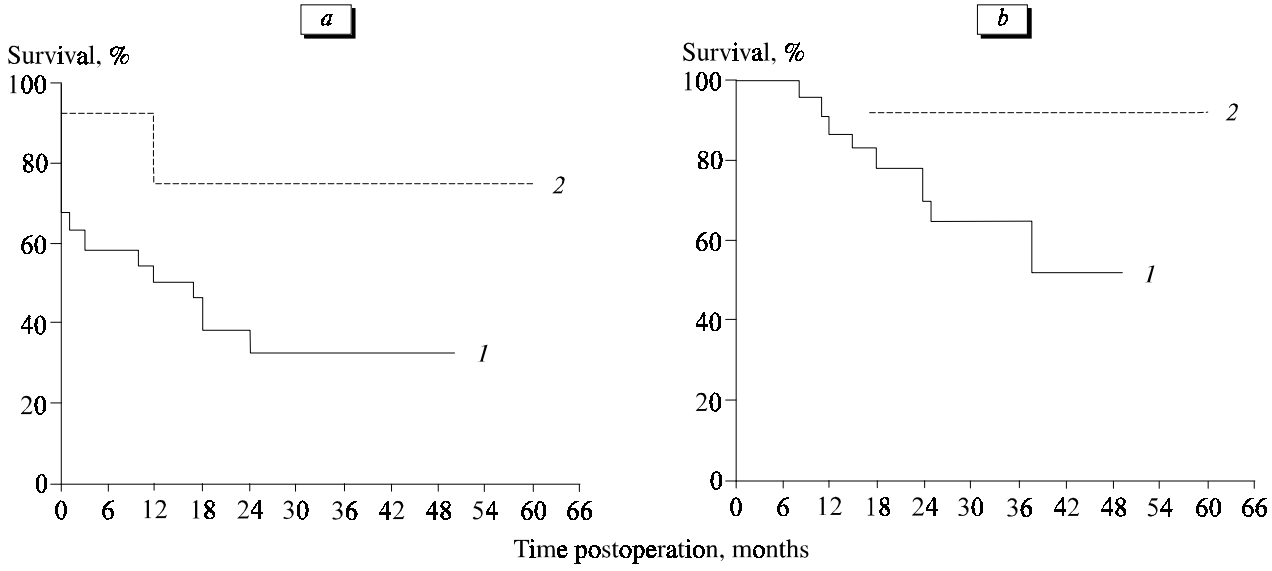

Fig. 1. Relapse-free (*a*) and total (*b*) survival of patients with ovarian cancer and concentration of uPA in primary tumor. Concentration of uPA: *1*) above 0.45 ng/mg protein; *2*) below 0.45 ng/mg protein.

tPA: the highest concentration of the enzyme was detected in patients with stage I and the lowest at stage IIIa. In disseminated process (stages IIIb and IV) the concentrations of tPA were virtually the same as during the early stages of the disease.

As the tumor grew, the concentrations of uPA gradually decreased, the difference between tumors corresponding to T1 and T4 being significant (Table 1). The concentration of PAI-1 increased significantly from T1 to T3 and decreased in T4. By contrast, the concentration of tPA decreased significantly during tumor progression from T1 to T3 and increased in T4. As for the lymph nodes, the highest concentrations of uPA and PAI-1 and the lowest concentration of tPA in primary tumor were observed in solitary metastases to the axillary lymph nodes on the involved side (N1) (Table 1).

Hence, changes in the concentrations of uPA and PAI1, on the one hand, and of tPA, on the other, was opposite, depending on the stage of BC. Judging from the level and ratio of the studied components of the plasminogen activation system, tumors with early metastases are characterized by the highest invasive activity and metastatic potential. At these stages the tumors are probably the best targets for therapy with uPA inhibitors.

Urokinase type plasminogen activator was detected in all samples of ovarian tumors and its concentration varied in a wide range (0.01-6.79 ng/mg protein). The mean content of uPA in the cytosol gradually increased with transition from normal ovaries to benign, borderline, and malignant tumors (Table 2). This increase was clearly seen when comparing the medians, the differences being significant (p=0.006).

The content of uPA in the contralateral ovary in patients with bilateral OC was lower than in the primary node, but it was significantly higher than the in the contralateral ovaries of patients with unilateral OC (p<0.05, Table 2). Similarly, the concentration of uPA in the omentum with metastases was twice higher than in intact omentum. The content of uPA in OC metastases to the greater omentum was virtually the same as in the primary tumor (Table 2). Therefore, malignant processes in the ovaries and adjacent tissues are characterized by increased tissue concentration of uPA.

No significant correlation between the concentration of uPA and the stage of OC was found, but the median of this parameter trended to increase with tumor progression. The highest concentrations of uPA was characteristic of medium-sized tumors (5-20 cm) (Table 3).

No clear-cut correlation between uPA concentration in the primary tumor and histological type or degree of differentiation of OC, presence of peritoneal carcinomatosis, ascites, metastases to the omentum, liver, or retroperitoneal lymph nodes was observed.

Hence, the content of uPA in OC does not correlate with clinical morphological characteristics. Nonetheless, comparing the relapse-free and total survival rates in patients with uPA above and below the median value (0.45 ng/mg protein) we found that in patients with high tumor content of uPA both values were significantly worse than in the patients with low uPA content (Fig. 1, p<0.05). In the multifactorial analysis including parameters of the disease, patient age and menopausal status, histological type of the tumor and differentiation degree, uPA retained its pro-

gnostic value (p=0.0021). Therefore, uPA is an important independent prognostic factor in OC.

In patients with NSLC, significant differences between the tumor and normal tissue were detected for all parameters: the levels of uPA were 2.58±0.31 and 0.42±0.05 ng/mg (p<0.01), PAI-1 6.90±0.95 and 2.26± 0.25 ng/mg (p<0.01), and tPA 1.90±0.38 and 4.45± 0.54 ng/mg, respectively (p<0.05). The differences were most pronounced for uPA: the concentrations of uPA in normal tissue surpassed the median for tumors in only 2 patients, and none of the "normal" values reached the upper quartile for tumors (3.44 ng/mg protein).

Further paired comparison of the studied parameters in tumors and histologically intact tissues showed that in 93% patients with NSLC the concentration of uPA in the tumor 1.3-43.0-fold surpassed that in adjacent intact lung tissue. Similar ratio was observed for PAI-1 in 84% patients, while the concentration of tPA in the tumor was lower (maximally 121 times) than in intact lung tissue in 84% patients.

A strict direct correlation was revealed between PAI-1 levels in the tumor and histologically intact lung tissue (r=0.52; p<0.05); for tPA this correlation was weak (r=0.27), but significant (p<0.05), while for uPA no correlation was detected. A direct correlation was revealed between tumor concentrations of uPA and PAI-1 (r=0.35; p<0.05). The level of tPA did not correlate with other parameters.

Hence, high uPA concentration in the tumor is the most pronounced shift in the plasminogen activation system in NSLC.

No significant relationships between tumor concentrations of uPA, tPA, and PAI-1 and clinical stage of NSLC, patient age and sex, or clinical form were detected. However, the contents of both plasminogen activators were significantly higher in small noninvasive tumors (T1) than in large (T2-T3) tumors (p<0.05; Table 4). The concentration of PAI-1 did not depend on the tumor size. On the other hand, the level of PAI-1 was significantly increased in primary tumors with numerous metastases to lymph nodes (N3) (Table 4). For uPA these differences presented as a trend. No relationship between distant metastases and test parameters was detected.

There were virtually no differences between the main histological types of NSLC, squamous-cell carcinoma and adenocarcinoma. On the other hand, there

TABLE 4. Cytosol Concentrations of uPA, PAI-1, and tPA (ng/mg Protein) in Tumors in Patients with NSLC Depending on Process Dissemination and Tumor Differentiation

Clinical morphological factors		uPA	PAI-1	tPA
Tumor size	T1 (n=5)	4.91±1.76	8.88±3.55	6.03±3.24
		(1.04-11.10)	(3.3-22.9)	(1.66-18.71)
	T2 (n=31)	2.32±0.35**	6.69±1.31	1.68±0.37*
		(0.12-7.92)	(0.77-35.70)	(0.0-6.09)
	T3 (n=14)	2.73±0.77	4.37±1.35	1.35±0.47**
		(0-9.76)	(1.13-17.56)	(0-6.83)
	T4 (n=4)	2.10±0.79	10.85±4.39	0.84±0.46
		(0.71-4.33)	(3.93-23.33)	(0-2.14)
Lymph node involvement	N0 (n=25)	2.46±0.51	7.47±1.63	2.64±0.80
		(0.22-6.25)	(0.98-35.71)	(0.0-18.71)
	N1 (n=10)	2.39±0.64	5.82±2.24	1.61±0.58
		(0.04-13.24)	(0.77-23.33)	(0.21-6.10)
	N2 (n=18)	2.86±0.60	5.22±1.09	1.13±0.33
		(0.22-6.25)	(1.13-16.45)	(0.0-5.73)
	N3 (n=1)	6.10	17.56+	2.13
Differentiation degree	high (n=9)	1.87±0.80	8.78±3.90	3.34±0.77
		(0-7.91)	(0.99-35.71)	(0.26-6.83)
	moderate (n=34)	2.60±0.40	6.44±1.02	1.89±0.59
		(0.12-11.10)	(0.77-23.33)	(0-18.71)
	low (n=7)	7.00±1.24	5.59±2.04	1.03±0.42
		(0.22-9.75)	(0.88-12.81)	(0-2.90)

Note. *p<0.01, **p<0.05 *vs.* T1; +p<0.05 *vs.* N2 and N1.

was a pronounced trend to an increase of uPA concentration and decrease of tPA concentration in NSLC of higher differentiation (Table 4).

Interestingly, the uPA/tPA ratio in NSLC cytosol increased from 0.95 to 9.11 with decreasing the degree of tumor differentiation, while for intact lung this ratio was only 0.19. Hence, the uPA/tPA ratio seems to be the most promising predictor for NSLC.

On the whole, changes in the content of uPA and PAI-1 are similar in NSLC and breast cancer: intensive expression of these proteins corresponds to a more aggressive tumor process. At the same time, the pattern of tPA changes in the tumor are opposite.

Hence, a marked increase in the content of uPA and PAI-1 is a universal characteristic of various malignant tumors. The content of tPA is decreased in the majority of malignant tumors, but the correlation between the expression of plasminogen activation system components and clinical morphological features of tumor process and probably its prognosis depends on the type of tumor. However uPA is a perspective and selective target for antimetastatic antitumor therapy in various malignancies.

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