ONCOLOGY

Analysis of NM23 Protein and Components of Plasminogen Activation System in Tumors of Patients with Stomach Cancer with Consideration for Disease Clinical Picture and Morphology

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Immunohistochemical analysis of the expression and distribution of nm23 protein and biochemical analysis of the main components of plasminogen activation system in tumors were carried out in stomach cancer patients. The data indicate that the expression of nm23 protein in malignant epithelial gastric tumors is heterogeneous, characterized by cytoplasmic and nuclear immunoreactivity. Reduced expression of the marker is more typical of poorly or undifferentiated tumors. The expression of nm23 protein positively correlated with tPA level (r_s=0.4; p=0.01) and did not correlate with the content of uPA and PAI-1 in tumors. High PAI-1 values in tumors (>0.5 ng/mg protein) significantly correlated with lower 3-year overall survival of stomach cancer patients. These data confirm the role of the studied proteins in invasion and metastases of malignant tumors and suggest a relationship between changes in the expression of nm23 protein and mechanisms of stomach cancer progress.

Key Words: *stomach cancer; nm23 protein; plasminogen activation system; metastases; prognosis*

The course of stomach cancer (SC) is determined by many clinical and morphological factors closely related to abnormal expression and functioning of molecular biological markers in the tumor, which characterize the aggressiveness of tumor process. The development of accessory (immunohistochemical and biochemical) methods for measurements of these factors in the tumor is in progress now. They are intended to serve as prognostic tests in clinical oncology and to be used for evaluation of tumor sensitivity to therapy.

The markers determining the invasive characteristics and metastatic potential of tumor cells [4,5, 7,11] constitute a special group. For example, the interaction during tumor progress between nm23 metastasis suppressor protein and various components of plasminogen activation system, which serve as indicators of invasive activity of the tumor, is an interesting problem, heretofore little studied for stomach cancer.

Non-metastatic cells protein (nm23) or nucleoside diphosphate kinase protein (NDP) is involved

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in cell proliferation and differentiation processes and metastasis regulation [4,5,7]. Abnormal expression of nm23 in malignant tumors of the stomach is associated with an unfavorable prognosis [9-11].

Urokinase and tissue plasminogen activators (uPA and tPA serine proteases) are the key components of the proteolytic cascade [1,2]. Protease activities are suppressed by the serpin peptidase inhibitor (PAI-1). The concentrations of uPA and PAI-1 are increased significantly in malignant tumors, while the content of tPA is low in comparison with normal tissues [1,3,6,8].

We studied the relationship between the expression of nm23 protein and content of plasminogen activation system components in tumors of SC patients with consideration for the main clinical morphological characteristics and disease prognosis.

MATERIALS AND METHODS

The postoperative material from 108 patients with stages I-IV SC (72 male and 36 female) aged 36-75 years was studied.

Histological study of gastric tumors showed adenocarcinomas of different differentiation degree in 80-74.1% and signet ring cell carcinomas in 28-25.9% cases.

Immunohistochemical study of nm23 protein expression was carried out by the biotin-streptavidine immunoperoxidase method on serial paraffin sections with monoclonal antibodies to nm23 protein (Dako; Clone 36B5; working dilution of antibodies 1:50). The reaction was visualized by DAB$^+$ diaminobenzidine (Dako). Cell nuclei were poststained with Mayer hematoxylin. Protein expression was evaluated by the semiquantitative method with consideration for staining intensity and number of antigen-positive cells (negative expression: no reaction; low expression: less than 25% stained cells; high expression: more than 25% cells with medium and high staining intensity).

Urokinase (uPA) and tissue (tPA) plasminogen activators and their inhibitor (PAI-1) were assayed by ELISA. The concentrations of the markers were measured in the cytosol fraction of stomach cancer specimens using kits created at laboratory headed by Prof. T. Bernraad (Neumegen, the Netherlands).

The data were statistically processed using Statistica 6.0 software.

RESULTS

Immunohistochemical study showed accumulation of nm23 protein in tumor cell nuclei and cytoplasm. Protein expression presenting as diffuse homogenous staining of the cell cytoplasm was detected in tumors of 68 patients with SC (63%) (Fig. 1, *a*). High expression of nm23 protein in cell cytoplasm was detected in 25.9% (28 of 108) tumors. The ex-

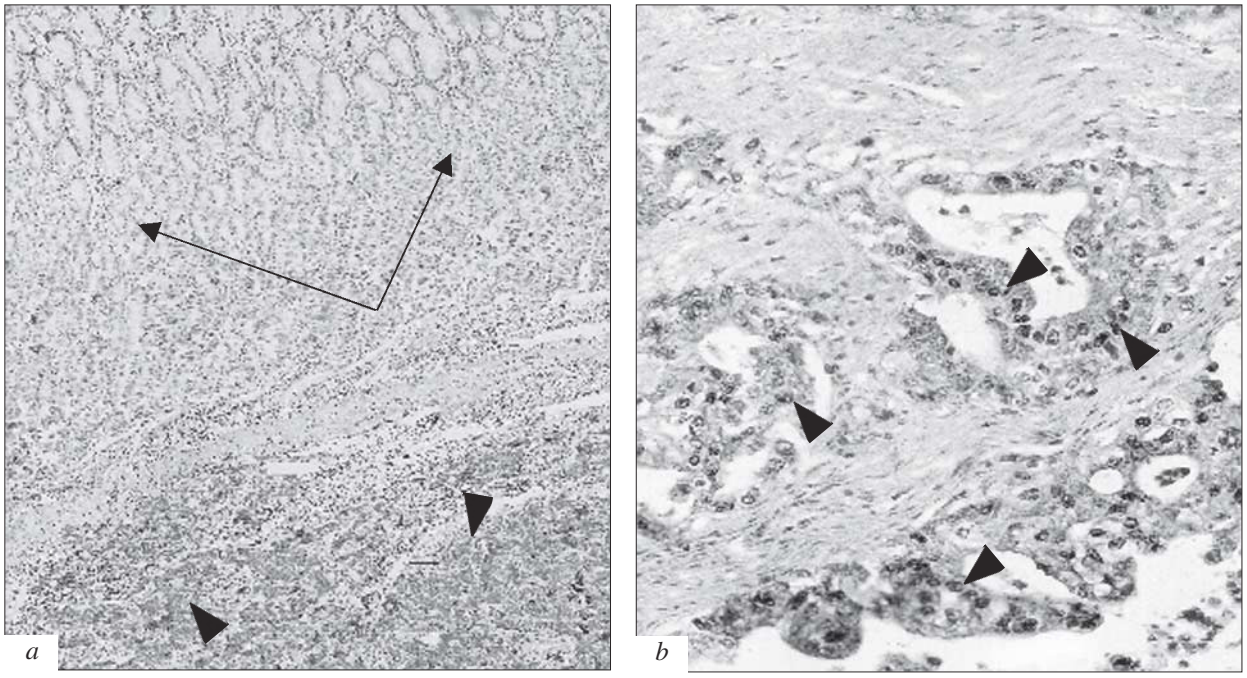

Fig. 1. Expression of nm23 protein in tumors of SC patients. *a*) specific reaction in poorly differentiated adenocarcinoma cells (wide arrows) and in the gastric mucosal epithelium (thin arrows), ×150; *b*) intense specific staining of the majority of cell nuclei (arrows) in a moderately differentiated adenocarcinoma of the stomach, ×400.

pression of the marker was low in the majority of tumors (40 of 108; 37.04%) or zero in the cytoplasm (12 of 108 cases; 11.11%). Staining of the tumor cell nuclei with antibodies to nm23 protein was detected in the tumors of 24 (22.2%) SC patients (Fig. 1, *b*). The epithelium adjacent to the tumor demonstrated slight cytoplasmic staining.

No relationship between the expression of nm23 protein in tumor cell cytoplasm and nuclei and size of primary tumors (T parameter of the TNM system) and status of the local lymph nodes (N parameter of TNM system) was revealed (p=0.14 and p=0.6, respectively). The expression of the marker increased with increasing the depth of tumor invasion into the gastric wall, though the differences did not reach the level of statistical significance.

The expression of nm23 protein in SC cells depended on the histological structure and differentiation degree of the tumor (p=0.06). A trend to lower expression of the protein or complete absence of cytoplasmic and nuclear reaction was observed for poorly and undifferentiated tumors. For example, 50% tumors with histological structure of signet ring cell carcinoma demonstrated negative expression. High cytoplasmic expression of the marker was significantly more incident in adenocarcinoma cells (30%) than in signet ring cell carcinoma (16.7%). No nuclear expression of the protein was detected in tumors with histological structure of signet ring cell carcinoma, while in adenocarcinomas it was detected in 30% cases (p=0.03).

The content of uPA and tPA in the cytosolic fraction of tumors varied within a wide range (from 0.01 to 1.34 and from 0.01 to 2.36 ng/ml protein, respectively). The content of uPA increased in advanced stages of cancer, while tPA values were virtually the same.

No relationship between changes in uPA and tPA values in the tumor and levels of nm23 protein expression in cell nuclei and cytoplasm was detected (p=0.27 and p=0.33, respectively). On the other hand, the medians for these parameters increased with increasing the level of cytoplasmic immunoreactivity (Table 1). The uPA median reached the maximum level (0.23 ng/mg protein) in SC patients with high level of nm23 protein expression in tumor cell cytoplasm.

Analysis of correlations between the levels of the studied components of the plasminogen activation system in tumor cytosols of SC patients showed their close relationship. A strong correlation between uPA and PAI-1 levels in the tumor was detected (r=0.74; p<0.01). The relationship between the expression of tPA in tumor tissue and the levels of uPA (r=0.36; p<0.01) and PAI-1 (r=0.33; p<0.01) was less pronounced. No correlation between uPA and nm23 levels in the tumor was detected for the entire group of SC patients and for groups distinguished with consideration for the main clinical morphological characteristics of the disease. On the other hand, a significant correlation between tPA values and nm23 protein expression in tumor cell cytoplasm of SC patients was detected for the total group of SC patients (r=0.34; p=0.01; Fig. 2).

Elevated contents of PAI-1 in tumors of SC patients were observed during advanced stages of the disease. The increase in nm23 protein expression in tumor cell cytoplasm and nuclei was paralleled by reduction of PAI-1 production by these

TABLE 1. Content of uPA in the Tumor and Expression of nm23 Protein in SC Cell Cytoplasm ($M\pm m$)

Level of nm23 protein expression in cell cytoplasm	Number of patients	uPA, ng/mg protein	uPA range	uPA median
None	40	0.26±0.05	0.07-0.81	0.17
Low	40	0.33±0.10	0.01-1.34	0.11
High	28	0.32±0.09	0.05-1.06	0.23

TABLE 2. Content of PAI-1 in the Tumor with Consideration for nm23 Protein Expression in SC Cell Cytoplasm ($M\pm m$)

Level of nm23 protein expression in cell cytoplasm	Number of patients	PAI-1, ng/mg protein	PAI-1 range	PAI-1 median
None	40	0.51±0.07	0.12-1.19	0.45
Low	40	1.36±0.65	0.00-9.76	0.29
High	28	0.56±0.12	0.15-1.24	0.32

cells (p=0.9 and p=0.8, respectively; Table 2). Nuclear expression of nm23 protein did not depend on PAI-1 median in the total group of patients.

Analysis of overall survival of SC patients with consideration for the above characteristics showed that high level of PAI-1 in the tumor was an unfavorable prognostic factor correlating with low survival (Fig. 3). The 3-year overall survival values was higher in patients with the tumor PAI-1 level below 0.5 ng/mg protein (47.6% *vs.* 0% in the presence of more than 0.5 ng/mg protein PAI-1); a trend to reduction of nm23 protein production by tumor cells was detected in the same group.

Tumor concentrations of uPA and tPA and the level of nm23 protein expression virtually did not correlate with the overall survival of SC patients.

Studies of the relationship between the expression of nm23 protein and clinical course and prognosis of SC gave contradictory results [5,7,9-11]. Our results indicate that the expression of nm23 protein in malignant epithelial tumors of the stomach is heterogeneous and is often low or zero. Specific immunoreactivity is detected in tumor cell cytoplasm and nuclei (63 and 22.2%, respectively). High level of cytoplasmic expression of the protein was detected in just 25.9% tumors and did not depend on tumor size and involvement of the regional lymph nodes in the tumor process. It is noteworthy that low expression or the absence of nm23 protein in SC cells was more characteristic of poorly or undifferentiated tumors. Cytoplasmic immunoreactivity was low and nuclear one was zero in tumors with the signet ring cell carcinoma structure, characterized by extremely unfavorable clinical course and prognosis.

The levels of uPA, PAI-1, and tPA in tumor tissue are closely related in SC patients. No relationship was detected between uPA and PAI-1 concentrations in the tumors of SC patients and expression of nm23 protein in tumor cell cytoplasm and nuclei. On the other hand, a positive correlation between tPA concentration in the tumor and level of cytoplasmic expression of nm23 protein was revealed (r_s=0.4; p=0.01).

Evaluation of the prognostic significance of the studied regularities showed that only high level of PAI-1 in the tumor (>0.5 ng/mg protein) significantly correlated with reduction of the 3-year overall survival of SC patients. High levels of PAI-1 in tumor tissue of SC patients can be regarded as prognostically unfavorable and be taken into consideration in planning adjuvant therapy.

Hence, our findings indicate a relationship between reduced expression of nm23 protein in tumor cells, on the one hand, and low level of histological

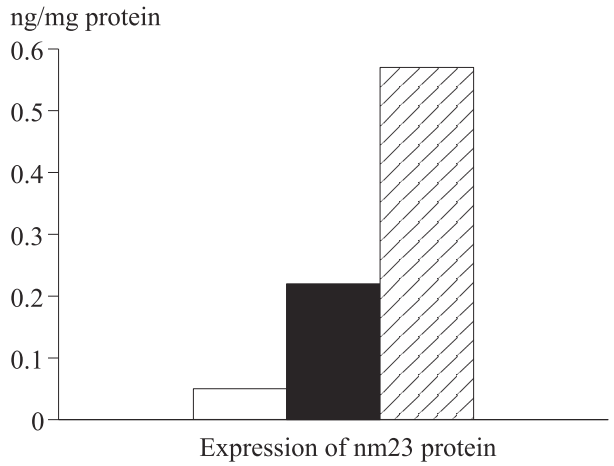

Fig. 2. Concentrations of tPA with consideration for the levels of nm23 protein expression in tumor cell cytoplasm in SC patients. Light bar: no expression; dark bar: low expression; cross-hatched bar: high expression.

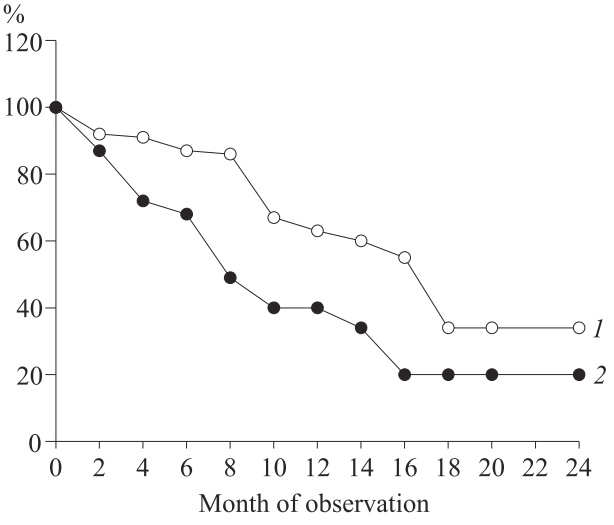

Fig. 3. Analysis of overall survival of SC patients with consideration for PAI-1 level in the tumor. *1*) <0.5 ng/mg protein PAI-1; *2*) >0.5 ng/mg protein PAI-1.

differentiation of the tumor and low concentration of tissue plasminogen activator (tPA), on the other, which characterizes the tumors with high malignancy potential. The decrease in tPA content paralleled by elevation of uPA and PAI-1 concentrations is in line with recent notions on changed proportion of these enzymes in malignant transformation of cells and subsequent increase of the invasive and metastatic potentials of the tumor [1-3,6,8].

Our data confirm the role of the studied proteins in invasion and metastasizing of malignant tumors and suggest a relationship between changed expression of nm23 protein and mechanisms of SC progress. Further studies of these relationships will contribute to the development of accessory methods for prediction of SC clinical course.

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