Urokinase and Tissue Plasminogen Activators and Their PAI-1 Inhibitor in Tumors of Patients with Oral Mucosal Cancer: Relationship with the Main Clinical Morphological Factors

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Enhanced activation of plasminogen by the urokinase pathway (uPA elevation) in patients with cancer of the oral mucosa paralleled by an increase of PAI-1 level in the tumor compared to the adjacent mucosa was shown by enzyme immunoassay. No statistically significant associations of the level of the studied proteins in the tumor with such prognostic factors as location, growth form, histological structure, differentiation degree, size, and dissemination of the primary tumor, involvement of the regional lymph nodes, and stage of the disease were detected.

Key Words: *urokinase plasminogen activator; tissue plasminogen activator; plasminogen activator inhibitor-1; cancer of the oral mucosa*

Oral and oropharyngeal squamous-cell carcinoma is one of the most prevalent malignant head and neck tumors, which ranks second by incidence after laryngeal cancer. Though the oral cavity is easily accessible for visual examination, more than two-thirds of patients apply for specialized care with stages III or IV of the tumor process, and subsequent therapy is therefore extremely difficult and often ineffective. Many scientists think that treatment efficiency can be improved by not only rational use of combined methods, but also development of principally new pathogenetic therapeutic methods based on modern progress in biochemistry and molecular biology of tumors.

Invasion into the adjacent tissues and metastasizing into distant organs are, no doubt, the basic properties of malignant tumors; one of the main mechanisms underlying these processes is destruction of the basal membrane and extracellular matrix by tumor-associated proteases, involved also in neoangiogenesis, which promotes the growth of a new vessels. Several protease classes are involved in invasion and metastasizing. These are primarily the proteolytic cascade of plasminogen activation with plasmin formation and subsequent activation of matrix metalloproteinases destroying collagen and other components of the tumor stroma. Plasminogen activation system includes two types of activators : urokinase (uPA) and tissue (tPA). Their activity is suppressed by two protein inhibitors: PAI-1 and PAI-2 [5]. uPA is secreted in the form of inactive single-chain precursor, which, after binding to specific receptors (uPAR) on the cell surface is transformed (under the effect of plasmin and some other proteolytic enzymes) into active two-chain molecule. Active uPA, in turn, catalyzes plasminogen transformation into plasmin. Hence, the entire plasmin formation process can be presented as cyclic amplification regulated by the feedback mechanism.

The level and proportion of the expression of various components of the plasminogen activation system in tumor tissue can serve as markers of metastatic and

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invasive activities of the tumor, and hence, they are biologically significant prognostic factors in various tumors [2,6]. Measurements of uPA and PAI-1 are now used in some countries as obligatory tests for detection of prognostically unfavorable subgroups among patients with early stages of breast cancer [7]. Moreover, inhibition of urokinase activation of plasminogen is one of perspective approaches to the development of new protocols of antimetastatic therapy of various tumors, including cancer of the oral mucosa [10,14,15].

On the whole, the role of plasminogen activation system in tumors of the oral mucosa is little studied, though some clinical laboratory studies demonstrated unfavorable role of uPA and PAI-1 and of uPAR for the results of therapy for various forms of this disease [3,8,9,11-13].

We studied the content of uPA, tPA, and PAI-1 in tumor tissue and intact oral mucosa and evaluated the relationship between the levels of these proteins in the tumors and clinical and morphological peculiarities of the disease.

MATERIALS AND METHODS

The study was carried out in patients with cancer of the oral mucosa treated at N. N. Blokhin Cancer Research Center in 2007-2009. Forty patients (30 men and 10 women) aged 32-84 years (median 54 years) were examined. Cancer of the mobile part of the tongue was diagnosed in 12 patients, of the root of the tongue in 5; cancer of the bottom of the oral cavity was detected in 7 patients, of the alveolar process and retromolar region in 6 and 5 patients, respectively. Cancers of other locations were solitary. Disseminated stages (III and IV) were diagnosed in 32 (80%) patients. By the histological structure, the majority of tumors (32 cases) were squamous-cell nonkeratinizing cancer.

The tumors and histologically intact oral mucosa were analyzed in all patients. Material for analysis was collected during surgery. Tissue fragments (100-200 mg) were delivered in the ice from the operation room to laboratory and stored at -70°C until the study.

The concentrations of uPA, PAI-1, and tPA in the cytosols, isolated as described previously [1], were measured by direct enzyme immunoassays with standard kits (Technoclone GmbH) according to manufacturer's instruction. The concentrations of analyzed proteins were expressed in ng/mg cytosol protein. Protein was measured by the method of Lowry.

The values were compared using nonparametric Mann–Whitney, Kruskal–Wallis, and Wilcoxon tests. The relationships between the parameters were evaluated by Spearman ranked correlation test (R). The differences and correlations were considered significant at $p<0.05$. The data were statistically processed using Statistica 7.0 software.

RESULTS

Measurable levels of uPA and PAI-1 were detected in 90% tumors; uPA was detected in only 3 (7%) specimens of histologically intact oral mucosa, PAI-1 in 20 (50%). The concentrations of uPA and PAI-1 in tumor tissue were significantly higher than in mucosa samples in 93 and 85% patients, respectively ($p<0.0001$). The concentrations of these proteins in tumor cytosols varied (Table 1). Measurable, though very low levels of tPA were detected in all analyzed specimens of tumor and intact tissue (Table 1). The content of this protease in intact mucosa was higher than in the tumor in 70% patients, the medians being virtually the same.

A positive correlation between tPA concentrations in tumor tissue and intact mucosa was detected ($R=0.40$; $p<0.01$). The concentration of PAI-1 in tumor tissue did not correlate with that in normal oral mucosa; virtually no uPA was detected in intact mucosa. On the other hand, a high positive relationship between uPA and PAI-1 levels in tumor tissue was detected ($R=0.84$, $p<0.001$). These regularities indicate that the increase in uPA and PAI-1 expression in tu-

TABLE 1. Content (ng/mg Protein) of Plasminogen Activation System Components in the Cytosols of Tumors and Adjacent Histologically Intact Tissues of Patients with Cancer of the Oral Mucosa

Parameter	Tumor (T)		Mucosa (N)		T>N, %
	range of values	median (25-75%)	range of values	median (25-75%)	
uPA	0-14.6	1.6*** (0.88-2.6)	0-1.4	0 (0-0)	93
PAI-1	0-113.8	18.6*** (4.2-50.8)	0-34.7	0.05 (0-1.2)	85
tPA	0.14-5.0	0.30 (0.23-0.41)	0.13-1.8	0.35 (0.28-0.45)	30

Note. ***$p<0.0001$ compared to intact mucosa (paired Wilcoxon test).

mor tissue does not depend on their initial level in the oral mucosa, while the expression of tPA in the tumor directly depends on the basal content of this enzyme in patient's mucosa.

Hence, a significant coordinated increase of uPA and PAI-1 concentrations in oral mucosal cancer tissue in comparison with the adjacent mucosa was observed. The level of tPA in malignant tumors was usually significantly below the normal. These results are in line with published data on tumors of the oral cavity [4,8], also obtained by enzyme immunoassays, and with our data on tumors of different histogenesis [2].

In order to evaluate clinical significance of measurements of these markers in the tumors of patients with cancer of the oral mucosa, we analyzed the relationship between these parameters and the main clinical morphological characteristics of the disease (Table 2). No clear-cut statistically significant associations between the levels of the studied proteins in the tumors and such prognostic factors as primary tumor location, growth type, histological structure, differentiation degree, size, and dissemination (T), degree of the regional lymph node involvement (N) were detected. We can speak about just some trends, which can acquire not only statistical, but also biological significance in studies on larger groups of patients. The levels of uPA and PAI-1 were the highest in stage II process, the difference in comparison with stage III being statistically significant for uPA ($p<0.05$). These data are in good agreement with our results obtained in studies of some other tumors (breast cancer, lung cancer, ovarian cancer) and indicate the highest invasive and metastatic potential of the tumors (judging by the levels of the analyzed components of plasminogen activation system) at the early stages of invasion and metastasizing [1]. In ad-

TABLE 2. Relationship between the Content of Plasminogen Activation System Components in the Cytosols of Tumors and Adjacent Histologically Intact Tissues in Patients with Cancer of the Oral Mucosa and the Main Clinical Morphological Characteristics

Sign	Grade	N	Concentration, ng/mg protein (median, range)		
			uPA	PAI-1	tPA
Stage	I	2	1.4 (0-2.9)	30.0 (0-59.9)	0.18 (0.14-0.22)
	II	5	3.2* (0.8-14.6)	36.0 (0-63.4)	0.34 (0.23-0.43)
	III	10	1.0 (0-7.2)	7.6 (0-79.1)	0.26 (0.19-1.25)
	IV	22	1.7 (0-11.5)	18.0 (0-113.8)	0.32 (0.14-0.91)
T	T_1	2	1.4 (0-2.9)	30.0 (0-59.9)	0.18 (0.14-0.22)
	T_2	9	2.6 (0-14.6)	10.6 (0-63.4)	0.33 (0.23-0.43)
	T_3	13	1.6 (0-7.2)	23.4 (0-98.3)	0.26 (0.14-1.25)
	T_4	15	1.7 (0.3-11.5)	18.6 (0-113.8)	0.31 (0.16-0.91)
N	N_0	17	1.6 (0-14.6)	23.5 (0-79.1)	0.30 (0.14-1.25)
	N_1	9	1.5 (0-2.6)	10.6 (0-108.4)	0.26 (0.16-0.64)
	N_2	14	1.8 (0-11.5)	24.8 (0-113.8)	0.32 (0.14-0.91)
Growth form	Exophytic	4	4.2 (0.3-8.4)	35.0 (27.4-79.1)	0.21 (0.16-0.43)
	Endophytic	7	1.8 (0-3.6)	14.0 (0-98.3)	0.25 (0.14-0.62)
	Mixed	28	1.5 (0-14.6)	14.0 (0-113.8)	0.32 (0.14-1.25)
Morphology (squamous-cell cancer)	Keratinizing	32	1.5 (0-14.6)	18.0 (0-108.4)	0.28 (0.14-1.25)
	Nonkeratinizing	7	1.8 (0-11.5)	23.4 (0-113.8)	0.33 (0.22-0.91)
Differentiation	High	30	1.6 (0-11.5)	18.0 (0-113.8)	0.30 (0.14-1.25)
	Moderate	9	1.6 (0.3-14.6)	23.4 (0.3-63.4)	0.29 (0.2-0.46)
Continuing tumor growth	Present	13	1.4 (0-11.5)	18.6 (0-113.8)	0.31 (0.14-1.25)
	None	14	1.7 (0-14.6)	9.0 (0-79.1)	0.25 (0.16-0.84)

Note. *$p<0.05$ (Mann-Whitney test) compared to stage III.

dition, uPA and PAI-1 medians in exophytic growth of cancer of the oral mucosa proved to be more than 2-fold higher than in endophytic growth; PAI-1 level was also 2-fold increased in cases with continuing tumor growth ($p>0.05$ in all cases).

Hence, previously detected regularity [1,2] according to which a significant and coordinated increase of the uPA and PAI-1 expression occurs in the majority of malignant tumors of different histogenesis in comparison with the homologous normal tissue was confirmed for cancer of the oral mucosa. This observation can be important from both theoretical and practical points of view. On the one hand, it suggests that enhanced activation of plasminogen by the urokinase type paralleled by strengthening of tumor cell defense from self-destruction (due to increase of PAI-1 level) is a universal sign of malignant degeneration. On the other hand, intensive expression of uPA in the tumor in comparison with the adjacent tissue makes this enzyme a prospective selective target for antimetastatic therapy for cancer of the oral mucosa characterized by poor sensitivity to standard chemotherapy. The absence of clear-cut relationship between uPA and PAI-1 concentrations and the main clinical morphological characteristics of the disease does not preclude its potential role as an independent prognostic factor for relapse-free and overall survival. This can be proven in longer observation of the patients on a larger group.

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