Expression of Maspin in Papillary Ta/T1 Bladder Neoplasms

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Abstract. *Background: The aim of the present study was to evaluate maspin expression in bladder urothelial papillary neoplasms and test the results for correlation with clinicopathological parameters. Patients and Methods: A total of 111 urothelial neoplasms from 66 patients were evaluated: pathological examination of primary tumours disclosed 48 pTa and 18 pT1. Fourteen additional biopsies, negative for neoplasm, were collected as control biopsies. For each of the 111 neoplastic samples and for the 14 control cases maspin and MIB1 immunoreactivity was evaluated. The immunohistochemical reactions of the 66 primary neoplasms were used for statistical analysis when the disease-free interval, presence and number of relapses, and progression of the disease were tested, whereas all of the 111 tumors were used when the association between the maspin pattern and histological grade and/or pT were evaluated. Thirty-three patients with primary pTa papillary neoplasms (68.7%) and 11 out of eighteen with pT1 (61%), had subsequent relapses of disease. For maspin immunoreactivity the presence/absence of nuclear staining and the pattern of staining were considered. Four patterns of reactivity were recognized and were used for statistical analyses. Results: A statistical association was found between the maspin pattern and pT, histological grade and nuclear staining. Conclusion: In papillary urothelial neoplasms, maspin has a pattern of distribution that is associated with the histological grade and pathological stage, and this probably reflects its different activities in the neoplastic process.*

Maspin, a 42 kDa protein belonging to the serpin family, has been shown to have a tissue distribution expression in normal mammary epithelial cells, in the placenta, prostate, thymus, testes, oral cavity, small intestine, skin and cornea (1, 2). It has been demonstrated that maspin is the product of a tumour suppressor gene (3).

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Although the mechanisms underlying its biological activity are considered to be largely unknown, maspin has been shown to act on the cell membrane both directly and indirectly, affecting cell adhesion and inhibiting cell motility and invasion (4). Chen *et al.* (5) demonstrated that maspin causes alterations in proteins associated with the actin network and a consequent reduction in cell motility, has a pro-apoptotic effect and influences the ubiquitin proteasome pathway of protein degradation.

Additionally, a p53-dependent regulatory pathway of maspin in human cancer has been reported (6). Maspin expression has been correlated to p53 protein expression providing additional information on the possible regulatory influence of the wild-type p53 protein (6-8).

Maspin protein moreover, is present as a secreted, cytoplasmic, nuclear and as a cell surface-associated protein, and its specific subcellular localization seems to be associated with distinct progression pathways (9-11).

Although maspin has been evaluated in many human types of cancer, only three reports are present in the literature on maspin expression in bladder carcinomas (12-14). In the first report, Friedrich *et al.* investigated the expression of maspin in non-invasive bladder carcinomas (pTa/pT1), testing the results for correlation with neo-angiogenesis and the disease-free interval or recurrence (12). The authors concluded that maspin expression did not statistically correlate with the disease-free interval or recurrence of disease.

In a subsequent study Sugimoto *et al.* (13) evaluated maspin expression in a series of muscular invasive and non-muscular invasive urothelial carcinomas and found that maspin expression was restricted to the muscular-invasive carcinomas, so having a worse prognostic meaning, in contrast with other studied human carcinomas.

Beecken and colleagues (14) very recently studied the impact of maspin expression on the growth pattern of TCC of the bladder, in bladder carcinoma cell lines and 51 patient samples of TCC of the bladder. Expression was detected by RT-PCR and immunohistochemistry. They found maspin expression in high quantities in normal urothelium and that it was preserved in superficial bladder cancer but significantly diminished in invasive carcinomas.

The aim of the present study was to evaluate maspin expression in bladder papillary urothelial neoplasms and compare its expression with clinicopathological parameters.

In particular we evaluated: i) the differences of maspin expression in papillary urothelial neoplasms of low malignant potential (PUNLMP), papillary low-grade (LG) and papillary high-grade (HG) urothelial carcinomas; ii) the value of the considered parameters in predicting recurrence and/or progression of disease as independent or combined factors.

Patients and Methods

Patients. A total of 111 samples of urothelial neoplasms were collected from 66 patients, selected from 1997 to 2004, from the archival material of Institute of Pathological Anatomy of Padova University. Fourteen additional biopsies negative for neoplasm, obtained from patients who underwent cystectomy for invasive carcinoma, were collected as control biopsies.

Fifteen patients were women and fifty-one men (male/female ratio, 3:1). Among women the age ranged from 63 to 90 years (median age, 76.1 years); among men the age ranged from 36 to 91 years (median age, 69.1 years).

The pathological examination of primary tumours disclosed 48 pTa and 18 pT1, with a grading distribution of 14 PUNLMP, 31 low-grade papillary urothelial carcinomas (LG) and 21 high-grade papillary carcinomas (HG).

Thirty-three patients with primary pTa papillary neoplasms (68.7%) and eleven out of eighteen with pT1 had subsequent relapses of disease. All the considered cases had only one neoplasm at the time of initial presentation. The follow-up ranged from 12 to 84 months (median value: 35.31 months, standard deviation: 15.288). Only four patients had a shorter follow-up and five patients were lost to follow-up. Patients were followed up by urinary cytology or histological biopsy.

Taking into account primary and subsequent relapses, in total 80 Ta and 31 T1b cases were examined, according to the TNM classification, VI edition, 2002. pTa/pT1 neoplasms were classified as: PUNLMP (24 cases, all pTa), LG (49 cases, pTa/pT1 44/5 respectively), and HG (38 cases, pTa/pT1 11/27), according to the WHO/ISUP (2004) classification of urothelial neoplasms (15). Table I lists the detailed patient's clinicopathological data and the immunohistochemical results.

Therapy. The following clinical strategy was used for papillary neoplasms, in agreement with the European guidelines (16): post-operative adjuvant therapy with chemotherapy (mitomycin C) or immunotherapy (Bacillus Calmette Guerin (BCG)) was administered to all patients with non-muscle invasive papillary carcinomas, except those with small singular pTa, low grade tumors.

Follow-up investigations were performed by urologists, with a cystoscopy every 3 months. All tumor recurrences were histologically confirmed.

Immunohistochemistry. All tissues were fixed in 10% formalin and embedded in paraffin wax. For each sample, maspin and MIB1 reactivity was evaluated. Formalin-fixed, paraffin-embedded 5 μm sections were cut for all 111 neoplastic samples and for the 14 normal control cases, and pre-treated in a microwave oven (750 watt) for 20 min in a citrate buffer (10 mM , pH 6.0).

The antibodies used were: for maspin: mouse monoclonal antibody, clone G167-70, diluted 1:500, BD-Biosciences, PharMingen International, San Diego, CA, USA, and for Ki-67: mouse monoclonal antibody, clone MIB-1, diluted 1:100, DAKO, Glostrup, Denmark.

The Automate Staining System (Biogenex-San Ramon, CA, USA) was used. For maspin the sections were pre-incubated with super block (Biogenex) for 5 min to block non-specific background staining, incubated for 45 min at room temperature and then incubated with EnVisionSystem HRP labelled polymer anti-mouse (DAKO) for 30 min. For MIB1, after pre-incubation the sections were incubated with biotinylated pre-diluted antibody for 20 min (Biogenex) and then with streptavidin-peroxidase complex (Biogenex) for 20 min. Between incubations, the sections were washed in phosphate-buffered saline (PBS) (pH.7.00) for 3 min. The colour was developed using 3,3'-diaminobenzidine (DAB; DAKO) for 4 min. Sections were counterstained with Mayer's haematoxylin.

Normal breast tissue was used as a positive control for maspin. As a negative control breast tissue was included substituting the primary antibody with PBS.

Data analysis

Immunohistochemistry. For MIB1 two dedicated pathologists each evaluated 10 randomised non overlapping frames throughout each neoplasm, at x20 magnification, counting a total minimum of 600 cells and expressing the number of positively labelled cells as a percentage out of the total number of cells. Only nuclear staining was considered as positive, independently of staining intensity.

For maspin immunoreactivity (IR) a double count was performed: i) the presence of nuclear staining and its percentage of positivity, and ii) the pattern of staining together with the intensity of staining, divided into weak (+/–) and strong (+). Both nuclear and cytoplasmic staining were evaluated as positive.

The percentage of positivity varied from 10% to 100%, with a median value of 60%. Considering the non-normal distribution of maspin values ($p=0.001$) the median value was used when the time of first relapse and the progression of disease were tested.

Immunoreactivity for maspin of non-neoplastic control cases was used as reference. Normal urothelium of control cases showed no maspin IR or a weak cytoplasmic reactivity at the basal and supra-basal layers.

Starting from the staining pattern of normal urothelium of the control case tissue, maspin IR was then classified into four patterns: i) negative staining (designated a) (Figure 1A); ii) a normal-like pattern (designated b) (Figure 1B); 3) an accentuated pattern, showing stronger intensity of staining throughout all layers of the epithelium (designated c) (Figure 1C); and 4) a border pattern, showing a strong intensity of staining in the deeper portion of the urothelium (designated d) (Figure 1D).

Statistical analysis. The following tests were used when appropriate: Fisher's exact test, Cramer's correlation coefficient for not ordered and Kendall's test for ordered categorical data, respectively.

The following multi-parametrical models were used when appropriate: Cox's proportional hazards (HR) model for survival time (time-to-event) outcomes on one or more predictors; logistic regression model expressed as odds ratio (OR) to predict disease progression.

Table I. *Clinicopathological parameters of the 66 patients and immunohistochemical results.*

Case No.	Age/Gender	pT	Grade†	Maspin pattern‡	Mib1%	Maspin%	Follow-up months	DFI	Number of relapses	Progression
1	70/M	1	HG	d	10	30	49	9	1	Yes
2	83/M	0	PUNLMP	b	2	60	46	46	0	No
3	64/M	0	LG	c	5	90	49	23	2	No
4	57/M	0	HG	c	10	100	lost			
5	72/F	0	LG	c	10	90	4	4	1	No
6	77/M	1	HG	c	30	90	46	46	0	No
7	69/M	0	LG	c	1	90	17	3	1	No
8	65/M	0	HG	c	1	90	46	2	2	Yes
9	72/M	1	HG	a	5	0	lost			
10	63/M	0	HG	d	0	90	48	12	1	No
11	76/M	1	HG	d	5	90	43	41	2	Yes
12	71/F	0	HG	b	40	0	44	4	1	No
13	87/M	0	PUNLMP	b	5	80	44	44	0	No
14	73/M	0	LG	c	10	70	48	22	1	No
15	75/M	0	PUNLMP	a	2	0	45	36	1	No
16	63/F	0	LG	c	10	100	45	11	1	No
17	61/M	0	LG	b	2	70	44	44	0	No
18	87/F	0	LG	b	20	90	45	4	1	No
19	67/M	0	PUNLMP	a	5	0	51	6	1	Yes
20	70/M	0	LG	b	5	70	10	5	2	No
21	79/M	0	LG	b	2	80	49	19	4	No
22	66/M	0	LG	b	10	90	14	5	3	Yes
23	79/M	0	PUNLMP	a	7	0	47	13	1	Yes
24	59/M	0	LG	b	1	70	50	50	0	No
25	77/M	0	HG	c	5	0	lost			
26	68/M	0	LG	b	5	90	42	5	2	No
27	66/M	0	PUNLMP	b	2	70	22	22	1	No
28	77/F	0	LG	b	5	90	51	5	3	No
29	36/M	0	LG	b	7	70	51	3	1	No
30	74/M	0	PUNLMP	b	1	80	47	29	2	No
31	89/F	1	HG	d	5	20	36	5	3	Yes
32	56/M	0	LG	b	10	30	42	3	7	Yes
33	63/F	1	HG	c	30	90	14	14	1	No
34	79/M	0	PUNLMP	b	5	90	44	44	0	No
35	71/M	0	PUNLMP	b	0	90	22	44	0	No
36	48/M	0	PUNLMP	b	2	90	40	3	1	No
37	89/F	1	LG	c	10	90	47	28	1	
38	53/M	0	LG	b	10	30		4	4	Yes
39	69/M	1	HG	b	20	50	12	10	1	Yes
40	86/M	0	LG	c	10	100		0	0	No
41	89/M	0	LG	c	20	100	lost			
42	79/M	1	HG	c	20	90	47	47	0	No
43	71/F	1	HG	c	25	90		3	1	No
44	74/M	0	LG	b	0	70	42	24	2	Yes
45	90/F	1	HG	d	1	10	lost		0	No
46	57/M	0	LG	b	1	70	44	44	0	No
47	55/M	0	LG	b	1	20	47	47	0	No
48	70/M	1	HG	d	10	90	4	4	1	Yes
49	62/M	0	HG	b	20	0	2	2	1	Yes
50	91/M	1	HG	c	10	90	5	5	0	No
51	69/M	1	HG	c	5	80	10	4	1	No
52	79/M	0	LG	b	5	50	29	4	3	Yes
53	77/M	0	PUNLMP	b	2	0	48	5	2	No
54	64/F	0	PUNLMP	a	2	0	48	12	1	Yes
55	80/M	0	LG	b	1	70	48	48	0	No
56	75/M	1	LG	b	1	70	32	32	0	No
57	67/F	0	LG	a	1	0	30	11	5	No
58	65/M	0	PUNLMP	b	1	0	43	29	1	No

Table I. *continued*

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Case No.	Age/Gender	pT	Grade‡	Maspin pattern†	Mib1%	Maspin%	Follow-up months	DFI	Number of relapses	Progression
59	50/M	1	LG	c	10	100	27	2	3	Yes
60	83/F	0	LG	b	30	90	46	2	1	No
61	53/M	0	LG	b	15	0	16	4	2	Yes
62	89/F	1	HG	a	80	0	10	7	3	Yes
63	67/F	0	LG	b	30	90	38	3	1	Yes
64	57/M	0	PUNLMP	a	1	0	32	12	5	No
65	67/M	0	LG	b	1	0	19	6	3	No
66	76/M	1	HG	d	5	80	27	17	1	No

‡PUNLMP, papillary urothelial neoplasm of low malignant potential; LG, low grade urothelial papillary carcinoma; HG, high grade urothelial papillary carcinoma. †Pattern of maspin distribution: a) negative for maspin; b) normal-like pattern of distribution; c) accentuated pattern; d) marginal pattern. DFI, disease-free interval.

Before analyses, the continuous variables (maspin and MIB1) were spliced in a binary way (0-1), assigning the value 1 to maspin <60% and MIB1 >10%. STATA 8 (StataCorp, College Station, TX, USA) was used for evaluations. The immunohistochemical reactions of the 66 primary neoplasms were used for statistical analysis when disease free interval (DFI), presence and number of relapses, and progression of the disease were tested; all 111 tumors were used to determine the association between the maspin pattern and grade and/or pT. $P<0.05$ was considered to be significant; *p*-values between 0.15 and 0.05 were considered as indicating a trend.

Results

Immunohistochemical results. Normal urothelium of control cases did not stain for maspin, or showed a weak intensity of staining at the basal and supra-basal layers (Figure 2A, B).

Some papillary tumors did not express maspin at all (pattern a) (12%), most papillary neoplasms showed maspin-positive IR in the basal, supra-basal and sometimes intermediate layers, lacking the stain of the superficial layer, with a weak stain (+/–) intensity (pattern b) (50%), other cases showed positive reaction throughout epithelial layers with strong staining intensity (pattern c) (27%) and other cases showed IR at the margin line of neoplastic clusters (pattern d) (10%) (Figure 1A, B, C, D). Among the primary 66 cases, we observed: pattern a staining in 5 pTa and 3 pT1, pattern b in 31 pTa and 2 pT1 and pattern c in 6 pTa and 12 pT1; all 7 cases with pattern d were pT1.

Considering all the 111 cases, we observed: pattern a in 18 pTa and 8 pT1, pattern b in 49 pTa and 1 pT1, pattern c in 9 pTa and 15 pT1, and pattern d in 4 pTa and 7 pT1. Maspin staining was prevalently cytoplasmic, with only 14 cases showing positive nuclear reaction (3 PUNLMP, 7 LG and 4 HG). The intensity of stain was weak (+/–) for PUNLMP and for the great majority of LG, and strong (+) in HG. The detailed results of immunohistochemistry are listed in Table I.

Statistical results. A statistical association was found between the maspin pattern and pT (Fisher's exact test: $p<0.001$, Cramer's correlation coefficient: 0.580), maspin pattern and histological grade (Fisher's exact test: $p<0.001$, Cramer's correlation coefficient: 0.585) and maspin pattern and nuclear staining (Fisher's exact test: $p=0.018$, Cramer's correlation coefficient: 0.2).

Using Cox's regression model correlated to the time of first relapse, maspin <60% had an HR of 2.01 ($p=0.027$; 95% confidence interval [CI]: 0.810-3.701), while MIB1 had an HR of 2.53 ($p=0.02$; CI: 1.387-4.642).

The logistic regression model showed the following results for the parameters related to the progression of disease: maspin <60%, OR= 8.61 ($p=0.01$; CI: 1.501-4.701); MIB1 >10%, OR: 13.31 ($p=0.01$; CI: 1.901-9.802). No statistical significance was found for histological grade ($p=0.50$) or gender ($p=0.23$).

Table II summarizes the maspin pattern distribution as related to the histological grade. Figure 3 shows the time to first relapse. Figures 4 and 5 show the time for first relapse as a function of maspin staining and MIB1 staining respectively.

Discussion

Maspin directly induces endothelial cell apoptosis *in vitro* and this effect is maspin specific. In a recent study Li *et al.* showed as tumor neo-vessels became leaky after maspin treatment, whereas normal mature vessels were not affected by maspin treatment (17). Targeting tumor vessel endothelium therefore should serve as an effective therapy against tumor angiogenesis and metastasis (17).

Although maspin has been investigated in tumor progression and metastasis in many types of human cancer, maspin in human bladder cancer has not been found to be a promising prognostic marker in pTa/pT1 bladder cancer (12),

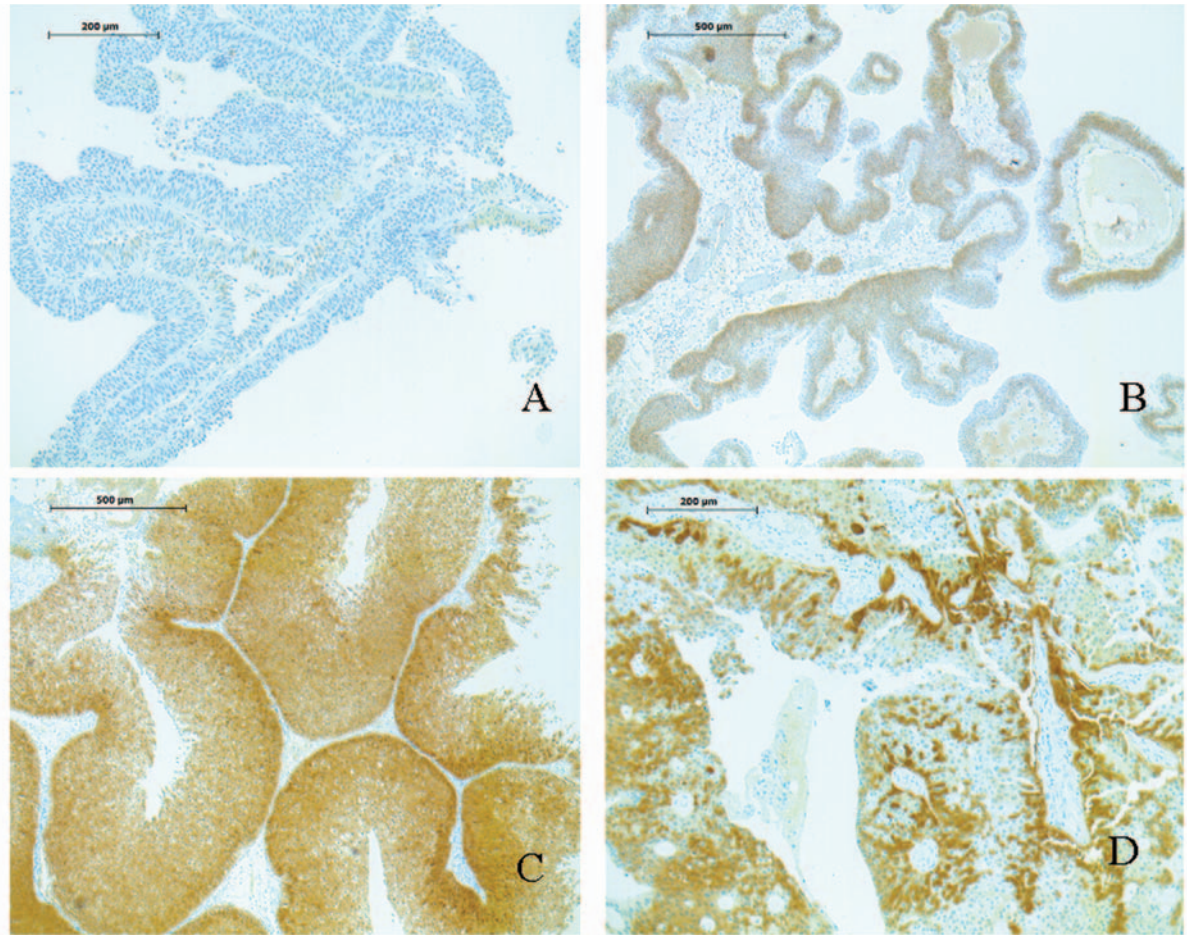

Figure 1. *Maspin immunoreaction in papillary urothelial neoplasms showing four different types of pattern staining. A) No reaction for maspin; B) a normal-like pattern of staining with a weak intensity, mainly at the lower layers of the urothelium; C) accentuated pattern with a strong intensity of staining concerning all the layers of the urothelium; D) marginal pattern with a strong intensity of staining at the lower layer of the urothelium. Maspin, A) x50; B) x50; C) x100; D) x100.*

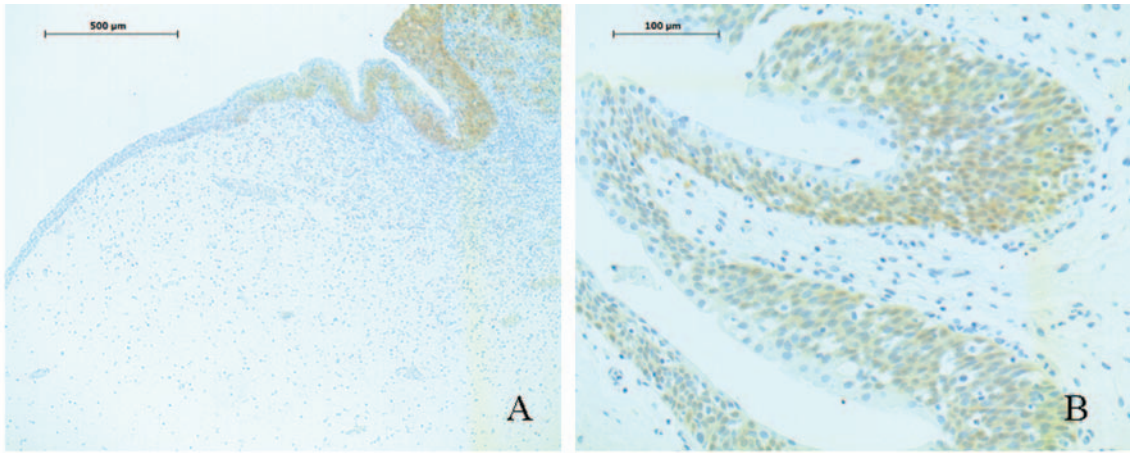

Figure 2. *Maspin immunoreaction in non-neoplastic bladder tissue shows no reaction or a weak reaction in the lower layers of the urothelium (A). A higher magnification showing greater detail (B). Maspin, A) x50; B) x200.*

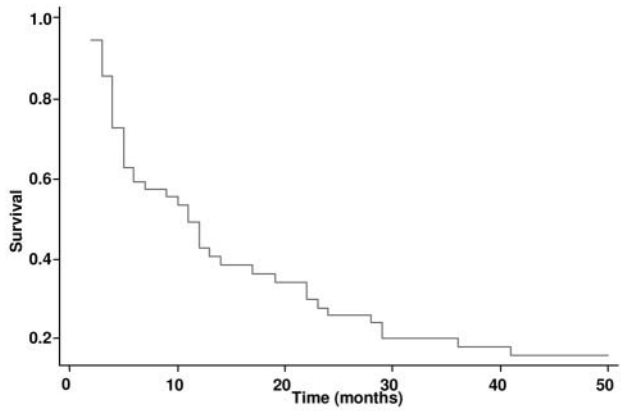

Figure 3. *Baseline survival function related to first relapse. Cox proportional hazard regression model.*

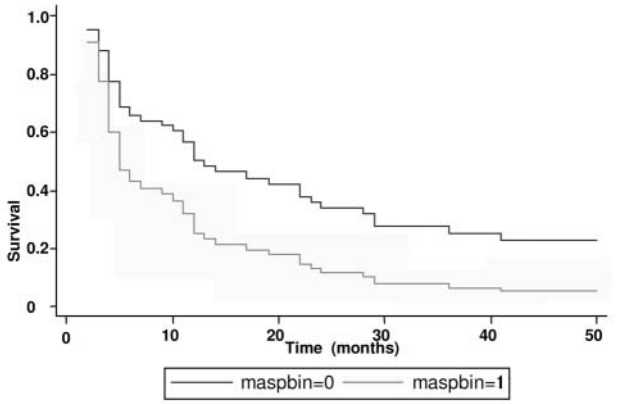

Figure 4. *First relapse by maspin survival functions. Upper curve: maspin >60% (0), lower curve: maspin <60% (1). Cox proportional hazards regression model.*

whereas it has been related to the development of muscle invasive carcinomas (13). It is interesting to note that the above mentioned authors found a positive correlation between increased maspin expression and the progression of disease, this being apparently in contrast with the results of other studies on human cancer that correlate the presence of maspin with a better prognosis. More recent evidence seems to correlate low maspin expression in bladder neoplasms to an increased tumor cell growth *in vivo* and *in vitro* (14). The authors observed that maspin expression was preserved in superficial bladder cancers but significantly diminished in invasive carcinomas. Within the group of invasive TCCs, the authors found that maspin expression was inversely correlated to the patient's prognosis and that low maspin expression levels were coupled to an increased tumor cell growth *in vivo*.

Very recently Lockett *et al.* (9) demonstrated the essential role of maspin in epithelial homeostasis and that a sub-cellular location of maspin seems to reflect a distinct tumor progression pathway. It has already demonstrated that maspin is present as a monomer, as a secreted, cytoplasmic and nuclear, as well as a cell surface-associated protein (11, 18-21). Evidence is emerging in the literature on the importance of maspin sub-cellular distribution in human malignancies (10, 11) and in this sense the present study is the first that considers the maspin pattern distribution rather than its percentage of positivity in bladder neoplasms. Increased evidence has proven that sub-cellular location of maspin has different role in regulating cellular homeostasis. In particular, the nuclear location of maspin seems to be the strongest predictor related to a patient's disease-free survival (10, 11, 22, 23).

The present results show that specific maspin patterns are associated with different histological degrees of differentiation and that nuclear staining is associated with neoplasms of lower histological grade.

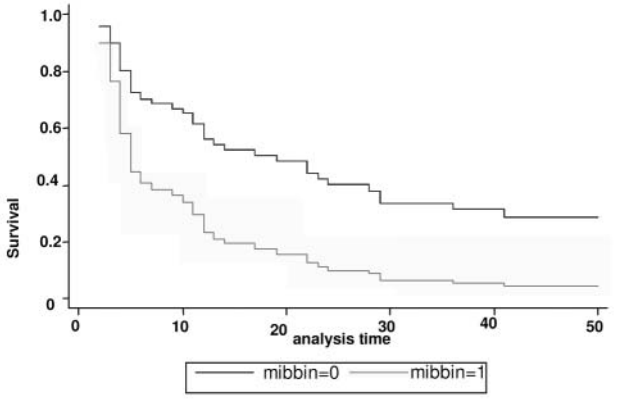

Figure 5. *First relapse by MIB1 survival functions. Upper curve: MIB1 <10% (0), lower curve: MIB1 >10% (1). Cox proportional hazards regression model.*

Table II. *Maspin distribution pattern as related to histological grade.*

Maspin pattern	HG	LG	PUNLMP	Total (number of specimens)
a	13	6	7	26
b	2	32	16	50
c	13	10	1	24
d	10	1	0	11
Total (number of specimens)	38	49	24	111

The staining distribution of maspin generally fell into two main types of expression: one similar to that of normal urothelium (pattern a or b) characterizing the great majority of PUNLMP (23/24), low grade papillary carcinomas (38/49)

and pTa stage tumors (67/80); and the other showing a stronger and/or diffuse staining (pattern c or d), seemingly associated with higher grade papillary carcinomas (23/37) and pT1 stage tumors (22/31).

Considering the number of relapses, maspin pattern b (expressing less than 60% maspin) had an HR of 2.01 and, using the logistic regression model for the progression of disease, an OR of 8.61.

Our results show that the strong expression of maspin is related to a better behaviour of papillary neoplasms and this is apparently in contrast with the absence of or reduced maspin staining observed in the neoplasms of a lower histological grade and at pTa stage. To explain our results, a possible hypothesis is that lower grade papillary neoplasms do not induce maspin activation, whereas high-grade neoplasms exert maspin action in an attempt to restrict tumor aggressiveness.

The same hypothesis may explain the presence of nuclear staining within the two categories of maspin pattern b and c, which identify the papillary neoplasms of lower histological grade (PUNLMP and LG).

Our results seem to show that the maspin distribution pattern can be more useful than the evaluation of its percentage of staining and this may be because its subcellular location reflects different functional activities. The present study shows that maspin maintains its protective role in papillary urothelial carcinomas and that it can be a helpful marker of behaviour in these tumors.

The present study shows that maspin staining pattern can be helpful in predicting, between LG and HG papillary urothelial neoplasms, the cases with a favourable behaviour with a low risk of relapse or progression of disease and a long disease-free interval.

More studies are needed to elucidate the exact implication of maspin subcellular location and what functions are precisely related to its different locations within the cell.

References

1 Blacque OE and Worrall DM: Evidence for a direct interaction between the tumor suppressor serpin, maspin, and types I and II collagen. J Biol Chem *27*: 10783-10788, 2002.

2 Ngamkitidechakul C, Warejcka DJ, Burke JM, O'Brien WJ and Twining SS: Sufficiency of the reactive site loop of maspin for induction of cell matrix adhesion and inhibition of cell invasion. J Biol Chem *278*: 31796-31806, 2003.

3 Kim DH, Yoon DS, Dooley WC, Nam ES, Ryu JW, Jung KC, Park HR, Sohn HR, Shin HS and Park YE: Association of maspin expression with the high histological grade and lymphocyte-rich stroma in early-stage breast cancer. Histopathology *42*: 37-42, 2003.

4 Bass R, Moreno Fernandez A-M and Ellis V: Maspin inhibits cell migration in the absence of protease inhibitory activity. J Biol Chem *277*: 46845-46848, 2002.

5 Chen EI, Florens L, Axelrod FT, Monosov E, Barbas CF 3rd, Yates JR 3rd, Felding-Habermann B and Smith JW: Maspin alters the carcinoma proteome. FASEB J *19*: 1123-1124, 2005.

6 Oshiro MM, Watts GS, Wozniak RJ, Junk DJ, Munoz-Rodriguez JL, Domann FE and Futscher BW: Mutant p53 and aberrant cytosine methylation cooperate to silence gene expression. Oncogene *22*: 3624-3634, 2003.

7 Zhang W and Zhang M: Tissue microarray analysis of maspin expression and its reverse correlation with mutant p53 in various tumors. Int J Oncol *20*: 1145-1150, 2002.

8 Machtens S, Serth J, Bokemeyer C, Bathke W, Minssen A, Kollmannsberger C, Hartmann J, Knuchel R, Kondo M, Jonas U and Kuczyk M: Expression of p53 and maspin protein in primary prostate cancer: correlation with clinical features. Int J Cancer *95*: 337-342, 2001.

9 Lockett J, Yin S, Li X, Meng Y and Sheng S: Tumor suppressive maspin and epithelial homeostasis. J Cell Biochem *97*: 651-660, 2006.

10 Marioni G, Blandamura S, Giacomelli L, Calgaro N, Segato P, Leo G, Fischetto D, Staffieri A and de Filippis C: Nuclear expression of maspin is associated with a lower recurrence rate and a longer disease-free interval after surgery for squamous cell carcinoma of the larynx. Histopathology *46*: 576-582, 2005.

11 Lonardo F, Li X, Siddiq F, Singh R, Al-Abbadi M, Pass HI and Sheng S: Maspin nuclear localization is linked to favorable morphological features in pulmonary adenocarcinoma. Lung Cancer *51*: 31-39, 2006.

12 Friedrich MG, Toma MI, Petri S, Cheng JC, Hammerer P, Erbersdobler A and Huland H: Expression of maspin in non-muscle invasive bladder carcinoma: correlation with tumor angiogenesis and prognosis. Eur Urol *45*: 737-743, 2004.

13 Sugimoto S, Maass N, Takimoto Y, Sato K, Minei S, Zhang M, Hoshikawa Y, Junemann KP, Jonat W and Nagasaki K: Expression and regulation of tumor suppressor gene maspin in human bladder cancer. Cancer Lett *203*: 209-215, 2004.

14 Beecken WD, Engl T, Engels K, Blumenberg C, Oppermann E, Camphausen K, Shing Y, Reinecke G, Jonas D and Blaheta R: Clinical relevance of maspin expression in bladder cancer. World J Urol *24*: 338-344, 2006.

15 Montironi R and Lopez-Beltran A: The 2004 WHO classification of bladder tumors: a summary and commentary. Int J Surg Pathol *13*: 143-153, 2005.

16 Oosterlink W: Guidelines on diagnosis and treatment of superficial bladder cancer. Minerva Urol Nefrol *56*: 65-72, 2004.

17 Li Z, Shi HY and Zhang M: Targeted expression of maspin in tumor vasculatures induces endothelial cell apoptosis. Oncogene *24*: 2008-2019, 2005.

18 Pemberton PA, Tipton AR, Pavloff N, Smith J, Erickson JR, Mouchabeck ZM and Kiefer MC: Maspin is an intracellular serpin that partitions into secretory vesicles and is present at the cell surface. J Histochem Cytochem *45*: 1697-1706, 1997.

19 Shao ZM, Nguyen M, Alpaugh ML, O'Connell JT and Barsky SH: The human myoepithelial cell exerts antiproliferative effects on breast carcinoma cells characterized by $p21^{WAF1/CIP1}$ induction, G2/M arrest, and apoptosis. Exp Cell Res *241*: 394-403, 1998.

20 McGowen R, Biliran H Jr, Sager R and Sheng S: The surface of prostate carcinoma DU145 cells mediates the inhibition of urokinase-type plasminogen activator by maspin. Cancer Res *60*: 4771-4778, 2000.

21 Chim SS, Tong YK, Chiu RW, Lau TK, Leung TN, Chan LY, Oudejans CB, Ding C and Lo YM: Detection of the placental epigenetic signature of the maspin gene in maternal plasma. Proc Natl Acad Sci USA *102*: 14753-14758, 2005.

22 Woenckhaus M, Bubendorf L, Dalquen P, Foerster J, Blaszyk H, Mirlacher M, Soler M, Dietmaier W, Sauter G, Hartmann A and Wild PJ: Nuclear and cytoplasmic maspin expression in primary non-small cell lung cancer. J Clin Pathol *60*: 483-486, 2007.

23 Dietmaier W, Bettstetter M, Wild PJ, Woenckhaus M, Rummele P, Hartmann A, Dechant S, Blaszyk H, Pauer A, Klinkhammer-Schalke M and Hofstadter F: Nuclear maspin expression is associated with response to adjuvant 5-fluorouracil based chemotherapy in patients with stage III colon cancer. Nuclear maspin expression is associated with response to adjuvant 5-fluorouracil based chemotherapy in patients with stage III colon cancer. Int J Cancer *118*: 2247-2254, 2006.

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