Relationship between Expression of NM23 Protein and Clinical Morphological Factors and Content of Plasminogen Activation System Components in Tumors of Patients with Gastric Cancer

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Immunohistochemical studies revealed a relationship between the expression of nm23 protein in gastric cancer cells and some parameters characterizing plasminogen activation system and clinical morphological factors. Expression of nm23 in the cytoplasm was detected almost 3-fold more often than in the nuclei; cytoplasmic expression more strictly correlated with patient's age, tumor location, and its histological structure than nuclear expression.

Key Words: *nm23 protein; plasminogen activation system; gastric cancer*

Surgery is now the main and virtually the only method for radical treatment of gastric cancer (GC) leading to complete cure in some cases. However, the 5-year survival of patients is a little higher than 30%, and the problem of predicting the disease course after radical treatment for GC remains unsolved. Additional molecular and biochemical markers characterizing aggressiveness of the tumor process and allowing individual approach to postoperative treatment are now studied and introduced into practice.

One of potentially important markers, involved in the processes of cell proliferation, differentiation, and metastasis control in GC, is nm23 protein (non-metastatic cells protein), nucleotide diphosphate kinase A, a product of nm23-H1 metastasis suppressor gene [9,11]. Hyperexpression of nm23 was detected in some human malignant tumors, such as lung, colon, breast, ovarian cancer, *etc.* [4,7,12]. This parameter was studied in GC [8-10,13] and it was hypothesized that high expression of nm23 in malignant tumors of this location can be associated with unfavorable prognosis [10,13].

On the other hand, numerous studies showed that the level and ratio of expression of various components of plasminogen activation system in tumor tissue is an informative biochemical indicator of metastatic and invasive activity of the tumor and hence, a biologically significant prognostic factor [5]. A significant increase in the production of urokinase type plasminogen activator (uPA) and its type 1 inhibitor (PAI-1) was demonstrated for the majority of malignant tumors of different histogenesis, in comparison with homologous normal tissues and benign tumors [2]. Preliminary studies of the expression of plasminogen activation system components in tumors and adjacent mucosa in GC patients showed that the expression of uPA and PAI-1 were already increased at the early stages of the disease [3].

Here we compared the expression of nm23 protein and the content of the main components of plasminogen activation system in tumors of GC

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patients and evaluated the relationship between this expression and the main clinical and morphological characteristics of the disease.

MATERIALS AND METHODS

The study was carried out in 108 patients (72 men and 36 women) aged 36-75 years (median 62.5 years) in whom GC was diagnosed for the first time. The patients presented with the following stages: 12 (11%) with IB (pT2N0M0) stage, 40 (37%) with stage II (pT2N1M0, pT3N0M0), 28 (26%) with stage IIIA (pT2N2M0, pT3N1M0, pT4N0M0), 24 (22%) with stage IIIB (pT3N2M0, pT4N1-2M0), and 4 (4%) with stage IV. The patients received no treatment before special laboratory and immunohistochemical studies.

Immunohistochemical study of the expression of protein marker nm23 in specimens of primary tumors from 54 patients operated for GC was carried out by the biotin-streptavidin immunoperoxidase method on serial paraffin sections using antibodies to nm23 protein (Dako; 1:50 working dilution). The immunohistochemical reaction was evaluated by the semiquantitative method, taking into consideration staining intensity and number of antigen-positive cells. Weak (+), moderate (++), and intensive (+++) staining were distinguished. The location of staining (cytoplasmic and/or nuclear) was evaluated in each case. If stained nuclei were detected, they were counted in 5 visual fields. The reaction was considered negative if staining was completely absent or there were just foci and solitary stained cells (<25%). Positive reaction in the cytoplasm varied by the level of the marker expression: moderate or intense staining of >50% GC cells was classified as high expression, while weak staining of the majority of tumor cells or moderate staining of 25-50% GC cells was considered as low expression.

The concentrations of uPA, PAI-1, and tissue plasminogen activator (tPA) were measured in tissue cytosols using standard ELISA kits (Catholic University of Nijmegen, Netherlands) [1,6].

The values in different groups were compared using parametric and nonparametric (Kruskal—Wallis) analysis of dispersions and nonparametric tests (Mann—Whitney and median tests). The rela-

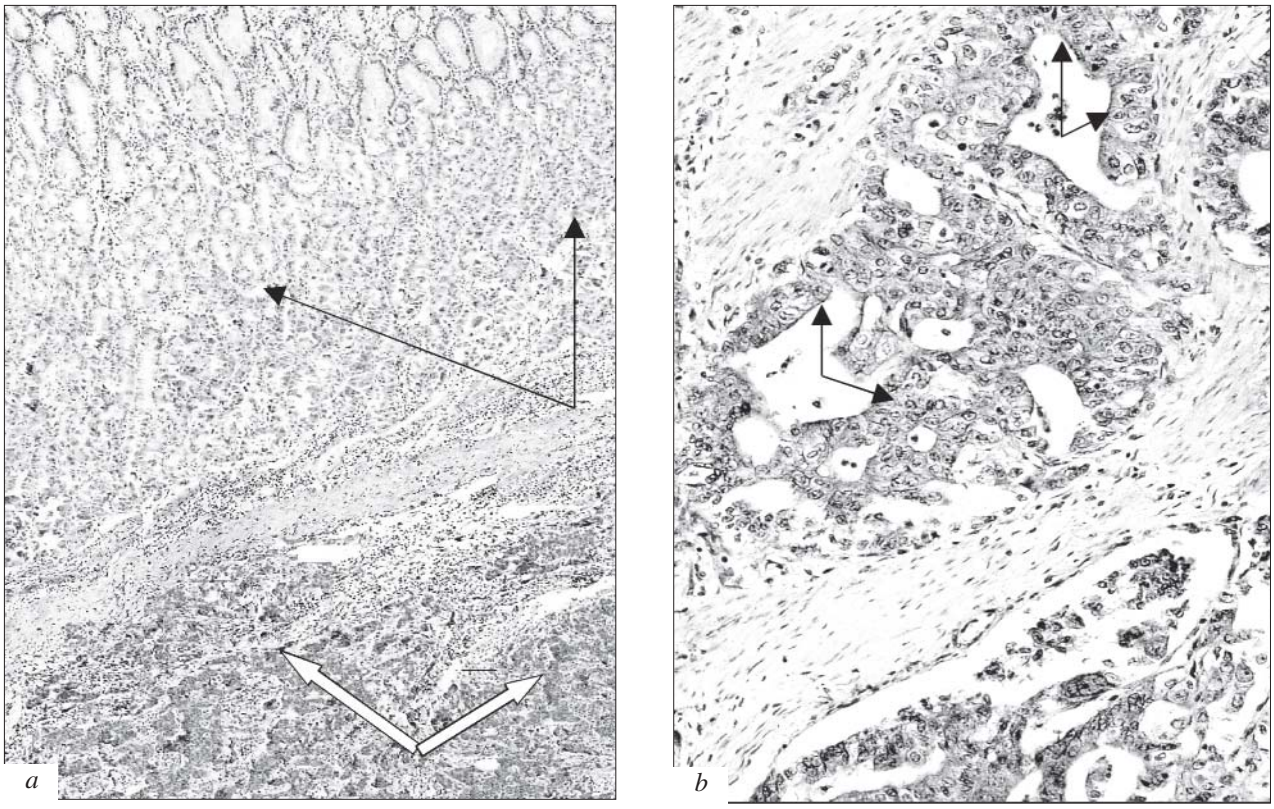

Fig. 1. Expression of nm23 protein in GC cell cytoplasm. *a*) specific reaction in poorly differentiated adenocarcinomas cells (light arrows) and in mucosa epithelium (dark arrows) of the stomach, ×150; *b*) protein accumulation in moderately differentiated adenocarcinoma cells (arrows), ×200. Here and in Fig. 2: biotin-streptavidin immunoperoxidase method, diaminobenzidine stain, cell nuclei post-stained by hematoxylin.

tionships between various parameters were evaluated using nonparametric Spearman rank correlation test. The significance of differences in the incidence of signs in different groups was evaluated by χ^2 test. The differences were considered significant at $p<0.05$.

RESULTS

The expression of nm23 protein was seen as diffuse homogeneous staining of GC cell cytoplasm (Fig. 1). nm23 protein (low or high expression) was detected in the cytoplasm of 34 (63%) tumors. Tumors with low expression were more frequent (37%), while high cytoplasmic expression of nm23 in tumors was detected in 26% GC patients.

No sex-related differences in cytoplasmic expression of nm23 protein were detected; the expression did not depend on T and N parameters. The incidence of different levels of nm23 protein expression in tumor cell cytoplasm was the same in cancer of gastric corpus (31.3%), while in patients with involvement of the antrum, the picture was different: high expression of nm23 protein in just 12.5% patients and no expression in 50% patients.

The level of nm23 protein expression in GC cell cytoplasm depended significantly on patient's age: the incidence of high expression in patients aged under 50 years was 67% *vs.* just 14% (almost 5-fold lower; $p=0.002$) in older patients. A trend to changes in the incidence of different levels of nm23 protein expression in GC cell cytoplasm, depending on histological structure of the tumors, was detected (Table 1). Expression of nm23 protein in the cytoplasm was detected in 70% tumors with adenocarcinoma structure (40 and 30% tumors with low and high expression, respectively). On the other hand, no nm23 expression was detected in 57% tumors with the structure of ring-cell carcinoma, while the incidence of high expression of nm23 protein was only 14%.

Staining with antibodies to nm23 was detected in 12 (22%) tumors (Fig. 2). No stained nuclei were detected in undifferentiated GC cells, while in adenocarcinomas, the nuclear expression of the protein was detected in 30% cases ($p=0.03$). On the other hand, no relationship was found between the degree of differentiation of gastric adenocarcinoma and nm23 protein-positive staining of GC cell nuclei.

Hence, expression of nm23 protein in the GC cell cytoplasm was detected in 63% patients, while nuclear expression of this protein was detected in tumors of 22% patients. Cytoplasmic expression was more often detected in adenocarcinomas than

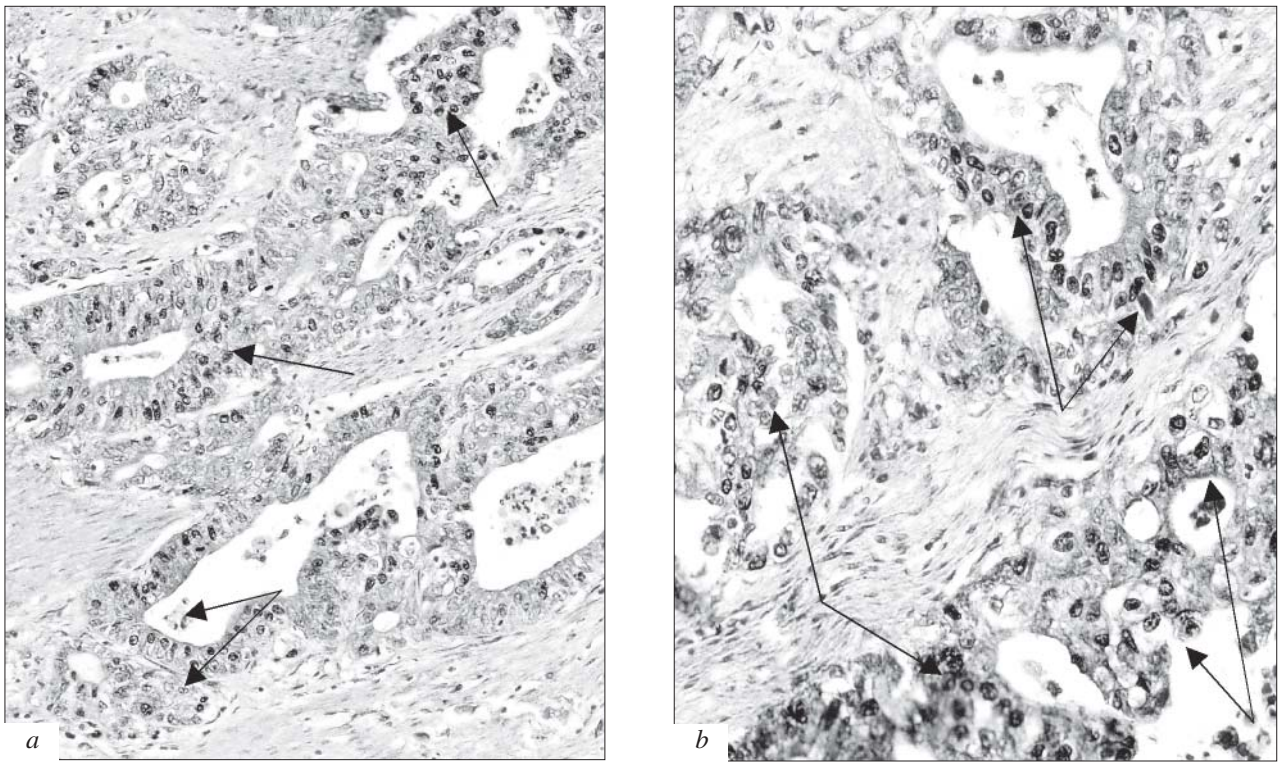

Fig. 2. Nuclear expression of nm23 protein in gastric adenocarcinoma cells. *a*) nuclear and cytoplasmic expression of the protein (arrows), ×200; *b*) intense specific staining of the majority of cancer cell nuclei (arrows), ×400.

in ring-cell tumors and in tumors of younger patients (<50 years). No significant relationship was found between the nuclear expression of nm23 protein and the main clinical morphologic factors; only the absence of nuclear expression in undifferentiated cancers is worthy of note.

The content of uPA in tumors did not significantly correlated with cell cytoplasm staining for nm23 protein, though the median of uPA level was higher in GC patients with high expression of nm23 protein. No correlation between uPA levels and nm23 protein expression in the cytoplasm was detected. The median of uPA content in tumors with nuclei not stained with antibodies to nm23 (0.16 ng/mg protein) did not differ significantly from the median of this parameter in tumors with stained nuclei (0.23 ng/mg protein). On the other hand, the medians of tPA levels increased with increasing the intensity of cell cytoplasm staining with antibodies to nm23 (Table 2). A positive correlation between tPA level and intensity of cytoplasm staining was noted (R=0.34; p=0.01). No relationship between tPA level in GC tissue and degree of the nuclei staining by antibodies to nm23 was detected. No significant relationship between the concentration of PAI-1 in the tumors of GC patients and the level of nm23 protein expression in tumor cell cytoplasm and nuclei was detected.

These data indicate a relationship between the levels of nm23 protein expression in GC cells, on the one hand, and some parameters characterizing plasminogen activation system and some clinical morphological factors, on the other. The expression of nm23 protein in the cytoplasm of GC cells was detected almost 3-fold more often than in the nuclei; the cytoplasmic expression more strictly correlated with such parameters as patient's age, tumor location, and histological structure. Further investigation of the detected regularities will presumably lead to creation of pathogenetic methods for postoperative treatment of GC patients and more valid approaches to this treatment.

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TABLE 1. Expression of nm23 Protein in Cell Cytoplasm of Gastric Tumors of Different Histological Structure

Expression of nm23 protein in the cytoplasm	Adenocarcinoma	Ring-cell cancer
Absent	24 (30%)	16 (57%)
Low	32 (40%)	8 (29%)
High	24 (30%)	4 (14%)

TABLE 2. Content of tPA in Gastric Tumors and Expression of nm23 Protein in Tumor Cell Cytoplasm

Expression of nm23 protein in the cytoplasm	Number of patients	Content of tPA, ng/mg protein	
		range	mediana
Absent	40	0-1.83	0.05
Low	40	0-2.25	0.22
High	28	0.12-2.36	0.57

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