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Maspin, VEGF and p53 Expression in Small Biopsies of Primary Advanced Lung Cancer and Relationship with Clinicopathologic Parameters

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Abstract Maspin, one of the serine protease inhibitors, has been shown to inhibit tumor progression and metastasis. We aimed to investigate maspin, p53 and VEGF expression in patients with squamous cell carcinoma (SCC), adenocarcinoma (AC) and small cell lung carcinoma (SCLC). The study included 28 SCC, 18 AC, 17 SCLC biopsy samples. We used the streptavidin biotin immunoperoxidase method to test for maspin, p53 and VEGF antibodies. Medical records of these patients were reviewed from archival files. Cytoplasmic maspin expression was detected in 89.3%, 77.8%, 52.9% of SCC, AC and SCLC, respectively. The rate was significantly higher in non-small cell lung cancer (NSCLC) and SCC than SCLC (p=0.013, p=0.021, respectively). The mean percentages of maspin expression were significantly higher in NSCLC, SCC and AC than in SCLC (p=0.0001, p=0.0001, p=0.038, respectively). In ACs, maspin and p53 expressions were correlated, although this was not statistically significant (p=0.053, r=0.464), and maspin positive cases had a significantly higher T status compared to negative cases (p=0.036). In SCC, the stage of disease was positively correlated with p53 (p=0.007, r=0.536) and negatively correlated with VEGF expression (p=0.013, r=−0.498). Multivariate analysis demonstrated that stage of disease was a significant independent prognostic parameter in NSCLC (95% confidence interval: 1.067–3.969; p=0.031). Although maspin expression is higher in SCC and AC, and is related with higher T status in AC, our data did not indicate its prognostic significance. Larger scale studies are needed to reveal the exact role of maspin in lung cancer pathogenesis.

Keywords Lung cancer · Maspin · VEGF · p53 · Biopsy · Immunohistochemistry

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Introduction

Protease and protease inhibitors play important roles in the progression of malignant tumors by promoting and/or antagonizing tumor invasion and metastasis. Maspin is a member of serine protease inhibitors (serpin), a large protein family, with diverse biological functions [1–4]. Maspin was discovered as a human mammary tumor suppressor gene [1], and studies have demonstrated its ability to inhibit cancer cell motility and invasion, and angiogenesis [1–5]. Some of the studies investigating maspin expression in non-small cell lung cancer (NSCLC) patients [6–14] have reported that enhanced maspin expression is a significant and independent factor predicting a favorable prognosis in squamous cell carcinoma (SCC) [6, 8, 9] and pulmonary adenocarcinoma (AC) [11, 12, 14]. However, other studies have reported contrary results. Hirai K et al. [10] have found maspin expression to be an independent negative prognostic factor for overall survival in NSCLC patients. Woenckhaus M et al. [7] have reported no correlation between maspin expression and patient's survival or the stage of disease. In addition, some studies have reported different subcellular localization of maspin expression, such as only cytoplasmic or nuclear and/or

cytoplasmic in NSCLC [3, 4, 6, 7, 11–14]. Overall, maspin status in lung tumors seems to be conflicting, and its exact roles in the development and progression of malignant tumors remain controversial and unclear.

Angiogenesis is an early step in tumor progression, and involved from the first stage of cancer development to the metastasis. At every stage of angiogenesis, one of the most important and effective factors is Vascular Endothelial Growth Factor (VEGF) [15–17]. It has been shown that overexpression of VEGF in the NSCLC is associated with increased metastatic potential and adverse prognosis [15, 17, 18]. p53 tumor suppressor gene mutation is one of the most common genetic changes in many tumors including lung cancer, and a mutated p53 gene or overexpression of its protein have been demonstrated in these tumors [17–21]. Studies have reported the importance of p53 in controlling angiogenesis by regulation of VEGF expression [17, 21–23]. It has been reported that mutant p53 induces VEGF expression, in contrast wild type p53 down-regulates it [23, 24]. Experimental studies have shown that VEGF activity is regulated by the p53 pathway, and also p53 activates the maspin promoter by binding directly to p53 consensus-binding site [3, 25]. In the literature, although the relationships of maspin expression with prognostic markers, such as micro-vessel density and proliferative rate of tumor cells [6–9] have been investigated in NSCLC, only a few studies have examined its association with p53 [6, 7, 11, 13, 14] and VEGF expressions [14]. Moreover, in these studies resected NSCLC materials have been used, and the clinical importance of maspin expression in small cell lung carcinoma (SCLC) patients and its relationship with VEGF and p53 remain unknown. Therefore, in this study we aimed to investigate maspin, p53 and VEGF expressions using immunohistochemistry in small biopsy materials of patients with primary SCC, AC and SCLC, and to assess their relationship with clinicopathological features.

Material and Methods

The Patients and Tissue Samples

The study included 63 primary lung cancer patients consisting of 28 SCC, 18 AC and 17 SCLC that had available tumor samples for immunohistochemical analysis. Paraffin-embedded archival material of bronchoscopic or trucut biopsies were used for this study. Histopathological diagnosis was performed according to WHO 2004 classification [20]. Only the biopsy specimens that histopathological subtypes were identifiable (SCC, AC) by light microscopic examination were selected to the study, the other not specified tumor samples were not included. Selected SCLC had been confirmed by immunohistochemistry including neuroendocrine markers during the previous histopathologic examination. No patients had had a diagnosis of lung carcinoma or received any treatment for lung carcinoma previously. For all patients, age, sex, tumor size, smoking status, and the data of radiological studies including chest X-ray, cranial, abdominal and chest computed tomography, and whole body bone scintigraphy were reviewed from the archival files. The performance status was evaluated according to ECOG (Eastern Cooperative Oncology Group) criteria [26]. The clinical course of the patients was identified from medical records and telephone inquiries. The clinical stages were defined according to the TNM [27] and modified VALG (Veterans Administration Lung Study Group) criteria [28] for NSCLC and SCLC, respectively. This study was reviewed and approved by the Ethics Committee, Faculty of Medicine, Suleyman Demirel University.

Immunohistochemical Analysis

The standard streptavidin biotin immunoperoxidase method was performed for maspin, p53 and VEGF antibodies (Table 1). The sections with a thickness of 4 μm were cut, deparaffinized in xylene, and dehydrated in descending dilutions of ethanol. Endogenous peroxidase activity was blocked using a 0.3% hydrogen peroxide solution for 20 min. For antigen retrieval, the sections were heated in citrate buffer solution (0.01 mol/L, pH6) for p53, and in ethylene diamine tetra-acetic acid buffer solution (EDTA) (1 mM, pH8) for both maspin and VEGF for 20 min. Background staining was minimized by incubation with goat serum (UltraVision HRP kit, LabVision, USA). The sections were incubated with a primary antibody followed by testing with a streptavidin-biotin-peroxidase kit (UltraVision Large Volume Detection System Anti-Polyvalent, HRP, LabVision, USA). Peroxidase activity was visualized with 3,3′-diaminobenzidine tetrachloride (DAB). Sections

Table 1 Antibodies used in the study and their dilutions

Antibodies	Dilution	Clone	Manufacturer	Place of production
Maspin	1/20	MS-1767 EAW24	LabVision	Fremont, CA, USA
P53	1/100	MS-186 DO-7	LabVision	Fremont, CA, USA
VEGF	1/100	MS-1467-P1 VG1	LabVision	Fremont, CA, USA

were counterstained with Mayer's hematoxylin, and mounted with mounting medium. Benign prostate, colon carcinoma and angiosarcoma tissues were used as positive controls for maspin, p53 and VEGF, respectively. Immunoreactivity was assessed only in the invasive tumor cells on the basis of the percentage of positive cells semi-quantitatively. The cases were classified as negative (−) if there was no staining for both maspin and VEGF. For p53, more than 5% positive nuclear staining in the tumor cells was accepted as aberrant expression, and quantified as the percentage of positive cells.

Statistical Analysis

Analysis was carried out using a software package for windows (SPSS, version 15.0. Chicago, Ill., USA). The association among biological factors was assessed by Mann Whitney *U* test and Spearman rank correlation coefficient. The Pearson square test was used to assess the association between clinicopathological and immunohistochemical data. Survival probabilities were estimated using the Kaplan–Meier method, with significance evaluated by two-sided log rank test. Overall survival was defined as the time from the date of pathologic diagnosis to death. Patients known to be still alive at the time of the analysis were censored at the time of their last follow up. Multivariate analysis of prognostic factors such as age, stage, performance status, and the positivities of maspin, VEGF and p53 were analyzed using Cox's regression model for each histological group. A value of $p<0.05$ was accepted as statistically significant.

Results

The study included 28 (44.4%) SCC, 18 (28.6%) AC and 17 (27%) SCLC. There were 58 males (92.1%) and 5 females (7.9%). The mean age was 61.4±12.4 (28–81) years in all cases, with 27 (42.9%) patients under 60 and 36 (57.1%) patients were 60 or older. All patients were symptomatic at the time of diagnosis. The mean size of tumor was 4.65±1.9 (range 2 to 9) in the measurable 38 NSCLC. Only 7 patients (13%) had never smoked, whereas 47 (87%) patients had smoked, with a mean 62.5±34.5 (15–150) pack-years. Performance status (ECOG) was stratified as 0–1 and 2–3 in 45 (81.8%) and 10 (18.2%) patients, respectively. In 40 NSCLC cases, there were 3 (7.5%) stage IIB, 2 (5%) stage IIIA, 9 (22.5%) IIIB, and 26 (65%) stage IV diseases. In SCLC patients, 2 (17.6%) had limited, and 14 (82.4%) cases had extensive disease.

Immunohistochemical Analysis

Cytoplasmic maspin expression was detected in 48 (76.2%) of all cases with a mean percentage of 38.2% (range 0–90%). Only three SCC cases showed a little scattered nuclear staining. As summarized in Table 2, 39 (84.8%) of NSCLC (25 SCC, 14 AC) and 9 (52.9%) of SCLC showed maspin immunoreactivity (Fig. 1a, b, c). The incidence of maspin positivity was significantly higher in the NSCLC and SCC patients than in SCLC cases ($p=0.013$, $p=0.021$, respectively). Mean percentage of maspin expression was highest in SCC (51.8%) and lowest in SCLC (17.1%). There were statistically significant differences in relation to the mean percentages of maspin expression between NSCLC and SCLC, and also between SCLC and SCC or AC ($p=0.0001$, $p=0.0001$, $p=0.038$, respectively), but not between SCC and AC ($p=0.092$).

Thirty-five cases (55.6%) showed nuclear p53 staining with a mean percentage of 21.9% (range 0–95%) in all cases. As seen in Table 2, 27 (58.7%) cases of NSCLC (15 SCC, 12 AC) and 8 (47.1%) cases of SCLC were positive for p53 (Fig. 2a, b, c). VEGF immunreactivity was seen in 59 (93.7%) cases, of which 43 (93.5%) were NSCLC (all SCC and 15 AC) and 16 (94.1%) were SCLC, with a mean percentage of 62.4% (range 0–95%) (Fig. 3a, b, c). There were no significant differences among the histological types regarding either the incidence of positivity or the mean percentages of p53 and VEGF expression ($p>0.05$), although the difference in incidence of VEGF positivity between SCC and AC approached significance ($p=0.054$) (Table 2).

The data were analyzed according to each histological type. In the NSCLC group, the positive correlation between the percentage of maspin expression and p53 expression approached significance ($p=0.054$, $r=0.286$). As summa-

Table 2 Maspin, p53 and VEGF expression according to histological subtypes of lung cancer

	n	Maspin expression		P53 expression		VEGF expression	
		n (%)	mean %	*n* (%)	mean %	*n* (%)	mean %
NSCLC	46	39 (84.8)*	46.0[#]	27 (58.7)	22.3	43 (93.5)	65.2
SCC	28	25 (89.3)[†]	51.8[§]	15 (53.6)	20.9	28 (100)[δ]	66.96
AC	18	14 (77.8)	36.9[‡]	12 (66.7)	24.5	15 (83.3)[δ]	62.50
SCLC	17	9 (52.9)*,[†]	17.1[#,§,‡]	8 (47.1)	20.6	16 (94.1)	54.71

*$p=0.013$ (NSCLC vs. SCLC), [†] $p=0.021$ (SCC vs. SCLC), [δ] $p=0.054$ (SCC vs. AC), [#] $p=0.0001$ (NSCLC vs. SCLC), [§] $p=0.0001$ (SCC vs. SCLC), [‡] $p=0.038$ (AC vs. SCLC)

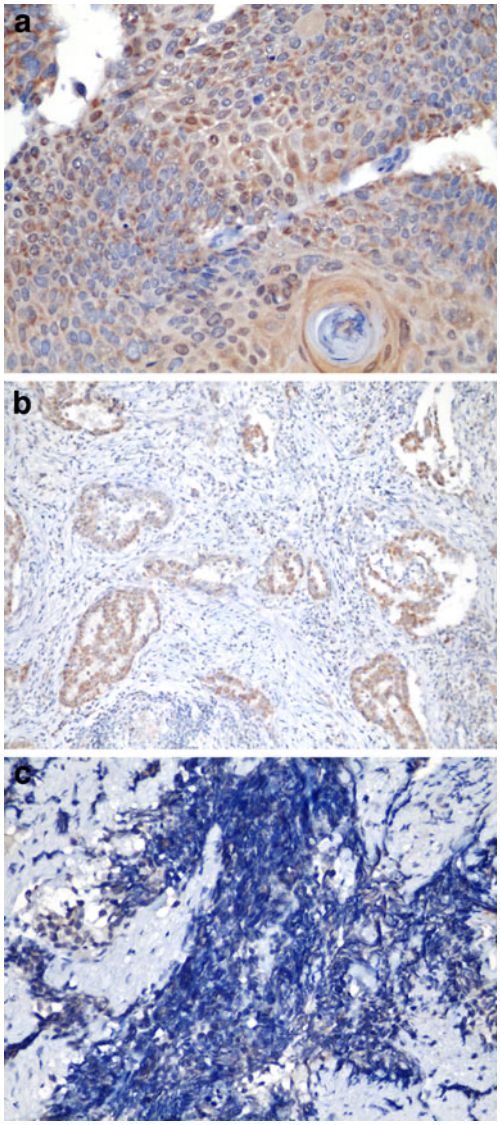

Fig. 1 Maspin immunoreactivity in the tumoral cells in **a** SCC, **b** AC, **c** SCLC

rized in Table 3, p53 positive cases had metastasis and a higher stage of disease, in contrast to the p53 negative ones (p=0.008, p=0.043, respectively). While p53 expression was positively correlated with the stage of the disease (p=0.034, r=0.335), VEGF expression was negatively correlated (p=0.046, r=−0.317). In AC patients, as found in NSCLC, the correlation between the percentages of maspin and p53 expression approached significance (p=0.053, r=0.464). In addition, 11 of 12 p53 positive AC cases were maspin positive, whereas 3 of 6 p53 negative cases were maspin negative (p=0.083). The maspin positive cases (n=13) had significantly higher T status (eight cases) compared to maspin negative cases (three cases) (p=0.036). In the SCC group, there was no significant association between maspin, p53 and VEGF expression. However, as found in NSCLC, more p53 positive cases had metastasis (10/12) and a higher stage of disease than negative patients (3/12) (p=0.006, p=0.033, respectively). The stage of disease was positively correlated with p53 expression (p=0.007, r=0.536) and negatively correlated with VEGF expression (p=0.013, r=−0.498). In the SCLC group, there were no significant relationships between maspin, p53 and VEGF expression, or with clinicopathological parameters.

Survival Analysis

Clinical follow-up data were obtained in only 54 (85.7%) of 63 patients. Of these 54, 11 (17.5%) were alive and 43 (68.3%) had died at the end of the study. The overall survival rate ranged from 10 to 800 days (mean 261.8± 212.46 days). This distribution varied by histological type:

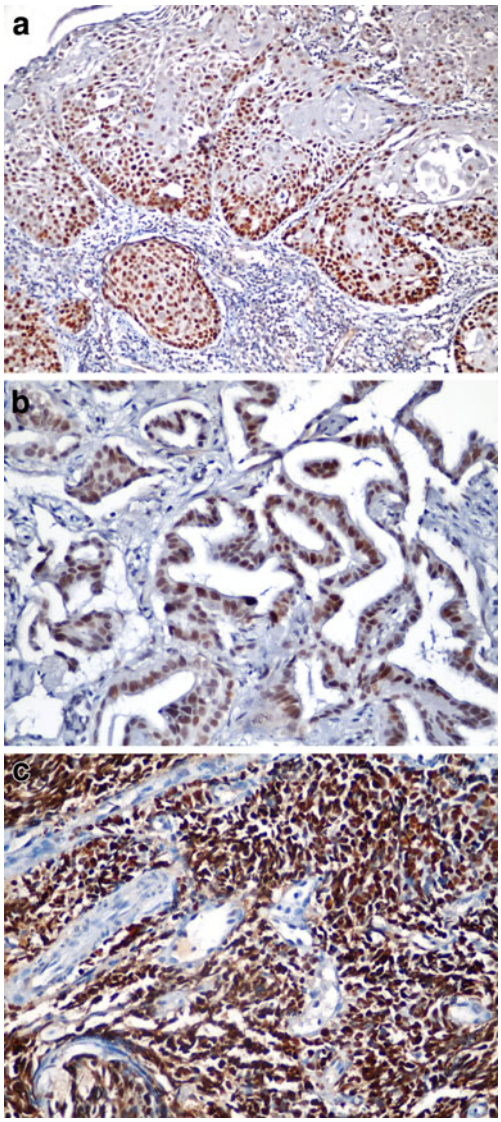

Fig. 2 p53 immunoreactivity in the tumoral cells in **a** SCC, **b** AC, **c** SCLC

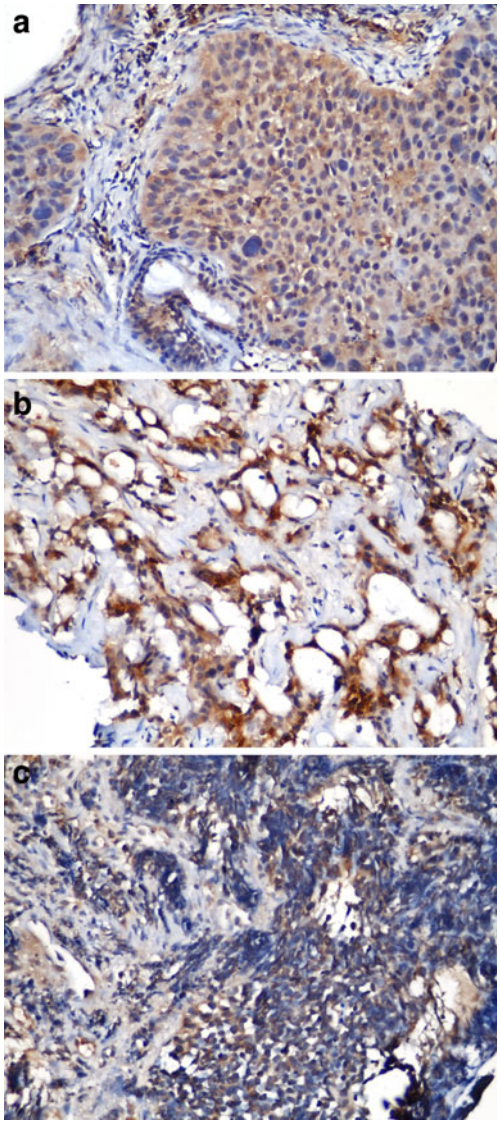

Fig. 3 VEGF immunoreactivity in the tumoral cells in **a** SCC, **b** AC, **c** SCLC

249.5±208.4 days (range 10–683) in 38 NSCLC patients; 259.1±223.1 days (range 21–675) in 21 SCC patients; 237.6±194.7 days (range 10–683) in 17 ACs; and 291.1± 226.0 days (range 19–800) in 16 SCLCs. Differences in overall survival rates were analyzed using the Kaplan Meier log rank test in each histological group, and any significant difference was found between immunopositive and negative patients for maspin, p53 and VEGF. The stage of disease was significant prognostic parameter affecting overall survival in NSCLC (p=0.030). Although its effect was not statistically significant, performance status appeared to be another prognostic parameter (p=0.058). In AC patients, stage of disease and presence of metastasis were significant parameters for survival (p=0.027, p= 0.007, respectively), as were ECOG and N status in SCC patients (p=0.043, p=0.037, respectively). Multivariate Cox's regression analysis demonstrated that stage of disease was a significant independent factor in predicting prognosis for NSCLC (hazard ratio: 2.058, 95% confidence interval [CI] 1.067–3.969; p=0.031) (Table 4).

Discussion

In the current study, we demonstrated that the incidence of maspin positivity in small biopsy materials was significantly higher in NSCLC and SCC cases than other types of lung cancer. Furthermore, the mean percentages of maspin expression in NSCLC, SCC and AC were also significantly higher than that of SCLC. It was also higher in SCC than AC, although this difference did not reach statistical significance. Several studies have reported that both the incidence of maspin expression and its staining intensity are significantly higher in resected lung SCC than the other types [6–8]. Katakura et al. [9] confirmed this finding by measuring maspin mRNA expression quantitatively in SSC cases. It has also been demonstrated that increased maspin expression is a significant independent factor in predicting a favorable prognosis in pulmonary SCC [6, 8, 9], and that it has prognostic significance in AC patients [11, 12, 14]. In contrast, Hirai et al. [10] showed that cytoplasmic maspin expression was an independent negative prognostic factor in NSCLC patients, in that maspin-positive patients had a poorer prognosis compared to negative ones. Other studies have not reported any significant association between maspin expression and clinicopathological variables or survival in NSCLC cases [6, 7, 9, 12]. In the current study, although both the incidence and mean percentage of maspin expression were significantly higher in SCC and NSCLC, we did not demonstrate a statistically significant prognostic difference between maspin positive and negative cases. This may be because our study included small number of cases and also small biopsy materials, not resected lung tissues. Although maspin expression was unrelated to the clinical features of SCC and SCLC patients, in AC group maspin positive cases had significantly higher T status than negative ones. From these conflicting results, the clinical significance of maspin expression in lung cancer seems to remain unclear. In addition, the finding of lower maspin expression in the SCLC subtype may suggest that maspin may have a different role in SCLC and NSCLC types.

Regarding imunohistochemistry, some studies have reported only cytoplasmic reactivity [6], whereas others have demonstrated nuclear and/or cytoplasmic expression [7, 11–14]. It has also been reported that these expression patterns were involved in the histogenesis of different lung carcinomas [11–13]. In the current study, we obtained cytoplasmic staining in all cases, except for three SCC cases in which a little scattered nuclear staining was also

Table 3 Maspin, p53 and VEGF expression in NSCLC cases

	n (%)	Maspin		*p*	P53		*p*	VEGF		*P*
		+	−		+	−		+	−	
All cases	46 (100)	39	7		27	19		43	3	
Age				ns			ns			ns
<60	20 (43.5)	16	4		11	9		19	1	
≥60	26 (56.5)	23	3		16	10		24	2	
Sex				ns			ns			ns
Male	42 (91.3)	35	7		24	18		39	3	
Female	4 (8.7)	4	0		3	1		4	0	
Tumor size				ns			ns			ns
<4 cm	16 (42.1)	13	3		10	6		15	1	
≥4 cm	22 (57.9)	20	2		12	10		21	1	
ECOG				ns			ns			ns
0–1	31 (79.5)	27	4		16	15		30	1	
2–3	8 (20.5)	7	1		6	2		6	2	
Tumor status				**0.061**			ns			ns
T1	0 (0)	–	–		–	–		–	–	
T2	16 (40)	13	3		8	8		15	1	
T3	4 (10)	2	2		2	2		3	1	
T4	20 (50)	19	1		13	7		19	1	
Node status				ns			ns			ns
N0	4 (10)	4	0		3	1		4	0	
N1	6 (15)	6	0		3	3		5	1	
N2	21 (52.5)	16	5		12	9		19	2	
N3	9 (22.5)	8	1		5	4		9	0	
Metastasis				ns			**0.008**			ns
Negative	14 (35)	13	1		4	10		14	0	
Positive	26 (65)	21	5		19	7		23	3	
Stage				ns			**0.043**			ns
IIB	3 (7.5)	3	0		1	2		3	0	
IIIA	2 (5)	2	0		0	2		2	0	
IIIB	9 (22.5)	8	1		3	6		9	0	
IV	26 (65)	21	5		19	7		23	3	
Maspin +	39				25	14	**0.091**	37	2	ns
P53 +	27	25	2	**0.091**				26	1	ns
VEGF +	43	37	6	ns	26	17	ns			

ns not significant

Table 4 Multivariate analysis of prognostic factors (Cox's regression model) in NSCLC patients

	B	SE	Exp(B)	*p*	95% CI	
					Lower	Upper
Age, years, < or ≥60	0.084	0.436	0.919	0.846	0.391	2.161
Stage, II–IIIA/IIIB–IV	0.722	0.335	2.058	**0.031**	1.067	3.969
ECOG, 0–I/II–IV	−0.993	0.515	2.699	**0.054**	0.983	7.411
Maspin, +/−	0.191	0.719	0.826	0.791	0.202	3.383
P53, +/−	0.140	0.530	0.869	0.791	0.308	2.454
VEGF, +/−	−0.897	0.857	2.452	0.296	0.457	13.166

present. Woenckhaus et al. [7] demonstrated that both cytoplasmic and nuclear maspin expressions were seen more frequently in SCC cases than other types. Zheng et al. [11] revealed that cytoplasmic staining was the lowest in AC and highest in SCC, and that nuclear maspin expression increased gradually from SCC, through AC and large cell carcinoma to SCLC. They also reported that the cytoplasmic pattern was a predictor of good survival only for AC cases [11]. On the other hand, some studies revealed that nuclear maspin expression was significantly correlated with lower histological grade, proliferative rate and prolonged survival in resected AC and NSCLC cases [13, 14, 29]. These immunostaining results may suggest that different subcellular distributions of maspin expression may have distinct molecular features in the carcinogenesis of lung carcinomas.

The p53 gene is the most studied gene in all types of cancer [18]. Its prognostic role in lung cancer is controversial [30]. Although some studies have found no prognostic significance, others, including a meta-analysis, have reported that p53 abnormalities had an adverse prognostic effect in NSCLC [18, 20, 30–32]. Tsao et al. [32] also found that p53 was a significant predictive marker for a differentially greater benefit from adjuvant chemotherapy in resected NSCLC patients. It has also been reported that p53 protein expression is significantly different among histological subtypes, in which AC has less positive results than SCC or large cell carcinoma [18, 31], and that p53 abnormalities may predict poor prognosis in patients with AC [20]. In our study, although more cases of AC were positive for p53, there was no significant difference between histological types. However, p53 expression was higher in patients with advanced stage, especially in the SCC subtype, but it was not related to overall survival. These results may confirm that p53 expression could be one of the adverse prognostic parameters particularly in SCC subtype.

It has been reported that p53 activates maspin by binding directly to the maspin promoter. DNA damaging agents and cytotoxic drugs induce endogenous maspin expression in cells containing wild type p53, and maspin expression is refractory to DNA damaging agents in cells containing mutant p53 [25]. Although some studies have found that maspin inhibits the development or progression of malignant tumors through some mechanisms such as a p53-dependent pathway and angiogenesis inhibition, its exact biological mechanism is unclear [5, 6, 25]. To date a few studies have investigated the relationship between maspin and p53 in NSCLC, and a significant positive correlation has been reported between increased cytoplasmic maspin expression and aberrant p53 expression [6, 11]. However, Woenckhaus et al. [7] demonstrated that positive p53 staining was significantly associated with nuclear maspin expression in NSCLC, but not with cytoplasmic expression. Recently, Frey et al. [14] revealed a correlation between the subcellular expression of maspin and p53 expression in resected pulmonary AC. On the other hand, Lonardo et al. [13] reported that nuclear maspin expression was correlated with negative p53 staining in pulmonary ACs. In the current study, cytoplasmic maspin expression in NSCLC and AC appeared to be positively correlated with p53 expression. These results may suggest that the biological effects of maspin seem to be linked to its subcellular localization.

VEGF is one of the most important angiogenic factors, and its expression is reported in approximately 40–90% of all NSCLC cases [15, 16, 21, 33, 34]. In our study, consistent with Yoo et al. [35], VEGF staining was detected in more than 90% of all cases. Most studies have revealed that VEGF expression is correlated with tumor stage, poor outcomes and lower disease-free rate in NSCLC patients [15–18, 36, 37], although some studies have not found any significant association between VEGF and prognosis or histopathological features [21, 35, 38]. It has been speculated that uniform VEGF expression may enhance or reflect the malignant potential of the tumor itself [37]. Nakashima et al. [39] noted that different members of VEGF family had different prognostic roles in the NSCLC subgroup. Yilmaz et al. [15] found that VEGF immunostaining was an independent prognostic factor in early stage NSCLC, although no significant correlation was found between VEGF and histopathological type. However, Liao et al. [38] reported a higher rate of VEGF expression in the early than advanced stages in resected NSCLC, although it was not statistically significant. They hypothesized that neoangiogenesis was more obvious in the early stage than the advanced stage, whereas other biological markers, such as p53, became more evident in the later stage. Our study did not demonstrate a significant association between VEGF expression and patient survival. However, it was inversely correlated with clinical stage in the SCC subtype, but not in AC or SCLC. These findings may support the hypothesis that neoangiogenesis and VEGF expression may increase in early stage SCC, and that its clinical significance may be different between lung cancer subtypes.

Several studies have shown that p53 is involved in controlling angiogenesis by regulation of VEGF expression, and mutant type p53 may enhance VEGF expression, whereas wild type p53 down-regulates VEGF promoter activity [21, 23, 24]. In lung cancer patients, it has been reported that presence of p53 gene mutation is significantly associated with VEGF expression [31, 40]. In present study, like Ludovini et al. [21], we did not find a significant association between VEGF and p53 expression immunohistochemically. On the other hand, several reports have indicated that maspin could function as an inhibitor of

angiogenesis [3, 5]. Zheng et al. [11] found that nuclear maspin expression was positively associated with levels of VEGF expression. Recently, Frey et al. [14] also showed that maspin expression was positively correlated with VEGF-A levels in lung AC patients. However, other studies did not show any correlation between maspin expression and tumor angiogenesis, intratumoral microvessel density, proliferative activity or apoptosis in NSCLC [6, 8, 9]. In our study, we did not demonstrate any significant relationship between VEGF and maspin expression. These results may suggest that more studies should be planned investigating the relationship between maspin, VEGF and p53 in lung cancer.

Although a variety of biological markers have been investigated in predicting treatment and prognosis in lung cancer, no prognostic marker has been established yet. Currently, the best treatment for NSCLC is surgery, and TNM stage is the most important prognostic factor for survival [20, 35]. Consistent with these findings, our study, despite using small biopsy materials, confirmed the importance of clinical stage for NSCLC. Performance status appeared to be another important parameter affecting overall survival for NSCLC and SCC. Our results suggest that p53 protein expression may predict a poor outcome in SCC. Maspin expression is higher in SCC and AC subtypes than SCLC and is related with higher T status in AC, although our results did not indicate its prognostic significance in lung cancer. These results may suggest that maspin may have different roles in SCLC and NSCLC types, and further studies are needed to reveal the exact role of maspin in lung cancer pathogenesis.

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Conflict of Interest Statement All authors declare that there are no conflicts of interest.

References

1. Zou Z, Anisowicz A, Hendrix MJ et al (1994) Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. Science 263:526–529
2. Chen EI, Yates JR (2006) Maspin and tumor metastasis. IUBMB Life 58:25–29
3. Bailey CM, Khalkhali-Ellis Z, Seftor EA et al (2006) Biological functions of Maspin. J Cell Physiol 209:617–624
4. Lockett J, Yin S, Li X et al (2006) Tumor suppressive maspin and epithelial homeostasis. J Cell Biochem 97:651–660
5. Zhang M, Volpert O, Shi YH et al (2000) Maspin is an angiogenesis inhibitor. Nat Med 6:196–199
6. Nakagawa M, Katakura H, Adachi M et al (2006) Maspin expression and its clinical significance in non-small cell lung cancer. Ann Surg Oncol 13:1517–1523
7. Woenckhaus M, Bubendorf L, Dalquen P et al (2007) Nuclear and cytoplasmic maspin expression in primary non-small cell lung cancer. J Clin Pathol 60:483–486
8. Takanami I, Abiko T, Koizumi S (2008) Expression of maspin in non-small-cell lung cancer: correlation with clinical features. Clin Lung Cancer 9:361–366
9. Katakura H, Takenaka K, Nakagawa M et al (2006) Maspin gene expression is a significant prognostic factor in resected non-small cell lung cancer (NSCLC). Maspin in NSCLC. Lung Cancer 51:323–328
10. Hirai K, Koizumi K, Haraguchi S et al (2005) Prognostic significance of the tumor suppressor gene maspin in non-small cell lung cancer. Ann Thorac Surg 79:248–253
11. Zheng HC, Saito H, Masuda S et al (2008) Cytoplasmic and nuclear maspin expression in lung carcinomas: an immunohistochemical study using tissue microarrays. App Immunohistochem Mol Morphol 16:459–465
12. Nakashima M, Ohike N, Nagasaki K et al (2004) Prognostic significance of the maspin tumor suppressor gene in pulmonary adenocarcinoma. J Cancer Res Clin Oncol 130:475–479
13. Lonardo F, Li X, Siddiq F et al (2006) Maspin nuclear localization is linked to favorable morphological features in pulmonary adenocarcinoma. Lung Cancer 51:31–39
14. Frey A, Soubani AO, Adam AK et al (2009) Nuclear, compared with combined nuclear and cytoplasmic expression of maspin, is linked in lung adenocarcinoma to reduced VEGF-A levels and in Stage I, improved survival. Histopathology 54:590–597
15. Yilmaz A, Ernam D, Unsal E et al (2007) Vascular endothelial growth factor immunostaining correlates with postoperative relapse and survival in non-small cell lung cancer. Arch Med Res 38:764–768
16. Bremnes RM, Camps C, Sirera R (2006) Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood. Lung Cancer 51:143–158
17. Boldrini L, Gisfredi S, Ursino S et al (2005) Interleukin-8 in non-small cell lung carcinoma: relation with angiogenic pattern and p53 alterations. Lung Cancer 50:309–317
18. Zhu CQ, Shih W, Ling CH et al (2006) Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation. J Clin Pathol 59:790–800
19. Hollstein M, Sidransky D, Volgestein B et al (1991) p53 mutation in human cancers. Science 253:49–53
20. Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC (2004) World Health Organization classification of tumours. Pathology and genetics of tumours of the lung, pleura, thymus and heart. IARC Press, Lyon
21. Ludovini V, Gregorc V, Pistola L et al (2004) Vascular endothelial growth factor, p53, Rb, Bcl-2 expression and response to chemotherapy in advanced non-small cell lung cancer. Lung Cancer 46:77–85
22. Freemantle SJ, Dmitrovsky E (2002) Clinical link between p53 and angiogenesis in lung cancer. J Clin Oncol 20:883–884
23. Yuan A, Yu CJ, Luh KT et al (2002) Aberrant p53 expression correlates with expression of vascular endothelial growth factor mRNA and interleukin-8 mRNA and neoangiogenesis in non-small-cell lung cancer. J Clin Oncol 20:900–910
24. Mukhopadhyay D, Tsiokas L, Sukhatme VP (1995) Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. Cancer Res 57:6161–6165
25. Zou Z, Gao C, Nagaich AK et al (2000) p53 regulates the expression of the tumor suppressor gene maspin. J Biol Chem 275:6051–6054
26. Oken MM, Creech RH, Tormey DC et al (1982) Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649–655

27. Mountain CF (1997) Revisions in the international system for staging lung cancer. Chest 111:1710–1717
28. Stahel RA, Ginsberg R, Havemann K et al (1989) Staging and prognostic factors in small cell lung cancer: a consensus report. Lung Cancer 5:119–126
29. Smith SL, Watson SG, Ratschiller D et al (2003) Maspin- the most commonly expressed gene of the 18q21.3 serpin cluster in lung cancer- is strongly expressed in preneoplastic bronchial lesions. Oncogene 22:8677–8687
30. Steels E, Paesmans M, Berghamans T et al (2001) Role of p53 as a prognostic factor for survival in lung cancer: a systematic review of the literature with a meta-analysis. Eur Respir J 18:705–719
31. Laudanski J, Niklinska W, Burzykowski T et al (2001) Prognostic significance of p53 and bcl-2 abnormalities in operable nonsmall cell lung cancer. Eur Respir J 17:660–666
32. Tsao MS, Aviel-Ronen S, Ding K et al (2007) Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer. J Clin Oncol 25:5240–5247
33. O'Byrne KJ, Koukourakis MI, Giatromanolaki A et al (2000) Vascular endothelial growth factor, platelet-derived endothelial cell growth factor and angiogenesis in non-small-cell lung cancer. Br J Cancer 82:1427–1432
34. Fontanini G, Boldrini L, Vignati S et al (1998) Bcl-2 and p53 regulate vascular endothelial growth factor (VEGF)-mediated angiogenesis in non-small cell lung carcinoma. Eur J Cancer 34:718–723
35. Yoo J, Yung JH, Lee MA et al (2007) Immunohistochemical analysis of non-small cell lung cancer: correlation with clinical parameters and prognosis. J Korean Med Sci 22:318–325
36. Ito H, Oshita F, Kameda Y et al (2002) Expression of vascular endothelial growth factor and basic fibroblast growth factor in small adenocarcinomas. Oncol Rep 9:119–123
37. Seto T, Higashiyama M, Funai H et al (2006) Prognostic value of expression of vascular endothelial growth factor and its flt-1 and KDR receptors in stage I non-small- cell lung cancer. Lung Cancer 53:91–96
38. Liao M, Wang H, Lin Z et al (2001) Vascular endothelial growth factor and other biological predictors related to the postoperative survival rate on non-small cell lung cancer. Lung Cancer 33:125–132
39. Nakashima T, Huang CL, Liu D et al (2004) Expression of vascular endothelial growth factor-A and vascular endothelial growth factor-C as prognostic factors for non small cell lung cancer. Med Sci Monit 10:BR157–BR165
40. Niklinska W, Burzykowski T, Chyczewski L, Niklinski J (2001) Expression of vascular endothelial growth factor (VEGF) in non-small cell lung cancer (NSCLC): association with p53 gene mutation and prognosis. Lung Cancer 34:S59–S64