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The nm-23 Protein Expression in Non-Small-Cell Lung Cancer*

Ekspresja proteiny nm-23 w niedrobnokomórkowym raku płuca

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Abstract

Background. *NM-23* gene is considered a metastasis suppressor gene. Expression of its product is reported to correlate inversely with metastatic potential and prognosis in some cancers, but its significance in lung cancer is unclear. Lung cancer is currently the most frequently diagnosed neoplasm in males and the second most frequent in females in Poland and most developed countries. The frequency and unfavorable prognosis in this malignancy justifies studies on prognostic factors.
Objectives. The aim of this study was to evaluate the prognostic value of nm-23 protein expression in non-small-cell lung cancer and to compare nm-23 protein expression with some clinico-pathological findings.
Material and Methods. nm-23 protein expression was examined in 95 patients (mean age: 59.48 ± 7.86 years) with non-small-cell lung cancer who had had surgical treatment. The histopathological diagnoses were squamous cell carcinoma in 59 patients, adenocarcinoma in 26, large cell carcinoma in 5, and non-small-cell lung cancer of unspecified type in 5. nm-23 expression was determined by immunohistochemistry on paraffin-embedded tissue using a polyclonal antibody to nm-23. All patients were observed for at least 24 months, at which time 49 patients were alive and 46 had died.
Results. Positive cytoplasmatic staining was observed in 69 of the tumor tissues (72.63%). No relationship was found between nm-23 expression and survival. No correlation was found between nm-23 expression and clinico-pathological parameters, including gender, histological type, tumor differentiation stage, and nodal involvement. Higher expression of nm-23 protein was found in patients with squamous carcinoma without tumor cell emboli in blood vessels ($p = 0.0161$).
Conclusions. These data suggest that the nm-23 protein expression probably has no prognostic value in lung cancer and limited significance in squamous cell lung cancer (**Adv Clin Exp Med 2008, 17, 3, 307–312**).

Key words: nm-23 protein, non-small-cell lung cancer, prognostic factors.

Streszczenie

Wprowadzenie. Gen *NM-23* jest zaliczany do genów antymetastatycznych. W części nowotworów stwierdzono odwrotną korelację między ekspresją jego produktu a zdolnością do tworzenia przerzutów i rokowaniem. Zależność ta i znaczenie genu *NM-23* w raku płuca pozostaje jednak niejasne. Rak płuca jest najczęściej występującym nowotworem złośliwym u mężczyzn i drugim u kobiet w Polsce i w większości rozwiniętych krajów świata. Rozpowszechnienie raka płuca i niekorzystne rokowanie skłania do badań nad czynnikami prognostycznymi.
Cel pracy. Ocena znaczenia rokowniczego ekspresji proteiny nm-23 w raku niedrobnokomórkowym płuca oraz jej porównanie z wybranymi wskaźnikami klinicznymi.
Materiał i metody. Ekspresję proteiny nm-23 oznaczono u 95 chorych na niedrobnokomórkowego raka płuca, u których przeprowadzono zabieg operacyjny. Średni wiek w badanej grupie 59,48 ± 7,86 lat. U 59 chorych rozpoznano raka płaskonabłonkowego, u 26 gruczolakoraka, u 5 raka wielkokomórkowego, a u 5 raka niedrobnokomórkowego bez określenia podtypu histopatologicznego. Ekspresję proteiny nm-23 oznaczono w materiale guza metodą immunohistochemiczną. Zastosowano przeciwciało poliklonalne Rabbit Anti Human nm-23 Protein DAKO A 0096. Pacjenci zostali poddani 2-letniej obserwacji. 2 lata przeżyło 49 chorych. 46 osób zmarło.

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Wyniki. Ekspresję proteiny nm-23 stwierdzono w 69 preparatach (72,63%). Nie wykazano związku proteiny nm-23 z przeżyciem ani z wybranymi wskaźnikami, takimi jak płeć, typ histopatologiczny, zajęcie węzłów chłonnych czy stopień zróżnicowania. Wykazano natomiast, że wyższą ekspresję nm-23 u chorych na raka płaskonabłonkowego bez zatorów z komórek nowotworowych w naczyniach.
Wnioski. Powyższe wyniki sugerują, że ekspresja białka nm-23 nie ma wartości prognostycznej w raku płuca. Ma ona tylko ograniczone znaczenie w raku płaskonabłonkowym płuca (**Adv Clin Exp Med 2008, 17, 3, 307–312**).

Słowa kluczowe: proteina nm-23, rak niedrobnokomórkowy płuca, czynniki prognostyczne.

Tumor metastasis, defined as the formation of progressively growing secondary tumor foci at sites other than the primary lesion, is responsible for a high degree of mortality in cancer patients [1, 2]. Metastasis suppressor genes, including *NM-23*, *KAI-1*, *KiSS-1*, *BrMS1*, *MKK4*, *RhoGDI2*, *CRSP3*, and *VDUP-1*, inhibit the formation of metastases without affecting the growth rate of the primary tumor [2, 3]. *NM-23* was identified in the murine K 1735 melanoma cell line in 1988 by P. Steeg and coworkers. They found an inverse correlation between *NM-23* gene expression and metastatic potential [4]. These observations were confirmed by other studies. Transfection of *NM-23* H1 cDNA into highly metastatic murine melanoma, rat mammary adenocarcinoma, human breast cancer, and melanoma cells reduced their invasiveness and metastatic potential *in vivo* [2]. *Nm-23* is located on chromosome 17q21[5]. Eight human homologues (*NM-23* H1-H8) have been described so far, but only H1 and H2 have been tested for their role in tumor spread.

The mechanism of metastasis suppression by *NM-23* gene is still unknown. Its gene products have been identified as nucleoside diphosphate kinases (NDPKs). The isoforms *NM-23* H1 and *NM-23* H2 encode 20.5-kDa and 18.5-kDa proteins. They have a highly homologous (88%) amino-acid sequence and are sometimes called kinase-A and kinase-B [6]. Kinase B was previously identified as a transcription factor of the c-Myc oncogene [7]. Roymans and coworkers identified rat nm-23R1/NDPK beta, a homologue of the human metastasis suppressor nm-23-H1, as a component of the centrosomal complex. They demonstrated that nm-23R1 is located in the centrosome of dividing and non-dividing cells. In addition, human nm-23-H1 was also shown to be present in the centrosome of different human cell types [8]. Kantor et al. reported that murine melanoma cell lines and human breast carcinoma cells that were stably transfected with nm-23-H1 lost their ability to migrate in response to a variety of chemoattractants, including serum platelet-derived growth factor and insulin-like growth factor 1 [9]. The protective activity on metastatic behavior was confirmed in some human neoplasms, mostly in breast cancer and melanoma. However, in other types of neoplasms (e.g. colorectal, gastric, renal, and lung cancer) the role of *NM-23* is still disputable [10,11]. The aim of this study was to evaluate the prognostic value of nm-23 protein expression in non-small-cell lung cancer and to compare the expression of nm-23 with some clinico-pathological findings.

Material and Methods

Ninety-five patients with pathologically confirmed non-small-cell lung cancer (65 men and 30 women, mean age: 59.48 ± 7.86 years) were evaluated. They had undergone surgery, i.e. lobectomy, bilobectomy, pneumonectomy, or exploratory thoracotomy. The histopathological diagnoses were squamous cell carcinoma in 59 patients, adenocarcinoma in 26 patients, large-cell carcinoma in 5 patients, and non-small-cell lung cancer of unspecified type in 5 patients. According to the TNM staging system, 9 patients were in stage IA, 20 in IB, 2 in IIA, 17 in IIB, 31 in IIIA, 7 in IIIB, and 9 in stage IV. Forty-two patients received chemotherapy, 24 of whom received neoadjuvant chemotherapy. Twenty-five patients had radiotherapy after surgical treatment. All patients were observed for at least 24 months at which time 49 (52%) of the patients were alive and 46 (48%) had died. The study was approved by the appropriate ethics committees related to the institution and all the patients gave informed consent to participate in the study.

Immunohistochemistry

Formalin-fixed well-preserved tumor-tissue blocks from surgically resected lung cancer specimens were used for immunohistochemical study. The 4-μm sections of formalin-fixed tissues were mounted on silanized slides, deparaffinized in xylene, and rehydrated through serial baths of alcohol to water. The hydrated sections were treated in 3% hydrogen peroxide for 10 minutes to eliminate endogenous peroxidase activity and washed in phosphate-buffered saline (PBS). The primary antibody used in this study was a polyclonal antibody to nm-23 gene product (Polyclonal Rabbit Anti Human nm-23 Protein DAKO A 0096). The antibody recognizes human nm-23-H1 and nm-

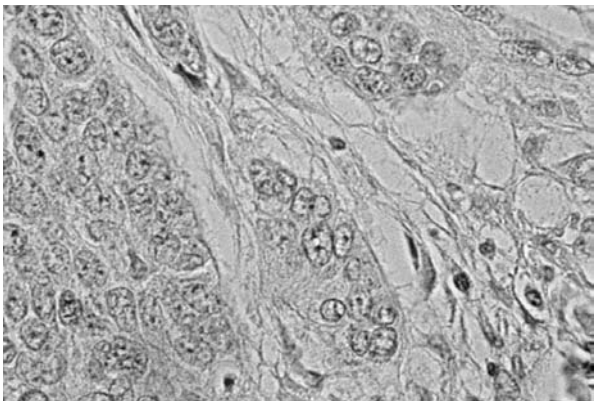
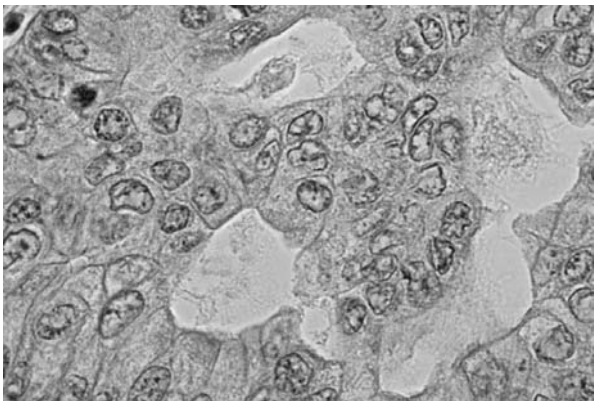
23-H2. The polyclonal antibody-treated slides were rinsed in PBS solution and incubated with a biotinylated secondary antibody. The slides were then washed in PBS and incubated with an avidin-biotin-peroxidase complex for 15 minutes. After washing with PBS, a chromogenic reaction was developed by incubating with 3,3-diaminobenzidine tetrahydrochloride (DAB+, Liquid K 3486 DAKO). Positive staining appeared as brown cell plasma. nm-23 protein accumulation was described as positive if more than 10% of the cells were stained.

Statistical Method

Statistical analysis was performed using CSS Statistica for Windows (version 5.0). The chi-squared test was used for two or multiple groups. Differences between samples were considered significant at $p < 0.05$. Survival curves were constructed using the Kaplan-Meier method.

Results

Sixty-nine of the 95 (72.63%) tumor tissue specimens were positive for nm-23 protein and 26 (27.36%) were negative. Figure 1 shows pictures of immunohistochemical staining. No statistical difference was found in nm-23 protein expression regarding gender, histopathological type, tumor staging, degree of cancer cell differentiation, or nodal involvement (Table 1). However, nodal involvement and degree of cancer cell differentiation were not determined in all

A   B

Ryc. 1. A. Brak ekspresji proteiny nm-23. B. Obecna ekspresja proteiny nm-23

Fig. 1. A. Negative immunostaining of nm-23 protein. B. Positive immunostaining of nm-23 protein

Table 1. Clinico-pathological findings and nm-23 protein expression

Tabela 1. Wybrane wskaźniki i ekspresja proteiny nm-23

A. Gender	nm-23 negative	nm-23 positive	Chi2	p
men	20	45	1.2	0.27
women	6	24		
B. Histological type				
squamous cell	19	40	1.5	0.22
adenocarcinoma	5	21		
C. Stage				
Stage I+II	11	37	0.97	0.33
Stage III+IV	15	32		
D. Degree of cancer cell differentiation (G)				
G 1-2	15	35	0.15	0.70
G 3	4	12		
G 1	0	3	0.95	0.33
G 3	4	12		
E. Metastases in lymph nodes (N)				
N 0	11	28	0.25	0.61
N 1-3	12	39		
N 0-1	14	42	0.02	0.88
N 2-3	9	25		

patients. The prognostic value of nm-23 protein expression in all the patients with non-small-cell lung cancer and separately in patients with squamous cell carcinoma and adenocarcinoma was analyzed, but there was no correlation between nm-23 protein expression and survival in any group (Table 2 and Figure 2). Higher expression of nm-23 protein was found in patients with

Table 2. Comparison of 24-month survival in all patients and in patients with squamous cell lung cancer and adenocarcinoma

Tabela 2. Porównanie 24-miesięcznego przeżycia z ekspresją proteiny nm-23 u wszystkich chorych oraz u chorych na raka płaskonabłonkowego i gruczolakoraka

Survival	nm-23 negative n (%)	nm-23 positive n (%)	Chi^2	*p*	Cox Mantel
A. All patients with NSCLC					
< 24 months > 24 months	13 (50%) 13 (50%)	33 (48%) 36 (52%)	0.04	0.85	0.62
B. Patients with squamous cell carcinoma					
< 24 months > 24 months	10 (53%) 9 (47%)	20 (50%) 20 (50%)	0.04	0.85	0.68
F. Patients with adenocarcinoma					
< 24 months > 24 months	11 (52%) 10 (48%)	2 (40%) 3 (60%)	0.25	0.62	0.85

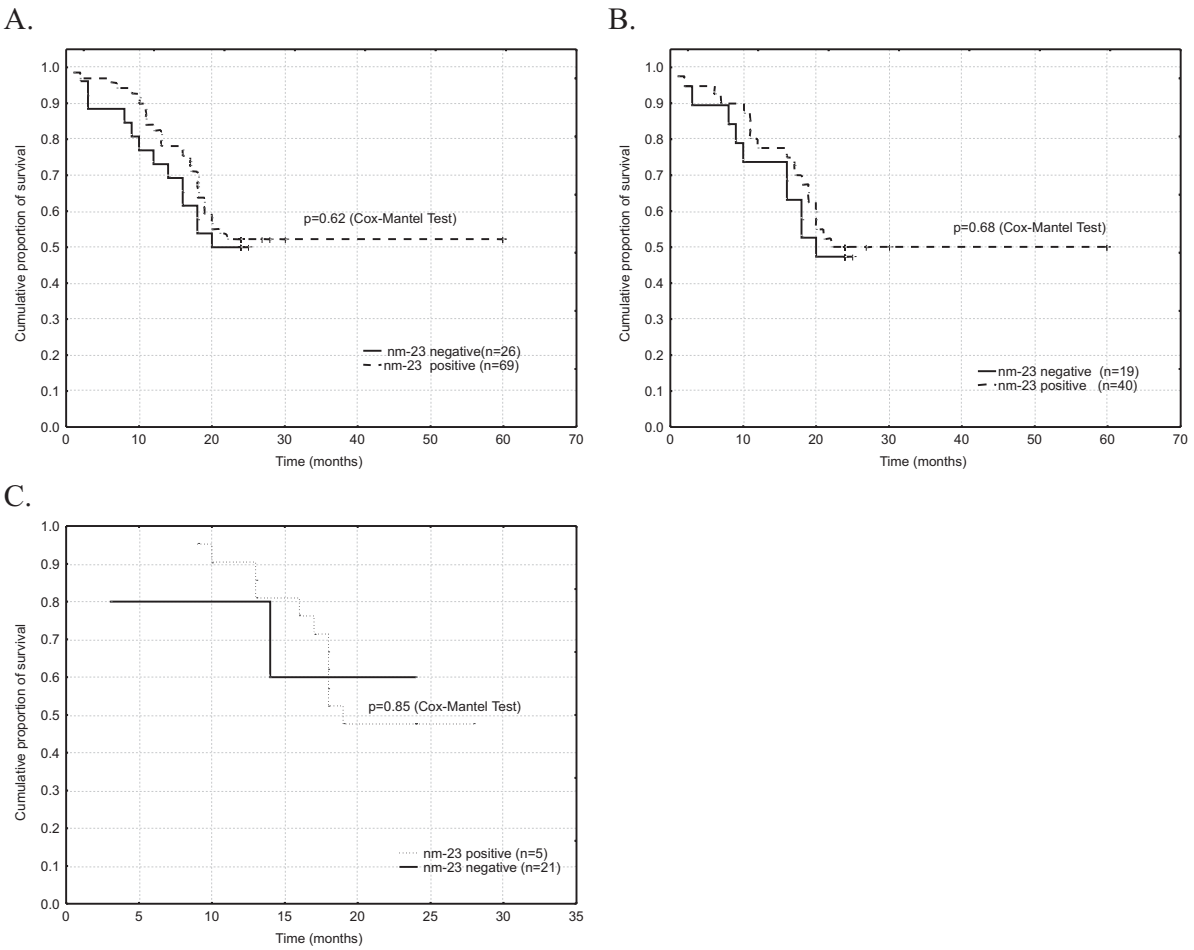


Fig. 2. Cumulative proportion of Kaplan-Meier survival according to nm-23 protein expression: A. In all patients with non-small-cell lung cancer, B. In patients with squamous cell lung cancer, C. In patients with adenocarcinoma

Ryc. 2. Skumulowana proporcja przeżywających Kaplana-Meiera w zależności od ekspresji proteiny nm-23: A. U wszystkich chorych na raka niedrobnokomórkowego, B. U chorych na raka płaskonabłonkowego, C. U chorych na gruczolakoraka

Table 3. Tumor cell emboli in blood vessels according to nm-23 protein expression and histological type

Tabela 3. Porównanie występowania zatorów z komórek nowotworowych z ekspresją proteiny nm-23 i typem histopatologicznym

	Tumor cell emboli in blood vessels		Chi^2	*p*
	present	not present		
A. Patients with NSCLC				
nm-23 positive	14	26	2.02	0.15
nm-23 negative	12	43		
B. Patients with squamous cell carcinoma				
nm-23 positive	14	26	5.80	0.0161
nm-23 negative	13	6		
C. Patients with adenocarcinoma				
nm-23 positive	1	10	1.26	0.26
nm-23 negative	4	11		

squamous cell carcinoma without tumor cell emboli in the blood vessels (81.25% vs. 51.85%, $p = 0.0161$) (Table 3).

Discussion

Lung cancer is currently the most frequently diagnosed cancer and the most common cause of cancer mortality in males. In women, only breast cancer occurs more frequently, but lung cancer remains the most common cause of cancer mortality in the majority of developed countries. Despite many studies, improved diagnostics and therapeutics, as well as supportive care options, the prognosis remains unfavorable and long-term survival has practically not changed in the last 50 years [12]. This situation justifies a search for prognostic factors in lung cancer.

The expression of the *NM*-23 gene product has been reported to correlate inversely with prognosis and metastatic potential in some, but not all, tumors. Despite many efforts, the antimetastatic significance of *NM*-23 gene in non-small-cell lung cancer remains to be established. Kawakubo et al. reported that reduced expression of nm-23 protein was associated with lymph node metastasis and poor patient survival in pulmonary adenocarcinoma, but correlation between nm-23 protein expression and other clinical findings, such as histological grade, presence of distant metastases, or primary tumor size, was not found [13]. Katakura et al. analyzed five-year survival in 117 patients with stage I non-small-cell lung cancer and showed that positive expression of nm-23 is a favorable prognostic factor [14]. Some data indicate that nm-23-H1 and -H2 isoform levels correlate with histological differentiation, but not with metastatic potential or stage of pulmonary adenocarcinoma [15].

Many other studies confirm the observations of the present study. Bosnar et al. found no correlation between nm-23 protein expression and TNM stage, grade, primary tumor size, or patient survival in squamous cell lung cancer [16]. Mac Kinnon et al. evaluated a group of 162 patients with adenocarcinoma and, based on immunohistochemical assessment of nm-23 protein, did not find any prognostic value [17]. Mirejovsky et al. found no relationship between *NM*-23 gene product expression and histological type, grade of differentiation, or metastasis [18].

The present study demonstrated higher expression of nm-23 protein in patients with squamous carcinoma without tumor cell emboli in the blood vessels ($p = 0.0161$). This suggests that nm-23 protein may have limited significance only in this type of lung cancer. Observations in thyroid carcinoma indicate that the biological significance of *NM-23* gene expression may be quite different not only in different tissues, but also in different neoplasms of the same organ. Zafon et al. confirmed a significant inverse relationship between *NM*-23 gene product immunoreactivity and metastatic disease and poor prognosis in follicular carcinoma, but in papillary carcinoma he observed no relationship between nm-23 protein immunoreactivity and survival or clinicopathological parameters [19]. Lei et al. suggest that in lung cancer, *NM-23* gene may play different roles in the pathogenesis and metastasis of squamous cell carcinoma and adenocarcinoma. He reported inverse correlation between positive staining and involvement of hilar or mediastinal lymph nodes only in patients with squamous cell carcinoma [20].

The association between *NM-23* and tumor cell emboli in blood vessels may be explained by the mechanism of metastasis suppression. Some studies indicate that *NM-23* gene may play a role in cell migration [9] and other observations show

that decreased *NM*-23 gene product expression could predict a risk of systemic micrometastasis in non-small-cell lung cancer [21]. The present study suggests that nm-23 protein expression has no prognostic value or antimetastatic role in lung cancer, but the present authors realize that the group of patients was heterogeneous (different histological types and stages), which decreases the value of these observations.

The authors concluded that nm-23 protein expression probably has no prognostic value in lung cancer. nm-23 protein expression has only limited significance in squamous cell lung cancer.

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