# ONCOLOGY

# Tissue- and Urokinase-Type Plasminogen Activators and Type 1 Plasminogen Activator Inhibitor in Melanomas and Benign Skin Pigment Neoplasms

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The content of urokinase- and tissue-type plasminogen activators and plasminogen activator inhibitor PAI-1 in the cytosol of primary and metastatic melanomas and benign skin pigment neoplasms was estimated by enzyme immunoassay. It was shown that local growth and invasion of melanomas are related to suppressed expression of tissue plasminogen activator. The content of urokinase plasminogen activator increases in patients with distant metastases and large thickness of the primary tumor.

**Key Words:** *urokinase plasminogen activator; tissue plasminogen activator; plasminogen activator inhibitor-1; melanoma; benign skin pigment neoplasms*

Skin melanoma is malignant human tumor, which often metastasizes. Melanoma results from neoplastic transformation of melanoblasts, melanocytes, and nevus cells and is the first local manifestation of the general disease of human melanocytic system. Evaluation of the invasive and metastatic potentials of melanoma, particularly at the early stage of tumor development, and the search for specific signs characterizing the risk for malignant transformation of benign pigment neoplasms (BPN) are important for the choice of therapy.

Recent studies showed that proteolytic cascade of plasminogen activation, which leads to the formation of plasmin, and a key agent of these reactions urokinase plasminogen activator (uPA) play an important role in metastatic growth and invasion of various malignant tumors [1,9,13]. uPA activity is regulated by various mechanisms, *e.g.*, this enzyme is inhibited by two protein serpin inhibitors PAI-1 and PAI-2 [3].

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*In vitro* experiments showed that plasmin formation on the cell surface induced by uPA is the major biochemical event, which promotes invasion of melanoma cells [11,12]. Expression of uPA and PAI-1 in human melanoma cells correlates with their ability to metastasize into the lungs after transplantation to athymic nude mice [14]. As differentiated from the majority of malignant tumors, melanoma cells are characterized by intensive expression and secretion of tissue plasminogen activator (tPA) [4,5,7]. tPA is usually produced by endothelial cells and possesses protective functions during various tumors. It remains unclear whether tPA plays a particular role during melanoma.

The levels and the ratio between various components of the plasminogen activation system in tumor tissues reflect their metastatic and invasive potentials and, therefore, can be used as prognostic criteria [1,2, 9]. There are ambiguous data on the expression of various components of the plasminogen activation system in human melanoma tissues [6,10].

Here we measured the content of uPA, tPA, and PAI-1 in primary and metastatic melanomas and skin

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**TABLE 1.** Concentrations of tPA, uPA, and PAI-1 (ng/mg Protein) in Cytosol of Skin Pigment Neoplasms

| Parameter | | Pigment nevus (*n*=19) | Melanoma | |
|---|---|---|---|---|
| | | | primary (*n*=24) | metastasis (*n*=20) |
| uPA | extreme values (median) | 0.10-2.35 (0.26) | 0.00-1.31 (0.23) | 0.00-1.35 (0.33) |
| | *M*±*m* | 0.49±0.14 | 0.34±0.07 | 0.43±0.09 |
| tPA | extreme values (median) | 0.00-0.37 (0.61) | 0.00-0.33 (0.00) | 0.00-0.84 (0.01) |
| | *M*±*m* | 1.17±0.32 | 0.50±0.27 | 0.51±0.22 |
| PAI-1 | extreme values (median) | 0.26-9.51 (1.29) | 0.00-5.72 (2.45) | 0.46-6.60 (2.70) |
| | *M*±*m* | 1.93±0.52 | 2.33±0.36 | 2.79±0.40 |

BPN and evaluated the relationship between their expression in malignant melanomas and clinical and morphological characteristics of the tumor process.

## MATERIALS AND METHODS

We examined 24 patients (11 men and 13 women) aging 22-85 years (average 53.4±3.1 years) with primary skin melanomas, 20 patients with metastatic melanomas, and 19 patients with skin BPN, including pigment nevi (*n*=11), seborrheic keratosis (*n*=5), and papillomas (*n*=3). In all patients, clinical diagnosis was confirmed by histological examination.

Tumor fragments (200-500 mg) were taken from each patient and stored at -70°C. We measured uPA, tPA, and PAI-1 concentrations in cytosols, which were prepared routinely [2] 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 obtained at the Catholic University Nijmegen (Netherlands) [2,8]. Quantitative analyses were performed on an ELX800 universal microplate reader (Bio-Tek Instruments) at 490/630 nm. Protein concentrations were expressed in ng/mg cytosol protein. Protein content was measured by the method of Lowry.

The results were analyzed by Student's *t* test and nonparametric median test. Correlation analyses were performed using Pearson (*r*) and Spearman (*R*) tests.

**TABLE 2.** Concentrations of tPA, uPA, and PAI-1 (ng/mg Protein) in Cytosol of Primary Melanomas from Male and Female Patients

| Parameter | Women (*n*=13) | Men (*n*=11) |
|---|---|---|
| uPA | | |
| extreme values (median) | 0.10-1.31 (0.28) | 0.03-0.55 (0.18) |
| *M*±*m* | 0.41±0.12 | 0.25±0.05 |
| tPA | | |
| extreme values (median) | 0.00-1.30 (0.00) | 0.00-6.33 (0.00) |
| *M*±*m* | 0.34±0.14 | 0.69±0.57 |
| PAI-1 | | |
| extreme values (median) | 0.00-5.72 (2.78) | 0.00-3.49 (0.98) |
| *M*±*m* | 3.19±0.49 | 1.32±0.34* |

**Note**. *$p$<0.01 compared to women.

## RESULTS

tPA was detected in 9 of 24 primary melanomas (37.5%), 17 of 19 skin BPN (89%), and 10 of 20 melanoma metastases (50%). uPA was found in 92, 100, and 95% tumors, respectively. PAI-1 was revealed in 95% BPN, 92% primary melanomas, and 100% metastatic melanomas. Thus, only benign and malignant

**TABLE 3.** Concentrations of tPA, uPA, and PAI-1 (ng/mg Protein) in Cytosol of Primary Melanomas Depending on Tumor Stage

| Parameter | | Stages I-II (*n*=10) | Stages III-IV (*n*-14) | Melanoma metastasis (*n*=20) |
|---|---|---|---|---|
| UPA | extreme values (median) | 0.00-0.49 (0.09) | 0.13-1.31 (0.28) | 0.00-1.35 (0.33) |
| | *M*±*m* | 0.15±0.05 | 0.47±0.11 | 0.43±0.09 |
| TPA | extreme values (median) | 0.00-6.33 (0.11) | 0.00-1.30 (0.00) | 0.00-3.84 (0.01) |
| | *M*±*m* | 0.99±0.62 | 0.19±0.10 | 0.51±0.22 |
| PAI-1 | extreme values (median) | 0.00-3.70 (1.23) | 0.40-5.72 (2.53) | 0.46-6.60 (2.70) |
| | *M*±*m* | 1.63±0.44 | 2.84±0.54 | 2.79±0.40 |

pigment neoplasms significantly differed in the incidence of tPA.

tPA content in BPN was much higher than in primary and metastatic melanomas (median test, Table 1). Mean and median concentrations of uPA and PAI-1 did not differ between various tumors. Nonparametric Spearman test showed that PAI-1 concentration in metastatic melanomas was higher than in BPN ($R$=0.25, $p$<0.05).

A positive correlation was found between uPA and PAI-1 contents in primary melanomas ($r$=0.73, $p$<0.001) and skin BPN ($r$=0.97, $p$<0.001). In patients with BPN we revealed a positive correlation between plasminogen activator concentrations ($r$=0.88, $p$<0.001) and tPA and PAI-1 concentrations ($r$=0.86, $p$<0.001). These correlations were not found in patients with primary melanomas. Correlations between the studied parameters were not revealed in patients with metastatic melanomas.

Thus, correlations between all studied parameters were found in patients with BPN, which probably maintains an equilibrium between oppositely directed proteolytic processes and prevents cell invasion. The correlation between uPA and tPA concentrations in patients with primary melanomas probably contributes to suppression of local melanoma growth. However, there are no correlations between the expression of plasminogen activators and their inhibitor in patients with metastatic melanomas. It should be emphasized that as differentiated from malignant tumors [1], uPA expression in primary and metastatic melanomas does not surpass that in benign neoplasms. Changes in the invasive potential are most likely associated with the decrease in tPA content and relative increase in PAI-1 concentration. According to current notions about the role of proteins, these changes protect tumor cells from self-destruction.

Clinical signs of skin melanomas differed between men and women. The mean concentration of PAI-1 in primary melanomas in women 2.4-fold surpassed that in men ($p$<0.01, Table 2). The mean content of uPA in primary melanomas in women was 1.6 times higher than in men (insignificant). Moreover, tumor tPA concentration in women was 2-fold lower than in men (insignificant).

The mean uPA concentration at the early stages of skin melanomas (I-II) was 3-fold lower than that in stages III-IV tumors ($p$<0.05). tPA concentration tended to increase at the early stage of tumor growth (Table 3). During tumor invasion, the tPA/uPA ratio decreased from 10.04 to 0.58 ($p$<0.01). A positive correlation was found between uPA concentration in primary melanoma and the stage of tumor growth ($R$=0.45, $p$=0.0196). uPA and PAI-1 contents in melanoma metastases were similar to those in primary invasive melanomas. However, tPA content and tPA/uPA ratio

**TABLE 4.** Concentrations of tPA, uPA, and PAI-1 (ng/mg Protein) in Cytosol of Primary Melanomas Depending on Primary Tumor Size

| Parameter | Diameter, cm | | | | Thickness, mm | | |
|---|---|---|---|---|---|---|---|
| | <1.4 ($n$=6) | 1.4-2.0 ($n$=8) | 2.0-3.5 ($n$=6) | >3.5 ($n$=4) | <3 ($n$=9) | 3-5 ($n$=2) | >5 ($n$=13) |
| uPA | | | | | | | |
| extreme values (median) | 0.03-0.31 (0.13) | 0.00-0.13 (0.27) | 0.07-0.76 (0.34) | 0.17-0.51 (0.33) | 0.00-0.47 (0.13) | 0.16-0.55 (0.35) | 0.00-0.31 (0.28) |
| $M\pm m$ | 0.36±0.20 | 0.30±0.13 | 0.37±0.11 | 0.33±0.08 | 0.17±0.05 | 0.35±0.19 | 0.45±0.11 |
| tPA | | | | | | | |
| extreme values (median) | 0.00-0.30 (0.63) | 0.00-0.33 (0.27) | 0.00-0.00 | 0.00-0.00 | 0.00-6.33 (0.81) | 0.00-0.45 (0.23) | 0.00-0.38 (0.00) |
| $M\pm m$ | 0.62±0.23 | 1.04±0.77 | — | — | 1.18±0.67 | 0.23±0.23 | 0.07±0.04 |
| PAI-1 | | | | | | | |
| extreme values (median) | 0.00-5.57 (0.83) | 1.34-5.72 (3.13) | 0.40-5.00 (1.11) | 0.67-2.66 (2.13) | 0.00-3.78 (1.34) | 0.53-2.53 (1.53) | 0.40-5.72 (2.62) |
| $M\pm m$ | 1.83±0.94 | 3.35±0.50 | 1.76±0.72 | 1.90±0.47 | 1.88±0.51 | 1.53±1.00 | 2.76±0.54 |

**TABLE 5.** Concentrations of tPA, uPA, and PAI-1 (ng/mg Protein) in Cytosol of Primary Melanomas Depending on Tumor Prominence above Undamaged Skin, Stage of Invasion, and Presence of Distant Metastases

| Parameter | Prominence, mm | | Stage of invasion | | | Metastases | |
|---|---|---|---|---|---|---|---|
| | below 5.42 ($n$=14) | above 5.42 ($n$=5) | III ($n$=13) | IV ($n$=8) | V ($n$=2) | without ($n$=15) | with ($n$=9) |
| uPA | | | | | | | |
| extreme values (median) | 0.00-1.31 (0.18) | 0.14-0.41 (0.21) | 0.00-0.31 (0.20) | 0.07-1.13 (0.19) | 0.25-0.76 (0.50) | 0.00-0.55 (0.21) | 0.14-1.31 (0.25) |
| $M\pm m$ | 0.35±0.11 | 0.24±0.04 | 0.29±0.10 | 0.35±0.13 | 0.50±0.25 | 0.23±0.05 | 0.51±0.15* |
| tPA | | | | | | | |
| extreme values (median) | 0.00-6.33 (0.00) | 0.00-0.32 (0.00) | 0.00-6.33 (0.22) | 0.00-0.38 (0.00) | 0.00-0.00 | 0.00-1.17 (0.00) | 0.00-6.33 (0.00) |
| $M\pm m$ | 0.69±0.45 | 0.06±0.06 | 0.87±0.48 | 0.09±0.06 | — | 0.24±0.11 | 0.94±0.69 |
| PAI-1 | | | | | | | |
| extreme values (median) | 0.00-5.72 (2.54) | 0.40-3.70 (1.34) | 0.00-5.57 (2.62) | 0.40-5.72 (1.75) | 1.66-5.00 (3.33) | 0.00-4.82 (2.37) | 0.40-5.72 (3.49) |
| $M\pm m$ | 2.5±0.5 | 2.08±0.57 | 2.49±0.50 | 2.06±0.61 | 3.33±1.67 | 1.94±0.35 | 2.98±0.74 |

**Note**. *$p$<0.05 compared to patients without metastases.

were intermediate and did not differ from those in primary tumors (independently on the stage).

The diameter of primary melanomas was 0.5-4.5 cm (mean 2.30±0.25 cm, median 2 cm). tPA was not found in large tumors (2.0-3.5 cm or larger, Table 4). The mean content of tPA did not differ between patients with metastatic melanomas and BPN. In patients with primary melanomas, a negative correlation was found between tPA concentration and tumor diameter ($R$=-0.58, $p$<0.01). We revealed no correlations between tumor diameter and uPA and PAI-1 concentrations in the cytosol.

Histological examination showed that the mean tumor thickness was 8.27±1.63 mm (1-30 mm). The mean concentrations of tPA, uPA, and PAI-1 did not differ between patients with various thickness of primary melanomas. However, uPA concentration tended to increase, and tPA content slightly decreased with an increasing the thickness of melanomas (Table 4). Spearman rank correlation test revealed significant changes in these parameters ($R$=0.42 and $p$<0.05 for uPA; $R$=-0.58 and $p$<0.01 for tPA). The tPA/uPA ratio decreased from 10.4 in 3-mm melanomas to 0.16 in 5-mm melanomas ($R$=-0.66, $p$<0.001).

Thus, tissue tPA concentration decreases with an increase in the diameter and thickness of melanomas. The increase in tumor thickness also correlates with the rise in uPA concentration. Our findings confirm the hypothesis that the decrease in tPA concentration in melanomas is the major factor promoting invasion and metastasizing of primary tumors.

Primary melanomas are elevated over undamaged skin. In all patients melanomas were elevated over the skin by 1-20 mm (mean 5.42±1.23 mm). All studied parameters were higher in patients with tumor prominence of less than 5.42 mm (insignificant, Table 5). No correlation was found between tumor prominence and tPA, uPA, and PAI-1 concentrations.

There are 5 stages of melanoma invasion: invasion of tumor cells into the epidermis, reticular layer, and papillary and reticular dermal layers, growth in the reticular dermal layer, and invasion into subcutaneous fat. Mean and median values of studied parameters did not depend on the stage of primary melanoma invasion (Table 5). Nonparametric Spearman rank test showed that tPA content decreased with an increase in the degree of tumor cell invasion ($R$=-0.42, $p$<0.05). tPA was not detected in 2 melanomas invading into subcutaneous fat.

Metastases were found in 9 patients. The mean uPA content in primary melanomas in patients with distant metastases was much higher than in patients without metastases (Table 5). In patients with distant metastases tPA and PAI-1 concentrations in primary melanomas were insignificantly higher than in patients without metastases.

Our study of the relationship between expression of various components of the plasminogen activation system and clinical and morphological characteristics of primary melanomas and comparative analysis of benign and malignant skin pigment neoplasms indicate that local growth and invasion of these tumors are associated with decreased tPA expression, which promotes lysis of tumor cells. tPA is not found in large tumors growing into the deep dermal layer. Expression of uPA and, particularly, PAI-1 insignificantly correlates with the development of primary melanomas. However, uPA content considerably increases in patients with distant metastases and large thickness of the primary tumors.

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