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Nuclear maspin expression as a good prognostic factor in human epithelial ovarian carcinoma

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Maspin, a protein belonging to the serpin superfamily, seems to exert tumour-suppressive activity. Its significance in ovarian cancer prognosis is currently under investigation. In the present work, immunocytochemical maspin expression in 132 invasive epithelial ovarian carcinomas was assessed independently in the nucleus and cytoplasm, in correlation with histopathological and clinical data. Positive maspin expression was found in 117 cases: nuclear/cytoplasmatic in 71, exclusive nuclear in 29, and only cytoplasmatic in 17 cases. Cytoplasmatic maspin expression was positively correlated with tumour grade ($p = 0.000$), FIGO stage ($p = 0.002$), and distant metastases ($p = 0.000$) but exhibited no significant correlation with tumour type ($p = 0.078$). Nuclear maspin expression showed negative correlation with tumour grade ($p = 0.025$), FIGO stage ($p = 0.05$), distant metastases ($p = 0.001$), and cancer remission ($p = 0.000$) but showed no significant relationship with the patients' age ($p = 0.948$) or cancer subtype ($p = 0.261$). Kaplan-Meier survival analysis showed that strong cytoplasmatic maspin expression was correlated with shorter disease-free survival ($p = 0.000$) whereas strong nuclear expression was correlated with longer survival ($p = 0.000$). In Cox regression analysis, low nuclear maspin expression (score 2 and 3) remained a significant independent prognostic factor ($p = 0.001$) with a relative death risk of 5.337. The obtained results suggest that maspin expression may be a significant marker in epithelial ovarian carcinoma prognosis with its nuclear expression being a good prognostic factor. (Folia Morphol 2010; 69, 4: 204–212)

Key words: maspin, immunohistochemistry, subcellular localisation, epithelial ovarian carcinoma, prognostic factors

INTRODUCTION

Ovarian cancer, being the sixth most frequently occurring cancer in the world, takes the second place (after cervical cancer) with respect to the occurrence of reproductive organ cancers, and is the fifth most common cause of death due to cancer in women. Annually over 3000 new cases are noted in Poland (reports based on data from the National Cancer Registry, Poland) [23].

Since the knowledge concerning ovarian cancer biology is still insufficient, it has become a subject of special interest among clinicians and scientists. The present studies are focused on the identification of new biomarkers which could find an application in early disease detection and monitoring, as well as targeting therapy design.

Maspin (mammary serine protease inhibitor), a protein belonging to serpin superfamily, has been initially

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identified and described as a product of a tumour-suppressing gene [38]. Its expression, except for in the mammary gland [10, 17], has been detected in numerous tissues both normal and cancerous, such as prostate, ovaries, testes, thymus, large and small intestine, stomach, pancreas, and lung [18, 21, 22, 30, 31, 32]. Experiments conducted on cancers of mammary gland and prostate suggest that the suppressive function of maspin in cancer disease may be multidirectional. Maspin has been shown to limit cancer cells mobility through the inhibition of the cascade of extracellular plasminogen activation [4, 6]. It also exhibits the capability of angiogenesis inhibition [36] and sensitisation of cancer cells to proapoptotic factors [11].

The biological role of maspin, as well as its mechanism of action in numerous cancers, has not been fully elucidated. Moreover, its clinical significance in many cases is ambiguous or even contradictory [35]. Whereas in some cancers (breast, prostate, stomach, and lung) the lack or decrease in maspin expression is associated with the formation of metastases and poor clinical prognosis, in others (pancreas and ovarian cancers) it is strong maspin expression which correlates with the increase in cancer invasiveness and worse survival rates. In ovarian glands maspin expression has been detected only in cancerous tissue (where its increase was connected with poor prognosis) and not in normal cells [32]. The significance of maspin as a prognostic factor is additionally complicated by the fact that its appearance in various cellular compartments may be associated with disparate biological functions and have different clinical implications. In invasive breast cancers nuclear maspin expression has been shown to correlate with good prognostic factors, whereas its cytoplasmic expression has been shown to correlate with poor prognostic factors [20]. Similar results have been obtained for lung cancer, where nuclear maspin expression correlated with good prognosis [15].

The aim of the present investigation was the elucidation of maspin expression significance assessed independently in the nucleus and cytoplasm of invasive epithelial ovarian cancer cells in correlation with histopathological data and clinical course of the disease.

MATERIAL AND METHODS

Patients and tissue samples

Tumour specimens were obtained during routine histopathological diagnosis (in the years 1992–2000) from 132 women with invasive epithelial ovarian carcinomas. All of the samples were collected in compliance with the requirements of the Institutional Review Board for the Protection of Human Subjects. Patients were treated at the Department of Gynaecology, II Chair of Gynaecology and Obstetrics at the Medical University of Wrocław, and were stu-died retrospectively. The slides for histopathological studies (H&E) were prepared from formalin-fixed paraffin wax blocks and the specimens for immunocytochemical studies from the adjacent cut fragments. Haematoxylin and eosin stained slides were subjected to histopathological evaluation by two pathologists. Tumour stage and grade were assigned according to the International Federation of Gynaecology and Obstetrics [28] and Silverberg grading system [26]. Clinicopathological variables are summarized in Table 1.

Table 1. Clinicopathological features of epithelial ovarian carcinoma patients

Patients	N	%
No. of cases	132	100
Age:		
≤ 65	63	47.7
> 65	69	52.3
Grade (G):		
G1	42	31.8
G2	46	34.8
G3	44	33.3
Stage (FIGO):		
I	32	24.2
II	25	18.9
III	41	31.1
IV	34	25.8
Distant metastasis:		
No	57	43.2
Yes	75	56.8
Remission:		
No	92	69.7
Yes	40	30.3
Histological subtype:		
Endometroid	50	37.9
Serous	35	26.5
Other	47	35.6

Immunohistochemistry

For immunocytochemical studies, paraffin sections were mounted on Superfrost$^+$ slides, dewaxed with xylene, and gradually rehydrated. Antigen retrieval was performed by boiling in citrate buffer (pH 6.0) for 15 min. Activity of endogenous peroxidase was blocked by 30 min incubation in 3% H_2O_2. Immunocytochemical reactions were performed using primary monoclonal mouse antibodies against human maspin (clone G167-7, BD PharMingen San Diego, CA). The antibodies were diluted 1:400 in antibody diluent with back-

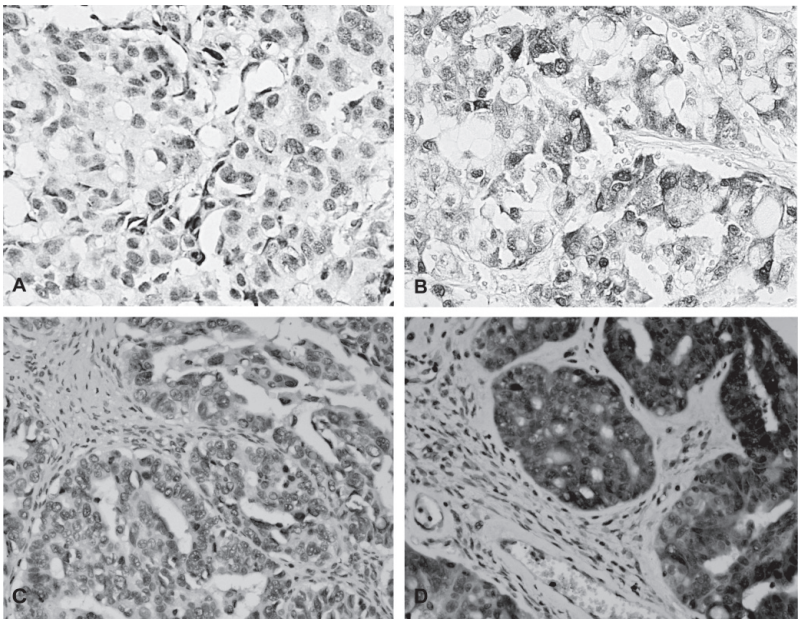

Figure 1. Immunohistochemical localisation of maspin in epithelial ovarian carcinoma. Representative immunohistochemical picture of nuclear (**A**) and combined nuclear–cytoplasmic expression of maspin (**B**). Representative immunohistochemistry of low (**C**) and high (**D**) expression level of maspin. The immunohistochemical signal was developed using DAB (brown). Picture C and D counterstained with haematoxylin.

ground reduction (DakoCytomations). The examined sections were incubated with primary antibodies for one hour at room temperature. Subsequently, incubations were performed with biotinylated secondary antibodies (15 min) and with streptavidin-biotinylated peroxidase complex (15 min). Diaminobenzidine (DAB) was used as a chromogen (LSAB+, HRP, DakoCytomations). Some sections were counterstained with Meyer's haematoxylin, and some were not counterstained to obtain a better estimation of the nuclear reaction. As a negative control of the reaction the first specific antibodies were substituted by Primary Mouse Negative Control (DakoCytomations).

Maspin expression was estimated semiquantitatively as the percentage of cells showing positive reaction, using the following scale [32]: 0 points: no positive cells; 1 point: 1–5% positive cells; 2 points: 6–50% positive cells; 3 points: > 50% positive cells. The staining intensity was rated as follows: 1 point: weak intensity; 2 points: moderate intensity; 3 points: strong intensity. The final assessment, performed as a sum of the percentage of positive cells and the reaction intensity, was categorised into four values (0–3 points): negative reaction ≤ 5% stained cells independently of the reaction intensity (0 points), weak reaction 1–2 (1 point), moderate reaction 3–4 (2 points), and strong reaction 5–6 (3 points). Maspin expression was estimated separately for the cytoplasm and nucleus of ovarian cancer cells using the same procedure.

Statistical analysis

Statistical analyses were performed using the SPSS for Windows version 17.0 software package (SPSS Inc., Chicago, IL, USA). The χ^2 tests were used to assess the statistical significance of association between cytoplasmic or nuclear maspin expression and prognostic variables. Univariate survival analysis was done according to the Kaplan-Meier model using the log-rank test. Survival time was estimated in months from the date of diagnosis to the date of death or last follow-up. Statistical significance was defined as a probability value less than 0.05. Finally, two Cox proportional hazards models (for nuclear and cytoplasmatic expression of maspin) were fitted to the data in order to assess the influence of maspin expression on hazards with control over other significant predictors of hazard, such as tumour stage, tumour grade, and age.

RESULTS

A positive reaction to maspin was found in 117 (88.6%) cases, whereas no reaction was observed in 15 (11.4%) cases. In the examined specimens of invasive ovarian cancer, maspin expression was present in the nucleus and/or cytoplasm. Nuclear and cytoplasmic distribution was observed in 71 (53.8%) cases, exclusive nuclear expression in 29 (22.0%), and in 17 (12.9%) cases the expression was limited to the cytoplasm. Representative patterns of maspin expression in ovarian cancers are demonstrated in Figure 1.

Table 2. Association between cytoplasmic maspin expression and clinicopathological variables

Patients	Negative (0)	Low (1)	Moderate (2)	Strong (3)	P	(0–1)	(2–3)	P
Age:					0.287			0.732
≤ 65	20 (45.5%)	13 (65.0%)	27 (46.6%)	3 (40.0%)		33 (51.6%)	30 (55.9%)	
> 65	24 (54.5%)	7 (35.0%)	31 (53.4%)	7 (60.0%)		31 (48.4%)	38 (44.1%)	
Grade (G):					0.000*			0.000
1	18 (40.9%)	10 (50.0%)	14 (24.1%)	0 (0%)		28 (43.8%)	14 (20.6%)	
2	19 (43.2%)	7 (35.0%)	20 (34.5%)	0 (0%)		26 (40.6%)	20 (29.4%)	
3	7 (15.9%)	3 (15.0%)	24 (41.4%)	10 (100%)		10 (15.6%)	34 (50.0%)	
Stage (FIGO):					0.002			0.000
I	14 (31.8%)	6 (30.0%)	12 (20.7%)	0 (0%)		20 (31.3%)	12 (17.6%)	
II	10 (22.7%)	9 (45.0%)	5 (8.6%)	1 (10.0%)		19 (29.7%)	6 (8.8%)	
III	11 (25.0%)	5 (25.0%)	20 (34.5%)	5 (50.0%)		16 (25.0%)	25 (36.8%)	
IV	9 (20.5%)	0 (0%)	21 (36.2%)	4 (40.0%)		9 (14.1%)	25 (36.8%)	
Distant metastasis:					0.000			0.000
No	24 (54.5%)	15 (75.0%)	17 (29.3%)	1 (10.0%)		39 (60.9%)	18 (26.5%)	
Yes	20 (45.5%)	5 (25%)	41 (70.7%)	9 (90%)		25 (39.1%)	50 (73.5%)	
Remission:					0.041			0.081
No	31 (70.5%)	9 (45.0%)	43 (74.1%)	9 (90.0%)		40 (62.5%)	52 (76.5%)	
Yes	13 (29.5%)	11 (55.0%)	15 (25.9%)	1 (10.0%)		24 (37.5%)	16 (23.5%)	
Histological subtype:					0.012			0.078
Endometroid	13 (29.5%)	5 (25.0%)	29 (50.0%)	3 (30.0%)		18 (28.1%)	32 (47.1%)	
Serous	16 (36.4%)	11 (55.0%)	13 (22.4%)	7 (70%)		27 (42.2%)	20 (29.4%)	
Other	15 (34.1%)	4 (20.0%)	16 (27.6%)	0 (0%)		19 (29.7%)	16 (23.5%)	

*Fisher's exact test

High cytoplasmic maspin expression (scores 2 and 3) was found in 68 (51.5%) cancers, whereas low or a lack of expression (scores 0 and 1) was seen in 64 (48.5%) cancers. High nuclear maspin expression was observed in 80 (60.6%) cases, whereas 52 (39.4%) cases exhibited low or a lack of maspin expression.

Using the χ^2 test, the relationships were examined between cytoplasmic maspin expression and age, tumour grade (G), disease stage (FIGO), distant metastases, cancer remission, and the histological subtype of the cancer. Cytoplasmic maspin immunoreactivity was positively associated with tumour grade (G) ($p = 0.000$), FIGO disease stage ($p = = 0.002$), and distant metastases ($p = 0.000$). No statistical associations were found between cytoplasmic maspin expression and the age ($p = 0.732$), disease remission ($p = 0.081$), and histological subtype of cancer ($p = 0.078$) (Table 2).

Nuclear maspin immunoreactivity was negatively associated with tumour grade (G) ($p = 0.025$), disease stage according to FIGO ($p = 0.05$), distant metastases ($p = 0.001$), and cancer remission ($p = = 0.000$) but showed no significant relationship with the patients' age ($p = 0.948$) or cancer subtype ($p = 0.261$) (Table 3).

In Kaplan-Meier's analysis, mean survival time was compared between groups showing lower (0–1) or higher (2–3) nuclear or cytoplasmic immunoreactivity score. Significantly shorter mean survival times characterised the cases with higher cytoplasmic maspin immunoreactivity (2–3) in comparison with the patients with lower (0–1) immunoreactivity ($p = 0.000$). In contrast, longer survival times characterised the cases with higher nuclear immunoreactivity scores (2–3) in comparison with patients exhibiting low immunoreactivity (0–1) ($p = 0.000$). Kaplan-Meier survival curves depicting the dependence between patient survival rates and maspin expression are shown in Figure 2.

Other prognostic factors in univariate survival analysis were age, tumour grade, FIGO stage, distant metastases, disease remission, and subtype of epithelial ovarian carcinoma. Tumour grade, FIGO stage, and distant metastases were significant survival factors, whereas the patients' age and subtype of cancer were non-significant survival factors (Table 4).

In the multivariate survival analysis (Cox regression), strong nuclear maspin expression (score 2 and 3) remained a significant independent prognostic factor ($p = 0.001$) with a relative risk of 5.337, but the cytoplasmic strong maspin expression was not significantly correlated with survival rates ($p = 0.104$; relative risk 1.583). Other independent factors in multivariate analysis were high tumour grade (G3)

Table 3. Association between nuclear maspin expression and clinicopathological variables

Patients	Negative (0)	Low (1)	Moderate (2)	Strong (3)	P	(0–1)	(2–3)	P
Age:					0.400			0.948
≤ 65	17 (58.6%)	8 (34.8%)	34 (47.2%)	4 (50%)		25 (48.1%)	38 (47.5%)	
> 65	12 (41.4%)	15 (65.2%)	38 (52.8%)	4 (50%)		27 (51.9%)	42 (52.5%)	
Grade (G):					0.007			0.025
1	6 (20.7%)	4 (17.4%)	25 (34.7%)	7 (87.5%)		10 (19.2%)	32 (40.0%)	
2	9 (34.4%)	10 (43.5%)	26 (36.1%)	1 (12.5%)		19 (36.5%)	27 (33.8%)	
3	14 (43.8%)	9 (39.1%)	21 (29.2%)	0 (0%)		23 (44.2%)	21 (26.3%)	
Stage (FIGO):					0.019*			0.004
I	4 (13.8%)	1 (4.3%)	25 (34.7%)	2 (25%)		5 (9.6%)	27 (33.8%)	
II	3 (10.3%)	5 (21.7%)	13 (18.1%)	4 (50%)		p8 (15.4%)	17 (21.3%)	
III	11 (37.9%)	9 (39.1%)	20 (27.8%)	1 (12.5%		20 (38.5%)	21 (26.3%)	
IV	11 (37.9%)	8 (34.8%)	14 (19.4%)	1 (12.5%)		19 (36.5%)	15 (18.8%)	
Distant metastasis:					0.005			0.001
No	7 (24.1%)	6 (26.1%)	38 (52.8%)	6 (75.0%)		13 (25.0%)	44 (55.0%)	
Yes	22 (75.9%)	17 (73.9%)	34 (47.2%)	2 (25%)		39 (75.0%)	36 (45.0%)	
Remission:					0.000			0.000
No	27 (93.1%)	22 (95.7%)	40 (55.6%)	3 (37.5%)		49 (94.2%)	43 (53.8%)	
Yes	2 (6.9%)	1 (4.3%)	32 (44.4%)	5 (62.5%)		3 (5.8%)	37 (46.3%)	
Histological subtype:					0.080			0.261
Endometroid	11 (37.9%)	13 (56.5%)	26 (36.1%)	0 (0%)		24 (46.2%)	26 (32.5%)	
Serous	6 (20.7%)	5 (21.7%)	19 (26.4%)	5 (62.5%)		11 (21.2%)	24 (30.0%)	
Other	12 (41.4%)	5 (21.7%)	27 (37.5%)	3 (37.5%)		17 (32.7%)	30 (37.5%)	

*Fisher's exact test

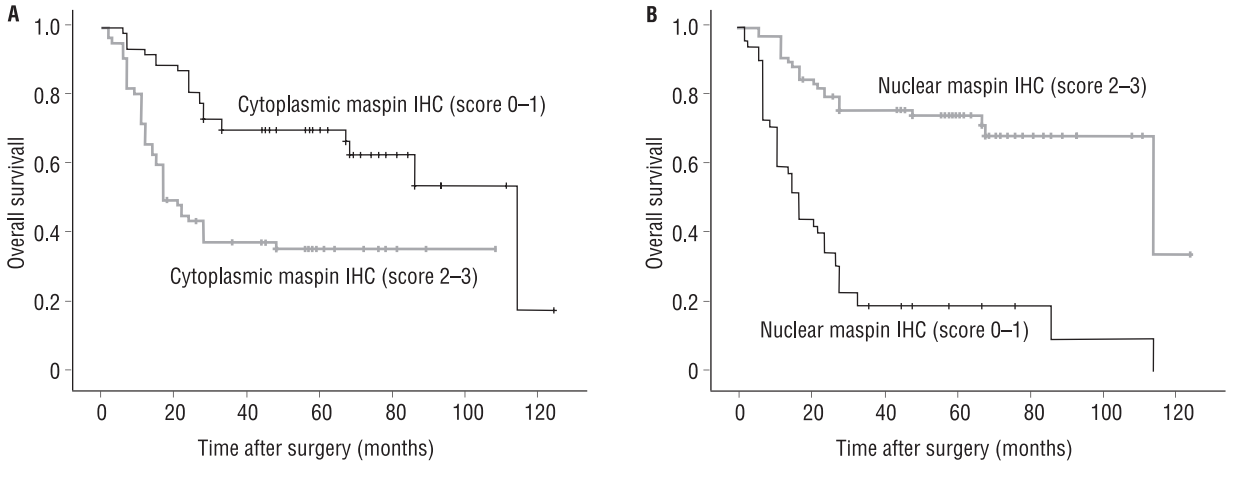

Figure 2. Overall survival plot in patients with epithelial ovarian carcinoma according to the Kaplan-Meier method; **A.** Patients having tumours with high maspin cytoplasmic expression (score 2 and 3) have a significantly poorer prognosis and survival rate than patients with low cytoplasmic expression (score 0 and 1); **B.** Patients with higher nuclear immunoreactivity (score 2 and 3) of maspin expression have an increased overall survival time and an increased progression-free time than patients with low (score 0 and 1) maspin nuclear expression.

and high tumour stage (FIGO III–IV). The patients' age was not a significant prognostic factor of relative risk of death (Tables 5 and 6).

DISCUSSION

There are several published studies on expression and clinical significance of maspin in human cancers. In this study a reversed correlation of intranuclear and cytoplasmic maspin fractions with invasive epithelial ovarian carcinoma stage was found, which is in agreement with the earlier literature concerning ovarian, breast, kidney, and lung cancers [7, 8, 20, 32].

Although maspin is a tumour suppressor which inhibits cell motility, invasion, and metastasis in

Table 4. Kaplan-Meier survival analysis

Patients	Cases	Events	Mean survival (Mo)	SE	P
Age:					0.391
≤ 65	63	32	70.215	6.571	
> 65	69	35	51.018	4.527	
Grade (G):					0.000
1	42	8	78.839	4.186	
2	46	27	64.476	6.815	
3	44	32	41.208	7.647	
Stage (FIGO):					0.000
1	32	2	104.938	4.151	
2	25	8	93.305	8.789	
3	41	25	42.747	5.219	
4	34	32	24.412	5.093	
Distant metastasis:					0.000
No	57	10	103.019	5.649	
Yes	75	57	37.587	5.102	
Remission:					0.000
No	92	66	45.521	4.720	
Yes	40	1	121.175	2.789	
Histological subtype:					0.146
Endometroid	50	31	57.500	7.437	
Serous	35	16	71.171	8.388	
Other	47	20	70.518	6.856	
Maspin cytoplasmic expression:					0.001
Negative	44	18	74.466	8.101	
Low	20	6	94.700	9.967	
Moderate	58	37	48.174	5.959	
Strong	10	6	34.800	9.612	
Maspin nuclear expression:					0.000
Negative	29	25	32.793	7.021	
Low	23	19	25.391	5.072	
Moderate	72	22	88.639	6.047	
Strong	8	1	84.375	8.068	
Maspin cytoplasmic expression:					0.000
Negative/low (0–1)	64	23	83.539	6.309	
Moderate/strong (2–3)	68	41	49.920	5.681	
Maspin nuclear expression:					0.000
Negative/low (0–1)	52	43	31.993	5.300	
Moderate/strong (2–3)	80	21	92.960	5.674	

Table 5. Hazard ratios (HR) for Cox proportional hazards model for nuclear maspin expression

	HR	95% CI for HR	P
Age: per year	0.992	0.976–1.008	0.320
Stage (FIGO):			
1–2	1.000		
3–4	5.193	2.365–11.401	< 0.001
Grade (G):			
1	1.000		
2	1.141	0.477–2.728	0.767
3	4.312	1.849–10.055	0.001
Maspin nuclear expression:			
Negative/low	5.337	2.915–9.771	0.001
Moderate/strong	1.000		

Table 6. Hazard ratios (HR) for Cox proportional hazards model for cytoplasmic maspin expression

	HR	95% CI for HR	P
Age: per year	0.993	0.973–1.004	0.141
Stage (FIGO):			
1–2	1.000		
3–4	6.117	2.924–12.797	0.001
Grade:			
1	1.000		
2	2.107	0.930–4.772	0.074
3	3.978	1.783–8.874	0.001
Maspin cytoplasmic expression:			
Negative/low	1.000		
Moderate/strong	1.583	0.910–2.753	0.104

some cancers, there are many contradictory reports about its expression and prognostic significance in other cancers [17, 38]. Downregulation of maspin expression is associated with the progression of breast, prostate, and colon carcinomas. On the other hand, in pancreatic, ovarian, and gastric cancers overexpression of maspin has been shown to correlate with cancer progression and poor prognosis [12]. In many studies maspin expression has been associated with the level of cancer differentiation and tumour grade. In pancreatic cancers, strong expression has been observed in poorly differentiated cancers (high-grade), whereas normal tissues as well as highly differentiated cancers (low-grade) have been characterised by a lack of maspin expression [16]. Pemberton et al. [22] found that maspin is a predominantly soluble cytoplasmic protein associated with secretory vesicles at the cell surface in mammary gland myoepithelial cells.

This study demonstrated maspin expression in almost 90% of invasive epithelial ovarian carcinoma cases. Similar expression in ovarian cancers has been exhibited immunocytochemically [32] and by using the immunoblotting technique [25]. In the present study, maspin staining was detected in the nucleus and cytoplasm. More than half of the examined ovarian carcinomas (52.08%) showed maspin expression both in the nucleus and in cytoplasm, 30% exclusively within the nucleus, and in only 6.25% of cases the expression was limited to the cytoplasm. Hence, the total nuclear expression was observed in 82.08% of examined cancers. Sood et al. [32] detected maspin expression in most of the examined ovarian cancers, and in the majority of the cases it was nuclear expression. Mohsin et al. [20], after immunocytochemical examination of 1068 invasive breast carcinoma cases, exhibited nuclear maspin reaction in 96% of cases, and cytoplasmic in 35%. Nuclear maspin localisation has also been shown in immunocytochemical studies on cancers of: prostate [18], lung [15], colon [5], and pancreas [16], as well as squamous cell carcinomas of the larynx [19].

In this work, the results obtained from immunohistochemical studies separately analysing nuclear and cytoplasmic maspin expression were compared with clinicopathological data. Nuclear maspin overexpression was correlated with lower tumour stage ($p = 0.004$), grade ($p = 0.025$), and higher remission rates ($p = 0.000$). No correlation was found between nuclear maspin expression and tumour type or women's age.

Cytoplasmic maspin expression also exhibited statistically significant correlation with tumour grade ($p = 0.03$) and FIGO stage, but this dependency demonstrated a reversed direction in comparison with nuclear expression: the greatest cytoplasmic levels of maspin were observed in high-grade, poorly differentiated cancers, and higher FIGO stage of disease (especially FIGO III and IV).

Kaplan-Meier's univariate survival analysis indicates that patients with high nuclear maspin expression (scores 2 and 3) have longer survival rates in comparison with those showing weak or no expression. The reversed pattern was observed in patients with high cytoplasmic maspin expression, whose survival rates were shorter. The performance of Cox regression analysis (multivariate) demonstrated that in the group of patients with low or no maspin expression the risk of death increased by over five-fold as compared with the group characterised by an average or high maspin expression ($HR = 5.337$; $p = 0.001$). Higher intensity of cytoplasmic maspin expression tended to correlate directly with risk of death; however, this effect proved to be statistically not significant.

These results are in agreement with the data obtained by Sood et al. [32] and Mohsin et al. [20], who discovered that in ovarian and breast cancers nuclear maspin expression is correlated with longer disease-free interval in patients. Cytoplasmic staining was associated with poor prognostic factors and shorter survival rates.

There is increasing evidence pointing to the disparate functions of maspin with regard to its subcellular localisation: whereas nuclear localisation is associated with good prognosis in many cancers, maspin presence in the cytoplasm is a bad prognostic factor [15]. Nuclear maspin fraction is thought to be a biologically active form playing a substantial role in cancer suppression, whereas the cytoplasmic fraction seems to be an inactive form [9]. Maspin accumulation in the cytoplasm may lead to its autoinhibition through polymerisation, resulting in activity decline [34]. This hypothesis would explain the high cytoplasmic maspin expression in ovarian cancers as well as its correlation with morphological and clinical features of tumours indicative of poor prognosis.

Maspin function, as well as mechanisms controlling its expression in ovarian cancers, has not been well explained. Studies on cell lines derived from normal and cancerous ovarian cells suggest that maspin expression is regulated epigenetically via methylation of maspin gene promoter. Cytosine methylation leads to the formation of a protein com-

plex containing methyl binding protein which inhibits gene transcription. Treatment of ovarian cells showing no maspin expression with DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine (5-aza-dC) abolishes epigenetic silencing of maspin gene [24]. Some studies show that p53 protein activates maspin expression through direct binding with the specific promoter region (consensus binding site), which stimulates nucleosome acetylation, chromatin relaxation, and gene activation [39].

The function of nuclear maspin is hardly known. It has been suggested that it participates in stress--induced apoptosis promotion, and probably affects some gene expression. Proapoptotic maspin action depends on the sensitisation of cells to exogenous factors inducing death. Maspin has been demonstrated to induce proapoptotic Bax protein, probably at the level of transcription and protein stability [14, 37]. Maspin increases the expression of transcription factors such as E2F1, antiangiogenic thrombospondin, and a complex of proteins rearranging chromatin SMARCA2, and decreases the expression of cytokines regulating inflammatory processes and cell proliferation [2]. Solomon et al. [29] demonstrated that nuclear maspin expression in ovarian cancer cells is associated with lower vascular endothelial growth factor expression and hence with diminished cancer vascularisation, which could explain the improved survival seen in patients with nuclear maspin expression.

Although direct molecular partners of maspin are not well known, recent studies suggest its interaction with oxidative stress-associated proteins (GST, glutathione S-transferase), heat shock proteins, histone deacetylase I (HDAC1), interferon regulatory factor 6 (IRF6), or transcription factors such as Egr1 and CGF2 (9). Maspin interaction with HDAC leads to its inhibition. In maspin-transfected prostate cancer cells the increase in the expression of genes downregulated by HDAC1, such as genes encoding p21 and Bax, has been observed [3]. Another molecule worth mentioning which is capable of interaction with maspin is IRF6, the protein which probably induces transition of the cells into the G0 phase. In breast cancers its level undergoes dynamic alteration resembling maspin changes; similarly to maspin, IRF6 expression decreases with cancer progression, while the induction of its expression leads to the cell cycle arrest [1].

CONCLUSIONS

In conclusion, maspin presence in two different cellular compartments may be associated with disparate function as well as with varied clinical implications. Numerous previous studies have suggested that nuclear maspin expression is a good prognostic factor in cancers derived from various organs including lung [27], mammary gland [20], and ovaries [32]. On the other hand, cytoplasmic maspin expression is associated with worse prognosis in the development of breast [20, 33] and lung [9, 13] cancers. The results obtained in this work suggest that nuclear and cytoplasmic maspin fractions may act in a reversed way in ovarian cancer. Positive correlation between nuclear maspin expression and histopathological and clinical features indicative of a good prognosis supports the hypothesis about its being a good prognostic factor in ovarian cancer [32]. Despite the fact that the mechanism of maspin action in ovarian cancer and its significance in cancer onset and progression are not explained, its expression and localisation may have potential prognostic significance and may be useful in planning future therapy.

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