Plasminogen Activators and Their Inhibitor in Bone Tumors and Tumor-Like Damages

N. E. Kushlinskii, A. I. Yusifov, E. S. Gershtein, Yu. N. Solov'ev, and N. N. Trapeznikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 8, pp. 197-199, August, 2001
Original article submitted August 2, 2001

Expression of urokinase- and tissue-type plasminogen activators and their inhibitor PAI-1 in the cytosolic fraction of 20 osteosarcomas, 20 chondrosarcomas, 13 giant-cell bone tumors, 5 Ewing's sarcomas, and 7 osteochondral exostoses was studied by enzyme immunoassay. The content of urokinase-type plasminogen activator increased, while the concentration of tissue-type plasminogen activator decreased in bone tumors of various histological compositions compared to osteochondral exostoses. A positive correlation was found between PAI-1 content and the volume of osteo- and chondrosarcomas. Expression of urokinase-type plasminogen activator increased in patients with primary osteosarcomas characterized by early generalization of the pathological process.

Key Words: *urokinase plasminogen activator; tissue plasminogen activator; plasminogen activator inhibitor-1; bone tumors*

Plasminogen, receptors, and enzymes regulating its activity play an important role in tumor invasion and metastatic growth [9,10]. Urokinase- (uPA) and tissue-type plasminogen activators (tPA) are present in normal and tumor tissues. Previous studies showed that uPA is involved in degradation of the intercellular matrix in normal and tumor tissues. tPA plays a role in thrombo- and fibrinolysis [10]. There are 2 specific plasminogen activator inhibitors (PAI). PAI-1 is produced by endotheliocytes and neoplastic cells and is present in the plasma and platelets. PAI-2 isolated from placental extracts is also formed in cultured monocytes and macrophages [3,6].

The intensity and ratio between expression of various components of the plasminogen activation system (PAS) in tumor tissues reflect their metastatic and invasive potentials and, therefore, can be used as prognostic criteria during various neoplasms [1,2,5,9]. Previous studies demonstrated that uPA and PAI-1 hold much prognostic significance in patients with breast cancer [5,9]. There is a correlation between the rise of tPA content in tumor tissues and the increase in relapse-free and total survival rates. However, high tPA—PAI-1 concentration is associated with poor prognosis [12]. The content and ratio between individual PAS components during the development of bone tumors remain unclear.

Here we compared the contents of uPA, tPA, and PAI-1 in primary bone tumors of various histological structure and evaluated the relationship between their expression and clinical and morphological characteristics of bone neoplasms.

MATERIALS AND METHODS

Samples of bone neoplasms were obtained from 65 patients (30 women and 35 men) treated at the N. N. Blokhin Russian Oncology Center (January 1999-May 2000). The age of patients was 9-62 years (average 25.7±11.5 years). Clinical and X-ray diagnosis of bone neoplasms was confirmed by morphological assay. We examined patients with osteosarcomas (n=20), chondrosarcomas (n=20), giant-cell bone tumors (n=13),

N. N. Blokhin Russian Oncological Center, Russian Academy of Medical Sciences, Moscow

0007-4888/01/1322-0780$25.00

Ewing's sarcomas (n=5), and osteochondral exostoses (n=7, control).

Tumor tissue fragments (200-500 mg) were taken immediately after surgical removal of neoplasms or during biopsy and stored at -70°C until isolation of the cytosolic fraction and enzyme immunoassay. We measured uPA, tPA, and PAI-1 concentrations in cytosols, which were routinely prepared and 10-fold diluted with K,Na-phosphate buffer (14 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8.1 mM Na_2HPO_4, pH 7.4) containing 0.1% Tween-20 and 1% bovine serum albumin. Enzyme immunoassay kits were developed at the Catholic University Nijmegen (Netherlands) [2,7]. The measurements were performed on an ELX800 universal microplate reader (Bio-Tek Instruments) at 490/630 nm. Protein concentrations were expressed in ng/mg cytosolic protein. Protein content was measured by the method of Lowry.

The results were analyzed by F test (dispersion analysis), Student's t test, and Wilcoxon test with correction for multiple comparisons. Correlation analysis was performed by Pearson test (r).

RESULTS

The highest content of uPA was found in giant-cell bone tumors and osteosarcomas and lowest in osteochondral exostoses (Table 1). uPA content in cytosols of osteochondral exostoses (benign tumor-like bone damages) was much lower than in osteosarcomas, chondrosarcomas, giant-cell bone tumors, and Ewing's sarcomas (p<0.05). The highest content of tPA was found in osteochondral exostoses and lowest in osteosarcomas and Ewing's sarcomas. These results confirm the hypothesis that malignant transformation is accompanied by changes in the ratio between plasminogen activators. uPA content is high in malignant tumors, while tPA prevails in normal tissues [1]. In our experiments uPA content was high, while tPA concentration was lowest in relatively benign giant-cell bone tumors.

The intensity of PAI-1 expression in osteochondral exostoses and bone tumors was similar. We found no significant differences between expression of PAS components in bone tumors of various histological compositions (Table 1).

There were no correlations between the contents of individual PAS components, as well as between their concentrations in the tumor tissue, age of patients, and histological variant of osteosarcomas. A positive correlation was found between PAI-1 content and the volume of primary osteosarcomas (r=0.72, p=0.014) and chondrosarcomas (r=0.64, p<0.05). In patients with chondrosarcomas, we revealed a positive correlation between the tumor volume and tPA content (r=0.67, p<0.05).

The patients were examined for 1-2.5 years. Metastases and local recurrences were found in 21.5 and 17% patients, respectively. The incidence of local recurrences was highest in patients with osteosarcomas and chondrosarcomas (30 and 25%, respectively). In these patients we revealed a correlation between the contents of uPA, tPA, and PAI-1 in primary tumors and efficiency of combination therapy.

Metastases into the lungs were found in 10 of 20 patients with osteosarcomas over the first 2 years after the start of therapy. We performed a retrospective comparative analysis of protease expression in patients with and without early generalization of the tumor process. uPA content in sarcoma cytosols in patients

TABLE 1. Content of PAS Components in Cytosols of Bone Tumors and Tumor-Like Damages (ng/mg Protein)

Parameter	Osteosarcoma (n=20)	Chondrosarcoma (n=20)	Ewing's sarcoma (n=5)	Giant-cell tumor (n=13)	Osteochondral exostoses (n=7)	Total sample
uPA						
extreme values	0-2.68	0-1.72	0.04–1.59	0-1.50	0-0.09	0-2.68
median	0.41	0.19	0.08	0.71	0.0	0.3
$M\pm s$	0.55±0.62	0.42±0.51	0.37±0.68	0.68±0.42	0.02±0.04	0.47±0.5
tPA						
extreme values	0-15.75	0-22.83	0.45-2.43	0-4.48	0-28.58	0-28.6
median	0.28	0.46	0.90	0.33	4.90	0.36
$M\pm s$	1.30±3.32	2.43±5.34	1.17±0.84	0.79±1.36	11.87±13.35	2.6±6.2
PAI-1						
extreme values	0.03-7.44	0.30-39.04	0.35-21.00	0.15-10.73	0-18.82	0-39.0
median	2.08	4.60	1.27	3.50	2.66	2.78
$M\pm s$	2.49±1.98	9.58±10.05	5.05±8.94	4.45±3.83	4.60±6.63	5.33±7.10

with early hematogenic metastases (median 0.50, 0.76± 0.73 ng/mg protein) 2-fold surpassed that in patients with osteosarcomas without distant metastases (median 0.24, 0.30±0.23 ng/mg protein, $p<0.05$). However, the content of other proteases in osteosarcomas characterized by early metastatic growth did not differ from that in non-metastasizing tumors (tPA median 0.29, 0.18 ng/mg protein; PAI-1 median 3.1, 1.73 ng/mg protein, $p>0.05$). In 7 patients with osteosarcomas local tumor recurrences were observed over the first 3 years after the start of therapy. We found no significant differences between the contents of proteases and their inhibitor in recurrent and nonrecurrent tumors.

Local recurrences were found in 5 of 20 patients with chondrosarcomas. We reveal no significant differences between the concentration of PAS components in tumor tissues in patients with recurrent and nonrecurrent chondrosarcomas.

Our findings suggest the relationship between the main proteases involved in plasminogen activation (primarily, uPA) and tumor aggressiveness in patients with bone neoplasms. This is confirmed by the intensive uPA expression in rapidly metastasizing osteosarcomas, the correlation between PAI-1 content and tumor volume, and suppressed uPA expression and high tPA concentration in osteochondral exostoses. These results are consistent with published data on tissue proteases in bone tumors [4,8,11].

Studies of PAS components in bone tumors should be aimed at the development of pathogenetic therapeutic approaches to the regulation of plasminogen activity, evaluation of prognostic significance, and the use of these parameters as criteria for high risk of malignant transformation of bone and cartilaginous tissues during tumor-like damages and benign bone neoplasms.

REFERENCES

1. E. S. Gershtein and N. E. Kushlinskii, *Byull. Eksp. Biol. Med*., **131**, No. 1, 81-87 (2001).
2. E. S. Gershtein, U. R. Mamedov, O. I. Kostyleva, and N. E. Kushlinskii, *Klin. Lab. Diagn*., No. 3, 16-21 (2000).
3. J. C. Castellote, E. Grau, M. A. Linde, *et al*., *Thromb. Haemost*., **63**, 67-71 (1990).
4. A. C. De Bart, P. H. Quax, C. W. Lowik, *et al*., *J. Bone Miner. Res*., **10**, 1374-1384 (1995).
5. M. J. Duffy, C. Duggan, H. E. Mulcahy, *et al*., *Clin. Chem*., **44**, 1177-1183 (1998).
6. L. A. Erickson, C. M. Hekman, and D. J. Lokutoff, *Proc. Natl. Acad. Sci. USA*, **82**, 8710-8714 (1985).
7. N. Grebenschikov, F. Sweep, A. Geurts, *et al*., *Int. J. Cancer*, **81**, 598-606 (1999).
8. C. Hackel, S. Krueger, H.-J. Grote, *et al*., *Cancer*, **89**, 995-1003 (2000).
9. M. P. Look and J. A. Foekens, *APMIS*, **107**, 150-159 (1999).
10. P. Mignatti and D. B. Rifkin, *Physiol. Rev*., **73**, 161-195 (1993).
11. J. Pollanen, O. Sakseba, E. M. Salonen, *et al*., *J. Cell Biol*., **104**, 1085-1097 (1987).
12. C. G. Sweep, J. Geurts-Moespot, N. Grebenschikov, *et al*., *Br. J. Cancer*, **78**, 1434-1441 (1998).