Maspin Expression in Renal Cell Carcinoma and Its Correlation with Clinicopathologic Parameters

Tahsin Turunc, Nebil Bal, Ayhan Dirim, Baris Kuzgunbay, Umit Gul, and Hakan Ozkardes

OBJECTIVES To investigate the relationship between maspin expression and prognostic parameters in renal cell carcinoma (RCC) with relevance to vascular endothelial growth factor (VEGF) expression and microvessel density.

METHODS One-hundred twenty-four patients with RCC of varying histologic types who underwent radical or partial nephrectomy were studied. The mean age of the patients was 59.4 years (range, 28-84). Maspin, VEGF, and microvessel density were studied by the universal avidin-biotin complex peroxidase method. Sections of 5-μm thickness were taken from paraffin blocks for immunohistochemical study. Cytoplasmic and/or nuclear staining were scored for maspin as negative and positive for all tumor cells.

RESULTS Cytoplasmic maspin expression was positive in 51 (41.1%) patients. Nuclear maspin expression was not seen in any of the materials. Maspin expression decreased as tumor size increased ($P = .036$) without any specific relation to tumor subtypes ($P = .583$), and decreased as the pathologic stages increased without reaching statistical significance ($P = .053$). There were no correlations between maspin positivity and either VEGF expression or microvessel density.

CONCLUSIONS In RCC, maspin expression is reduced with increased tumor size. Studies with larger series may be contributory in defining the role of maspin expression in RCC. Moreover, regulation of maspin expression genes appears to have the potential to lead to new treatment approaches. UROLOGY 76: 765.e8–765.e13, 2010. © 2010 Elsevier Inc.

Renal cell carcinoma (RCC) is one of the most malignant solid tumors and represents 2%-3% of all adult malignancies.[1] Although renal cancer can be diagnosed incidentally at early stages, resulting in a favorable prognosis, it still bears considerable mortality in many cases. Many pathologic findings related to RCC have been evaluated previously as prognostic factors, among which tumor stage, size, histologic subtype, and nuclear grade are prominent.[2] These markers may be predictive for prognosis, but indicators of invasive or metastatic potential are still needed.

Vascular endothelial growth factor (VEGF) is the primary proangiogenic growth factor, which contributes to the neovascularity of renal cancer.[3] VEGF is the most commonly studied factor due to its potent angiogenic traits throughout vasculogenesis and angiogenesis in embryologic and adult stages. It has been reported that VEGF expression is increased in patients with RCC.[4,5] Microvessel density (MVD) represents an estimate of tumor angiogenesis and has been associated with metastatic spread in patients with RCC.[6]

Mammary serine protease inhibitor (maspin) was first isolated and described by Zou et al. as a member of the serpin superfamily of protease inhibitors.[7] The regulatory mechanisms of maspin function, however, have not been fully described. Studies have demonstrated that maspin suppresses tumor growth and metastases by inhibiting tumor cell invasion and motility.[7,8] Zhang et al. have also demonstrated that maspin inhibits angiogenesis by reducing endothelial cell motility and MVD in cell cultures and rat cornea model systems.[9] In addition, although the mechanisms underlying its biological activities are still largely unknown, maspin seems to act on the cell membrane both directly and indirectly, affecting cell adhesion and inhibiting cell motility and invasion.[10] It has also been shown that maspin is highly expressed in various kinds of normal cells, whereas it displays decreased expression in several cancer cells.[8,11-13]

This study investigated the relation between maspin expression and the histologic subtypes, pathologic stages, nuclear grades, tumor sizes, and distant metastases of RCC. In addition, maspin expression in RCC was compared with that of VEGF and MVD for their relations with tumor angiogenesis.

From the Departments of Urology and Pathology, Baskent University Faculty of Medicine, Ankara, Turkey

Reprint requests: Baris Kuzgunbay, M.D., Baskent University Faculty of Medicine, Department of Urology, Adana Teaching and Medical Research Center, 01250 Adana, Turkey. E-mail: kuzgunbay33@yahoo.com

0090-4295/10/$34.00
doi:10.1016/j.urology.2009.12.077

MATERIAL AND METHODS

Clinicopathological Data

The clinical and pathologic findings of 124 RCC patients treated by radical or partial nephrectomy in our department between 2002 and 2008 were reviewed retrospectively. Of the 124 patients, 66.1% were male and 33.9% were female, with a mean age of 59.4 years (range, 28-84). The study was approved by the Baskent University ethical committee.

The tumors were staged according to the 2002 TNM classification and graded according to the Fuhrman grading system.[14,15] The histologic subtypes were assessed according to the consensus classification of renal cell neoplasia in the World Health Organization's 2004 classification system.[16]

The prognostic value of maspin was evaluated and maspin expression was correlated with the histologic tumor subtypes, pathologic stages, Fuhrman nuclear grades, tumor sizes, and the presence of distant metastases. In addition, the relation between maspin and VEGF expressions, and the relations between maspin expression and MVD were evaluated.

Immunohistochemical Evaluation

Immunohistochemistry was independently evaluated by a pathologist who was unaware of the clinical data. Sections of 5-μmthickness were obtained from paraffin blocks for immunohistochemical study. Maspin (monoclonal-mouse, clones EAW24, and MS-1767-R7; Lab Vision Corp./NeoMarkers, Fremont, CA), VEGF (clones VG1 and MS-1467-R7; Lab Vision Corp./NeoMarkers), and CD31 (clones JC/ 70A and MS-353-R7; Lab Vision Corp./NeoMarkers) were studied by using a universal avidin-biotin complex peroxidase (ultra-vision detection system, antipolyvalent; Lab Vision Corp./NeoMarkers) method. AEC (code K3469; Dako A/S, Glostrup, Denmark) was used as a chromogen. Cytoplasmic and/or nuclear staining for maspin and membranous and cytoplasmic granular staining for VEGF were evaluated in tumor cells.

Maspin staining was scored as negative (staining in 0%-5% of all tumor cells) or positive (staining in 6%-100% of all tumor cells). All tumor cells were evaluated for VEGF expression. Because of a lack of homogenous staining within the tumor, 2 parameters, as distribution and intensity of expression, were evaluated semiquantitively and scored separately.[17] The intensity of staining was determined as 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = heavy staining; and the distribution of staining was recorded as 0 = no staining, 1 = 1%-25%, 2 = 26%-50%, 3 = 51%-75%, and 4 = 76%-100%. The 2 scores were then multiplied to obtain a total score ranging from 0-12.

The MVD was measured by immunohistochemical staining with CD31 monoclonal antibody. The stained sections were screened at 100× magnification under a light microscope to identify 5 regions with the highest number of microvessels. The microvessels were then counted in these regions at 400× magnification. The final microvessel count was expressed as the mean number of vessels counted in 5 regions at high magnification.[6]

Statistical Analyses

The data were analyzed by using the SPSS for Windows software (version 16; SPSS, Inc., Chicago, IL). The relationship between maspin expression and categorical variables was compared by the chi-square test, or Fisher's exact probability test when appropriate. Nonclear cell tumors (papillary, chromophobe, and collecting duct) were evaluated together because of the small number of collecting duct tumors. The nonparametric Mann-Whitney *U* test was performed to compare maspin groups with VEGF score and CD31 counts. Survival curves were evaluated using the Kaplan–Meier method, and differences between groups were tested using the log-rank test. Cox regression analysis was used for multivariate analysis. A value of $P < .05$ was considered statistically significant.

RESULTS

Clinicopathological Findings

At clinical presentation, the tumor was incidental in 62 patients (50.0%) and flank pain was the main symptom in 29 patients (23%). The surgical treatment was radical nephrectomy in 106 patients and partial nephrectomy in 18 patients.

Of the 124 tumors examined, 68 (54.8%) were clear cell carcinomas (CC); 29 (23.4%) were papillary cell (PC); 23 (18.6%) were chromophobe cell (CPC); and 4 (3.2%) were carcinoma of the collecting ducts of Bellini (CDC). The mean tumor size was 6.1 cm (range, 1.2-15). The tumor size was ≤7 cm in 81 patients and >7 cm in 43 patients. Only Fuhrman grade 2 (n = 106, 85.5%) and grade 3 (n = 18, 14.5%) tumors were identified. No grade 1 tumor was identified in any of the pathologic specimens. Tumor stage was low (pT1, pT2) in 96 patients (77.4%) and high (pT3 and pT4) in 28 patients (22.6%). The demographic and clinicopathologic data of the 124 patients are shown in Table 1.

The mean follow-up period was 34.7 months (range, 3-120). The follow-up was based on a standard protocol. During the follow-up period, distant metastasis occurred in 25 patients. The metastasis of the lungs was the most frequent (n = 13). Other sites where metastases occurred were the bone (n = 7), brain (n = 3), peritoneum (n = 3), and liver (n = 3); in 4 patients, metastasis occurred at multiple sites. There were 3 patients with local recurrence in the operation sites. Six patients who were in pT3 stage previously and 19 patients in whom distant metastases developed were lost to follow-up.

Maspin Expression. Cytoplasmic maspin expression was detected in 51 (41.1%) patients. No nuclear expression of maspin was detected in any of the pathologic materials. Maspin expression was detected in 48.2% of the patients with a tumor size <7 cm, whereas the rate of maspin expression was 28% in patients with a tumor size >7 cm ($P = 0.036$). Maspin expression was detected in 65.2% of CPC, 38.2% of CC, 31% of PC, and 25% of CDC subtypes of RCC. There were no significant differences between the CC and nonclear cell subgroups with regard to maspin expression ($P = .583$). Moreover, no statistically significant differences were detected between CC and PC and between CC and CDC for maspin expression ($P > .05$), in the patients with CPC, the rate of maspin positivity was higher than that of the patients with CC ($P = .025$). Maspin staining of different histologic RCC subtypes is shown in Fig. 1. No significant

Table 1. The demographic and clinicopathologic data of the 124 patients

Variable	Value, n (%)
Age (yrs)	59.41 ± 11.1
Gender	
Male	82 (66.1)
Female	42 (33.9)
Clinical presentation	
Incidental	62 (50.0)
Symptomatic	62 (50.0)
Surgery	
Radical	106 (85.5)
Nephron-sparing	18 (14.5)
Tumor size	
≤7 cm	81 (65.3)
>7 cm	43 (34.7)
Localization	
Right	62 (50.0)
Left	62 (50.0)
Histologic subtype	
Clear cell	68 (54.8)
Papillary	29 (23.4)
Chromophobe	23 (18.6)
Collecting duct	4 (3.2)
Grade (Fuhrman)	
1	0 (0)
2	106 (85.5)
3	18 (14.5)
Pathologic stage	
Low (pT1, pT2)	96 (77.4)
High (pT3, pT4)	28 (22.6)
Distant metastasis	
Positive	25 (20.2)
Negative	99 (79.8)
Maspin	
Positive	51 (41.1)
Negative	73 (58.9)

correlation existed between maspin expression and tumor grades ($P = .301$). Maspin expression was demonstrated in 45.8% of the low stage (pT1, pT2) tumors, whereas its expression decreased to 25% in the tumors of higher stage (pT3, pT4) ($P = .053$). Maspin expression was 28% in the patients who developed metastasis in the follow-up, whereas it was 44.4% in the patients who were metastasis-free ($P = .174$).

There was no correlation between maspin positivity and either VEGF expression or microvessel density ($P = .204$ and $P = .768$, respectively). The statistical relationships between maspin expression and the clinicopathologic parameters of RCC are presented in Table 2.

Vascular Endothelial Growth Factor Expression and Microvessel Count. VEGF expression is predominantly observed in the cytoplasm of the cancer cells. No statistically significant correlations were found between the tumor subtypes and VEGF scores ($P = .757$). However, a statistically significant correlation was found between tumor stage and grade with VEGF score ($P = .006$ and $P = .008$, respectively). No statistically significant correlations were found between the survival rate and tumor size with VEGF scores ($P = .07$ and $P = .559$, respectively).

Tumor subtypes and MVD were not statistically correlated ($P = .526$). As the tumor stage, grade, and size increased, MVD levels statistically significantly decreased ($P = .01$, $P = .048$, and $P = .026$, respectively). Similarly, in the patients who died, the level of MVD decreased ($P = .002$).

No statistically significant relationship was found between VEGF scores and MVD ($P = .895$). VEGF and MVD positivites in RCC are shown in Fig. 2.

In Kaplan-Meier survival analysis, it was determined that with higher grade, size, and stage of the tumor, the survival was statistically significantly reduced (for each parameter $P < .001$). By contrast, maspin expression and tumor histologic subtypes and survival were not statistically significantly correlated ($P = .135$ and $P = .178$, respectively). Only the tumor stage was an independent predictor of cancer-specific survival in the multivariate analysis ($P < .001$).

COMMENT

Approximately 20%-30% of RCC cases are metastatic at the time of diagnosis.[18] The response of metastatic RCC to conventional chemotherapy, radiotherapy, and immunotherapy is inadequate. New prognostic markers of invasive and metastatic nature will be of great help in this regard. Characteristically, metastatic RCC is the most resistant malignancy to cytotoxic chemotherapy. Cytokine-based immunotherapy with interleukin-2 and/or interferon-alpha has been accepted as the standard treatment for patients with advanced RCC, although few patients benefit from the therapy. Recent advances in the understanding of molecular mechanisms and genetics of RCC have led to the identification of new targets, such as VEGF. Activation of VEGF pathway in RCC is associated with tumor angiogenesis, proliferation, and metastasis. Recently, multitargeted tyrosine kinase inhibitors (sunitinib, sorafenib) and mammalian target of rapamycin kinase inhibitors (temsirolimus, RAD001) targeting the VEGF pathway have shown clinical activity in metastatic RCC.[19]

Maspin (SerpinB5) is a member of the serine protease inhibitor superfamily, like plasminogen activator inhibitors 1 and 2, and $a1$-antitrypsin.[20] The structural homology of maspin to serpins led to the initial hypothesis that its tumor suppressor function might be attributed to its ability to inhibit proteolysis. This idea is supported by the fact that maspin was reported to inhibit the activity of tissue-type plasminogen activator.[21] Furthermore, it was shown that maspin can mediate the inhibition of urokinase-type plasminogen activator on the surface of prostate carcinoma cells.[22] Some studies revealed the proapoptotic effect of maspin and proposed that maspin might suppress tumor progression by enhancing cellular sensitivity to apoptotic stimuli.[23] Maspin is a discovered tumor suppressor gene whose tumor-suppressing mecha-

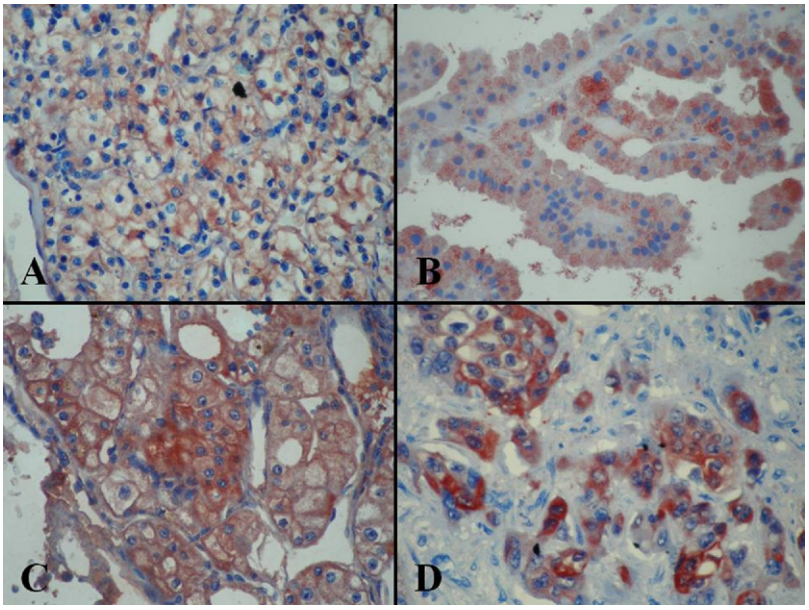

Figure 1. Maspin positivity in RCCs. **(A)** Clear cell carcinoma; **(B)** papillary renal cell carcinoma; **(C)** chromophobe RCC; **(D)** carcinoma of the collecting duct of Bellini. Original magnification 400×.

Table 2. Relationship between maspin expression and clinicopathological features of RCC

	Maspin Expression (n (%))		
	Negative	Positive	*P* Value
Tumor size			.036
≤7 cm	42 (51.8)	39 (48.2)	
>7 cm	31 (72)	12 (28)	
Histologic subtype			.583
Clear cell	42 (61.8)	26 (38.2)	
Nonclear cell			
Papillary	20 (69)	9 (31)	
Chromophobe	8 (34.8)	15 (65.2)	
Collecting duct	3 (75)	1 (25)	
Fuhrman Grade			.301
2	60 (56.6)	46 (43.4)	
3	13 (72.2)	5 (27.8)	
Pathologic stage			.053
Low (T1, T2)	52 (54.2)	44 (45.8)	
High (T3, T4)	21 (75)	7 (25)	
Distant metastasis			.174
Positive	18 (72)	7 (28)	
Negative	55 (55.6)	44 (44.4)	

nism seems to be as complex as the progression of the malignancy itself.[24]

Several recent studies have demonstrated that maspin is expressed in normal cells, downregulated in neoplastic cells, and absent in metastatic cells.[7,13] Tumor-suppressor effects of maspin have also been demonstrated before. However, conflicting data in nonsmall-cell lung and ovarian cancers are present, which show correlation with maspin positivity and poor prognosis.[25,26] In these studies, maspin showed cytoplasmic uptake. In a study investigating maspin in the urinary system and bladder cancer, it has been shown that cytoplasmic maspin expression is higher in normal bladder urothelium and superficial bladder tumors, whereas its expression is significantly reduced in invasive bladder carcinoma. Therefore, it has been suggested that maspin may be used as a marker in the prognosis of invasive carcinoma of the bladder.[12] In nonsmall-cell lung and ovarian cancers, cytoplasmic staining of maspin is a poor prognostic factor; in bladder cancer, it is a good prognostic factor.

The correlation between RCC and maspin markers has been investigated in only 1 study (Blandamura et al.), with the following findings. CC stained negatively with maspin, whereas positive staining was demonstrated in PC, CPC, and at least focally in CDC. Maspin nuclear reactivity was demonstrated in all patients examined; however, in only 1 CDC patient, cytoplasmic reactivity was detected. A negative correlation was found between the presence of metastasis in PC and CDC, and maspin.[27] In the present study, maspin staining was positive in 38.2% of CC patients, and in contrast to the aforementioned study, cytoplasmic maspin staining was observed in all tumor cells. Despite studies on maspin expression in RCC, the differences in the results of our study and the study by Blandamura et al. may be due to differences in the immunohistochemical methods used and use of different maspin rates in both studies. In our study, immunohistochemically ready Laboratory Vision Corporation/NeoMarkers was used, whereas Blandamura et al. used the Dako kit. The marker used in our study was previously used in studies on maspin expression,[11,26] and as in our study, in these studies, maspin showed cytoplasmic staining. In addition, the rates of maspin expression in different subtypes of RCC in both studies might have been due to different maspin cut-off values.

In some studies, cytoplasmic maspin staining in various tumors has been referred to as inactive maspin, and this

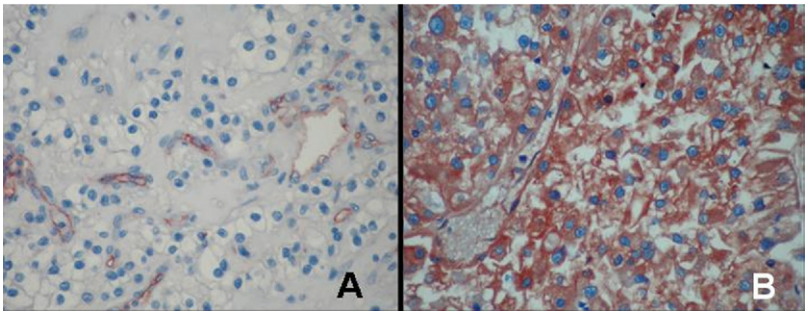

Figure 2. (A) Microvessel density positivity in clear cell carcinoma (original magnification 400×); **(B)** VEGF positivity in clear cell carcinoma (original magnification 400×).

has been interpreted as an indicator of poor prognosis. In contrast, nuclear maspin staining has been regarded as a good prognostic factor.[11,25,28] In our study, cytoplasmic maspin staining was evaluated as a good prognostic factor. These findings suggest that the presence of maspin in 2 different compartments of various tumor cells may have different biological and clinical implications. In a study by Mohsin et al., it was suggested that nuclear maspin staining might be associated with hormone receptor expression.[28] The presence of only cytoplasmic staining in our study may be a reflection of the hormonal inactivity of RCC in the absence of paraneoplastic syndromes. Another probability is the presence of already undefined isoforms of maspin, which may exert different biological activity in different cell types of either normal or tumoral origin. New evidence suggests that maspin's function is associated with its subcellular location. Therefore, targeted expression of maspin in tumor cells will provide tremendous insight into how maspin functions at different locations of the cell. Furthermore, to understand the ability to inhibit proteolysis, one of the suggested tumor suppressor functions of maspin,[21] it is important to identify interacting proteins in the context of the observed biological functions of maspin.[24] Therefore, further studies at a molecular level to investigate the presence or absence of maspin subgroups would provide clarity to these conflicting findings.

In our study, maspin was compared with VEGF and MVD because of their close relation with tumor angiogenesis. Zhang et al. have reported that maspin inhibited angiogenesis by decreasing MVD.[9] Bolat et al. have demonstrated the positive relation between VEGF expression and maspin, which is a poor prognostic factor in ovarian cancers.[26] Despite these studies, no relation was detected between maspin with VEGF and MVD in our study. This result might reflect that maspin displays its effect through mechanisms excluding angiogenesis.

The negative correlation between tumor size and maspin expression might be an indicator of reduced expression of this marker in more invasive tumors. In addition, this opinion was supported by the fact that there was maspin expression in 45.8% of the patients with low-stage tumors compared with 25% expression in the patients with higher-stage tumors. Similarly, maspin expression was 44.4% in patients without any distant metastasis, whereas it was only 28% in the patients who had distant metastases.

Despite recent increases in genetic and biological information and development of treatment strategies against cancer at the molecular level, effective and routinely used tumor markers, such as those used in the diagnosis of testis and prostate cancers, have not been defined for the follow-up of patients with RCC after treatment. Our study has some important limitations; the study is retrospective and the number of patients is quite low. If a higher number of cases had been included in our study, a negative relationship could have been found between tumor stage and maspin expression. Thus, in studies with larger series, results might be more objective. Moreover, maspin re-expression might become a therapeutic option in the treatment of invasive RCC. Further studies conducted on this subject will be enlightening.

Acknowledgement. This study was supported by a grant (KA06/145) provided by Baskent University School of Medicine.

References

1. Wein-Kavoussi-Novick-Partin-Peters. Renal tumors. In Campbell-Walsh: *Urology, Volume 2*, 9th ed. Location: Publisher; Year.
2. Frank I, Blute ML, Leibovich BC, et al. Independent validation of the 2002 American Joint Committee on cancer primary tumor classification for renal cell carcinoma using a large, single institution cohort. *J Urol.* 2005;173:1889-1892.
3. Gnarra JR, Zhou S, Merrill MJ, et al. Post-transcriptional regulation of vascular endothelial growth factor mRNA by the product of the VHL tumor supressor gene. *Proc Natl Acad Sci U S A.* 1996; 93:10589-10594.
4. Yildiz E, Gokce G, Kilicarslan H, et al. Prognostic value of the expression of Ki67, CD44 and vascular endothelial growth factor and microvessel invasion in renal cell carcinoma. *BJU Int.* 2004; 93:1087-1093.
5. Dirim A, Haberal AN, Goren MR, et al. VEGF, COX-2, and PCNA expression in renal cell carcinoma subtypes and their prognostic value. *Int Urol Nephrol.* 2008;40:861-868.
6. Fukata S, Inoue K, Kamada M, et al. Levels of angiogenesis and expression of angiogenesis-related genes are prognostic for organ-specific metastasis of renal cell carcinoma. *Cancer.* 2005;103:931-942.
7. Zou Z, Anisowicz A, Hendrix MJ, et al. Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. *Science.* 1994;263:526-529.
8. Sheng S, Carey J, Seftor EA, et al. Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells. *Proc Natl Acad Sci U S A.* 1996;93:11669-11674.

9. Zhang M, Volpert O, Shi YH, et al. Maspin is an angiogenesis inhibitor. *Nat Med*. 2000;6:196-199.
10. Bass R, Moreno FA-M, Ellis V. Maspin inhibits cell migration in the absence of protease inhibitory activity. *J Biol Chem*. 2002;277: 46845-46848.
11. Bal N, Kocer NE, Ertorer ME, et al. Maspin, E-selectin, and p-selectin expressions in papillary thyroid carcinomas and their correlation with prognostic parameters. *Pathol Res Pract*. 2008;204:743-750.
12. Beecken WD, Engl T, Engels K, et al. Clinical relevance of maspin expression in bladder cancer. *World J Urol*. 2006;24:338-344.
13. Maas N, Teffner M, Rosel F, et al. Decline in the expression of the serine proteinase inhibitor maspin is associated with tumour progression in ductal carcinomas of the breast. *J Pathol*. 2001;195:321-326.
14. Sobin LH, Wittekind C, eds. *TNM Classification of Malignant Tumors*, 6th ed. New York: Wiley-Liss; 2002.
15. Fuhrman SA, Lasky LC, Limas CL. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol*. 1982;6:655-663.
16. Eble JN, Sauter G, Epstein JI, et al. *World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of the Urinary System and Male Genital Organs*. Lyon: IARC Publishing; 2004.
17. Yilmazer D, Han U, Onal B. A comparison of the vascular density of VEGF expression with microvascular density determined with CD34 and CD31 staining and conventional prognostic markers in renal cell carcinoma. *Int Urol Nephrol*. 2007;39:691-698.
18. Lam JS, Shvarts O, Leppert JT, et al. Renal cell carcinoma 2005: new frontiers in staging, prognostication and targeted molecular therapy. *J Urol*. 2005;173:1853-1862.
19. Bukowski RM, Wood LS. Renal cell carcinoma: state of the art diagnosis and treatment. *Clin Oncol*. 2008;11:9-21.
20. Gettins PG. Serpin structure, mechanism, and function. *Chem Rev*. 2002;102:4751-4804.
21. Sheng S, Truong B, Fredrickson D, et al. Tissue-type plasminogen activator is a target of the tumor suppressor gene maspin. *Proc Natl Acad Sci U S A*. 1998;95:499-504.
22. McGowen R, Biliran H Jr., Sager R, et al. The surface of prostate carcinoma DU145 cells mediates the inhibition of urokinase-type plasminogen activator by maspin. *Cancer Res*. 2000;60: 4771-4778.
23. Liu J, Yin S, Reddy N, et al. Bax mediates the apoptosis-sensitizing effect of maspin. *Cancer Res*. 2004;64:1703-1711.
24. Chen EI, Yates JR. Maspin and tumor metastasis. *Life*. 2006;58: 25-29.
25. Hirai K, Koizumi K, Haraguchi S, et al. Prognostic significance of the tumor suppressor gene maspin in non-small cell lung cancer. *Ann Thorac Surg*. 2005;79:248-253.
26. Bolat F, Gumurdulu D, Erkanli S, et al. Maspin overexpression correlates with increased expression of vascular endothelial growth factors A, C, and D in human ovarian carcinoma. *Pathol Res Pract*. 2008;204:379-387.
27. Blandamura S, Giacomelli L, Leo G, et al. Nuclear maspin detection in renal cell tumours: possible diagnostic role and correlation with p53 status. *Histopathology*. 2006;49:274-282.
28. Mohsin SK, Zhang M, Clark GM, et al. Maspin expression in invasive breast cancer: association with other prognostic factors. *J Pathol*. 2003;199:432-435.