Comparative Study of Plasminogen Activators in Cancers and Normal Mucosae of Human Urinary Bladder

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ABSTRACT

We have developed a highly sensitive sandwich enzyme immunoassay for determination of urokinase-type plasminogen activator (u-PA) and tissue-type plasminogen activator (t-PA) antigen levels in extracts of human tissues. We determined antigen levels of PAs in extracts of 31 primary cancers and 15 normal mucosal tissues of the urinary bladder using this method. U-PA antigen levels in extracts of bladder cancers were significantly higher than those in normal tissues (p < 0.005). U-PA antigen levels significantly increased as histological grading of malignancy advanced. There was no correlation between t-PA antigen level and malignancy. These results indicate that an increase of u-PA antigen level may be a parameter of malignant transformation and may play an important role in invasiveness of cancer.

INTRODUCTION

PAs are serine proteases which convert plasminogen into an active form, plasmin. Two different types of PAs have been recognized in mammalian tissues, t-PA and u-PA. The principal physiological role of t-PA is to maintain the vascular tree free of fibrin (reviewed in Ref. 1). PAs have been identified in malignant tumor extracts (reviewed in Ref. 2). The plasminogen activation mediated by PAs is able to degrade the glycoproteins, fibronectin, and laminin occurring in both the basement membranes and the extracellular matrix (3-5). Antibodies which inhibit human u-PA activity have been shown to interfere with the metastatic propensity of a human cell line transplanted into chick embryos (6). Therefore, it has been thought that PAs may play an important role in expression of malignant potential and metastasis. A slight to extremely high activity of PA of nondetermined type was observed in bladder cancer (7). Other reports showed that bladder cancer cell lines secreted u-PA into the culture medium (8, 9).

Studies on PAs in tumor tissues have dealt up to now exclusively with the active enzyme (10-12). Several papers have reported PA antigen contents in extracts of tumors of 31 primary cancers and 15 normal mucosal tissues of the urinary bladder using this method. U-PA antigen levels in extracts of bladder cancers were significantly higher than those in normal tissues (p < 0.005). U-PA antigen levels significantly increased as histological grading of malignancy advanced. There was no correlation between t-PA antigen level and malignancy. These results indicate that an increase of u-PA antigen level may be a parameter of malignant transformation and may play an important role in invasiveness of cancer.

MATERIALS AND METHODS

Source of Tissues. Thirty-one primary bladder cancer tissues were obtained from patients undergoing transurethral resection at Miyazaki Medical College and Miyazaki Prefectural Hospital. None of the patients had previously received irradiation or chemotherapy. Their ages ranged from 46 to 87 yr (mean ± SD, 67 ± 10.5). Eight bladder mucosal tissues apart from cancer were transurethrally obtained. Seven mucosal tissues were obtained from cases autopsied within 6 h after death. These 15 normal bladder mucosal tissues were free of malignancy and infectious disease histologically. Their ages ranged from 36 to 74 yr (mean ± SD, 64 ± 10.7).

Grading and Staging. Cancer tissues were examined histologically to evaluate the histological grading and stage according to the General Rule for Clinical and Pathological Studies on Bladder Cancer (Japanese Urological Association and The Japanese Pathological Society, 1980) (17). The grading system is composed of well-differentiated tumors (G1), less differentiated ones (G2), and poorly differentiated ones (G3). The staging system is as follows: pTa for tumors restricted to the epithelium; pT1a,b for those that have invaded the lamina propria; pT2 for those that have invaded the superficial muscle layer; and pT3 for those that have invaded the deep muscle layer or spread into the juxtavesical tissues. Stage pT1a includes tumor without stalk invasion and pT1b with stalk invasion in papillary tumors.

Extraction of PA. The PA extraction procedure was adapted from that of Markus et al. (18). Briefly human tissues were rinsed 3 times with sterile Dulbecco's phosphate-buffered saline containing 250 mg per liter of kanamycin. Then the tissue samples were weighted out and minced with scissors. The sodium-detergent buffer was added to the tissue samples, and sample-buffer dilution was 10:1. This buffer described by Camiolo et al. (19) contained 0.075 M potassium acetate, 0.3 M NaCl, 0.01 M EDTA, and 0.25% Triton X-100, pH 4.2. The cut-up samples were homogenized. The homogenates were centrifuged at 3000 × g for 20 min at 4°C. The supernatants were stored at −80°C.

Sandwich EIA Technique for u-PA and t-PA. Anti-u-PA and t-PA rabbit sera were prepared in our laboratories by using pure u-PA (159,500 IU/ml, 0.99 mg/ml) and t-PA (1,040,000 IU/ml, 2.54 mg/ml) which were provided from Mochida Pharmaceuticals, Tokyo, Japan. Monomeric anti-u-PA and t-PA Fab'-peroxidase conjugates were prepared by the maleimide method (20). Standard u-PA or t-PA was diluted 0.1% (v/v) acetate/detergent buffer in Buffer A (0.1 M sodium phosphate buffer, pH 7.2, containing 0.1 M NaCl, 0.1% bovine serum albumin, and 0.1% sodium azide). Protein determination of tissue extracts was performed with a modification of the Lowry method using bovine serum albumin as standard (21).


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1 A preliminary report on this research was presented in poster form at the 46th Japanese Cancer Congress in Tokyo, Japan, 1987.
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2 The abbreviations used are: PAs, plasminogen activators; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; EIA, enzyme immunoassay.

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performed by the method of Bradford (21), and u-PA and t-PA antigen levels in tissue extracts were expressed as ng of PAs/mg of protein.

Statistical Analysis. Student t test (unpaired) was used for comparison of PA antigen levels in normal bladder mucosae and cancer tissues. P value less than 0.05 was taken to be statistically significant.

RESULTS

Standard curves for u-PA obtained by our assay method are shown in Fig. 1. Dose response of enzyme activity bound on the solid phase was observed between 0.003 and 0.3 ng of u-PA/0.15 ml (tube) by a 60-min incubation assay. Standard curves for t-PA obtained by our assay method are shown in Fig. 2. Dose response of enzyme activity bound on the solid phase was the same as that of u-PA by a 60-min incubation assay. Intra- and interassay coefficients of variation were 4.2% (n = 5) and 7.6% (n = 8) in the u-PA assay and 5.1% (n = 4) and 8.3% (n = 7) in the t-PA assay, respectively. With the addition of 1 and 0.1 ng of u-PA or t-PA/0.15 ml to the sample, the recovery was 95 to 103%.

The means of u-PA antigen levels in extracts of bladder cancers and normal mucosal tissues were 14.3 ± 2.47 and 1.39 ± 0.22 ng/mg of protein (mean ± SE), respectively, as shown in Fig. 3. u-PA antigen levels in extracts of cancers by grading were 5.58 ± 1.63 in G1, 11.0 ± 2.42 in G2, and 24.9 ± 5.28 ng/mg of protein (mean ± SE) in G3, respectively. The u-PA level in G3 cancers was significantly higher than that in G1 or G2 (P < 0.02), but no significant difference was observed between G1 and G2 cancers, or G2 and G3 ones.

There were 6 cases in pTa, 12 in pT1a, 7 in pT1b, and 6 in pT2 and pT3 (pT2+3) stage. u-PA antigen levels in extracts of cancers by stage were 5.92 ± 1.83 in pTa, 8.30 ± 1.69 in pT1a, 18.1 ± 2.71 in pT1b, and 30.1 ± 8.36 ng/mg of protein (mean ± SE) in pT2+3, respectively, as shown in Fig. 4. The u-PA level in pT1b or pT2+3 stage was significantly higher than that in pTa or pT1a (P < 0.01). There was no significant difference between pT1a and pT1b, or pT1b and pT2+3 stage.

t-PA antigen levels in extracts of normal and cancer tissues were 44.6 ± 6.02 and 42.9 ± 5.26 ng/mg of protein (mean ± SE), t-PA levels in cancers by grade were shown in Fig. 5. There was no significant difference between t-PA level and grade of malignancy (Fig. 5) or stage of cancer (data not shown).
We measured u-PA and t-PA antigen levels in extracts of bladder cancer and normal mucosal tissues. Although Tissot et al. (22) have demonstrated the production of inhibitors directly against u-PA in extracts of breast and colon carcinomas, our EIA method could measure the total u-PA antigen composed of free u-PA and u-PA-inhibitor complexes in tissue extracts (16). But we were not able to demonstrate the presence of PA-inhibitor complexes in bladder cancer extracts with zymogram (data not shown). Evers et al. (23) and Camiolo et al. (12) have shown zymograms which contain molecular weight bands corresponding to approximately 100 x 10^3, using the same PA extraction procedure. Thus, it seems that bladder cancer tissues may lack inhibitors to PA.

Studies on PAs in urinary bladder tumors have not been developed sufficiently until now. This matter might be due to tissue distortion by a resection procedure and difficulties in obtaining sufficient amounts of bladder tissues. Our tissue samples were obtained by a nonelectrical transurethral resection to prevent tissue distortion. We were able to measure antigen levels of PAs in a small amount of tissue using the sandwich EIA technique.

Our data have shown that the u-PA antigen level is significantly higher in primary bladder cancers than in normal bladder tissues. The results of quantitative analysis of u-PA antigen content reported here differ somewhat from previous ones of u-PA level between human malignant and normal tissues. The mean values of u-PA antigen contents in human kidney, colon, and breast tumors were 2.4-, 3.8-, and 4.0-fold higher than those in normal tissues, respectively (13-15). Sappino et al. (24) have recently reported that lung carcinomas displayed a mean 6-fold increase in u-PA mRNA levels over their nonmalignant counterparts. The u-PA antigen level in bladder cancers was 10-fold higher than that in normal bladder tissues in our series. This discrepancy might be due to differences in methodology and the organ specificity of these tumors.

Since it has been established that transformed cells secreted a large amount of PA (see review by Danø et al. (2)), our results described above suggest that the increased production of u-PA is associated with the malignant potential of tissues, and that determination of u-PA antigen level of tissue extracts may be useful as a tumor marker.

Some authors reported that melanoma cell lines and some other malignant tumor cell lines secreted t-PA into the culture medium (9, 25). Our data have revealed that there is no significant difference between t-PA levels in normal and cancerous bladder tissue. Since t-PA has been found in endothelial cells of the veins and small arteries with an immunohistochemical technique (16, 26), it should be always detectable in extracts of tissues.

Gelister et al. (14) reported that there was no relation between activity of PA and tumor grade or Dukes’ stage in colorectal cancer. In our study u-PA antigen levels in high grade and advanced stage of bladder cancer were significantly higher than those in low grade and early stage. This conflicting result may be due to different periods from the appearance of cancer to diagnosis and to differences in the biological characteristics of colon and bladder cancer. Hematuria, either gross or microscopic, is the most common and earliest symptom of bladder cancer, occurring in 85% of patients. The mean duration of symptoms before diagnosis of tumor has generally ranged between 3 and 8 mo (27). Then low-grade lesions of bladder cancers appear less likely to recur rapidly or to become invasive than high-grade lesions (28). Therefore, it is thought that histological grading of bladder cancers correlates with their stage in general.

Plasmin is thought to play a crucial role in the destruction of the extracellular matrix and the activation of matrix-degrading proteases (29, 30). Quigley et al. (31) have recently demonstrated that human u-PA cleaved fibronectin under plasminogen-free conditions yielding a limited number of high-molecular-weight cleavage products. Therefore, the production of u-PA in high grade and advanced stage might be an important factor contributing to the invasive quality of cancers.

Further work is being done to find a relationship between u-PA and prognosis of cancer.

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