Immunohistochemical Maspin Expression in Transitional Cell Carcinoma of the Bladder

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ABSTRACT

Purpose: Evaluation of Maspin marker in TCC of urine bladder, its grade, and stage, and to compare Maspin expression in normal transitional cell epithelium and TCC in patients admitted to Modarres Hospital from 2009 to 2012.

Materials & Methods: Paraffin blocks of bladder tissue, from total number of 76 patients with TCC, and normal epithelium were included. Slides were prepared, and stained by Immunohistochemical staining method for Maspin marker, and assessed by two independent pathologists.

Results: Maspin marker expression had been reduced in bladder tissue with TCC, which corresponded to the grade and stage. Normal urothelial mucosa was strongly Maspin-positive, while the intensity of staining in TCC was reduced as both the grade and stage were increased.

Conclusion: Maspin expression, by Immunohistochemistry method, is reduced in TCCs of the urinary bladder. There was a meaningful reverse relationship between the intensity of Maspin expression and tumor stage. It can be suggested that evaluation of the Maspin marker can be helpful as a prognostic factor and to determine the low and high grade TCC in difficult cases.

KEYWORDS: Maspin Expression, Transitional Cell Carcinoma, Bladder

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INTRODUCTION

Urinary bladder cancer is one of the most common malignancies, with a high morbidity and mortality rate [1]. Bladder cancer incidence rates rank 9th worldwide and it is the 7th and 17th most common malignancy in men and in women,

respectively [2]. Bladder cancer causes a significant clinical problem and has different histological types including transitional cell carcinoma (TCC), squamous cell carcinoma (SCC), adenocarcinoma and others.

Histologically, about 70% of the TCCs are superficial at the time of diagnosis, which do not invade to lamina or muscularis propria and are treated by transurethral resection of bladder tumor (TURB). However, 30% of all TCCs demonstrate highly malignancy with capacity of invasion into the muscular is propria and nearby structures, and lymph node and distant metastasis [3,4]

An about 64.20% increase in the relative frequency of TCC and a 28.72% decrease in SCC cases have been reported in clinicopathological parameters of 2003-2004 series [5] Grade and stage of tumor has been recognized as the most influential prognostic factor for bladder malignancy. However, other clinic pathological factors cannot predict the prognosis, accurately [6] Cystoscopy as a "gold standard" diagnostic technique is invasive, costly and detested. Although, urine cytology, as an adjunct to cystoscopy, is highly sensitive in detection of high-grade cancers, lacks the adequate sensitivity to detect low-grade ones. Thus, molecular markers study is helpful, for their potential predictive value, to achieve better screening, observation and therapeutic strategy [7]. Based on expression at the mRNA level, Maspin (Mammary serine protease inhibitor) was initially identified in normal mammary ducts using subtractive hybridization method [8]. Maspin has been demonstrated to suppress tumor growth and metastasis in several tumor types [9] Consequently, it may act as physiologic molecule to inhibit angiogneic factors which is concerned in the evolution of tumors in organs other than breast [4].

Although the precise mechanism of biological activity of Maspin are relatively unknown, it has been shown to express on the cell membrane, involving in intercellular adhesion, directly and indirectly, and consequently inhibits cell motility and invasion [10] The expression of Maspin has been reported in wide variety of cancers, but few articles are present in the literature on its prevalence in bladder malignancies [4]

It has already established that monomer of Maspin is presented in the cytoplasm, nucleus as well as in cell membranes [11] There are emerging evidences in the text books on the significance of sub-cellular distribution of Maspin in human cancers. Some studies consider the muslin's distribution pattern rather than its positivity proportion in the bladder neoplasms. Cumulative evidences are in favor of different roles of Maspin in regulating cellular homeostasis. Among them, the nuclear locality of Maspin is identified as the strongest predictor in disease-free survival of the patients [11,12]. Although, expression of Maspin mRNA was already found in urinary bladder of rat, lack of efficient assessment of Maspin expression and its clinicopathological correlation in human bladder cancer is seen [5] According to the results retrieved from various studies, the pattern of Maspin expression can predicts the different degrees of histological differentiation (Tumor grade), so nuclear pattern is seen more in lower histological grade of neoplasm [13].

In this report, we measured the Maspin expression in clinical specimens, including benign and malignant highly aggressive TCCs, to evaluate the correlation between patterns of Maspin expression with disease status.

MATERIALS AND METHODS

In this descriptive study, 49 patients with transitional cell carcinoma were recognized from the archives of Pathology ward. After cystectomy, the fresh normal bladder tissue samples and tumor tissue of TCC were taken. Then, information about age, gender, number of paraffin blocks and pathology diagnosis were recorded in the questionnaire. The slides for each patient was reviewed and the paraffin block containing tumor tissue, in total, were selected, and from these blocks, using a microtome, 3-5 micrometer thick representative sections of tumor samples of TCCs were prepared for immunohistochemistry study. Then, the sections were transferred on slides, deparaffinized in xylene, and dehydrated in graded ethanol series and heat-induced epitopes -retrieval were applied. The tissue was placed in 3% hydrogen peroxide and then washed with buffer. A little diluted blocking serum was poured on tissue and incubated in room temperature. The serum was removed from the tissue and slides were incubated with 1/80 diluted anti Maspin antibody (abcam-ab22354) for 45 minute at 4°C. The cuts were placed in avidin-biotin- peroxidase complex for another 30 minutes. Slides and cuts were washed and coated within Di- amino-benzidine and hydrogen peroxides for 10 minutes. Then, slides and cuts were floated in Mayer's hematoxylin solution for counterstain. Coverslip was placed on the slides and Maspin expression intensity was scored by a scaling system, ranging from 0 (no Maspin expression) to 4+ (maximum Maspin expression), and the percentage, ranging from 0 to 100 using an optical microscope. The study population was archival specimens of patients with bladder transitional cell carcinoma and normal tissue. Tissue samples were examined microscopically in pathology ward of Modarres Hospital of Tehran during 2009-2012, and their paraffin blocks were available in the archives of pathology ward. All quantitative variables were expressed as mean±standard deviation and qualitative variables were presented as percentage.

In order to compare quantitative variables in different ways, information regarding age, degree of differentiation, tumor stage, pathologic diagnosis and the frequency of Maspin expression was recorded. The statistical significance of the results is dermined by Mann-Whitney Rank Sum test. All statistical tests were performed two-tailed with significance level of 0.05 and the analysis was performed using SPSS 16 software.

RESULTS

This study contains 49 patients aged 25 to 85 years, mean age of 54 years. Patients were sectioned into two groups, according to the degree of tumor differentiation: 28 patients (57.1%) were assigned to participate in low grade transitional cell carcinoma group, 16 patients (32.7%) in high grade transitional cell carcinoma group. Five specimens (10.2%) were unremarkable bladder mucosa (used as control). In this study, none of the sample cases were urothelial papilloma or low potential malignant urothelial neoplasm.

The patients were divided into 5 groups, according to the tumor stage. Of these, 3 cases (6.1%) were in PTa group, 5 patients (10.2%) in PT1 group, 20 patients (40.82%) in PT2a group, 14 patients (28.58%) in PT2b group, 2 (4.1%) in group PT3 and 5 patients (10.2%) in unremarkable group (Table 1).

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Figures 1and 2: Positive diffuse and strong immunoreactivity for Maspin in normal urothelial mucosa
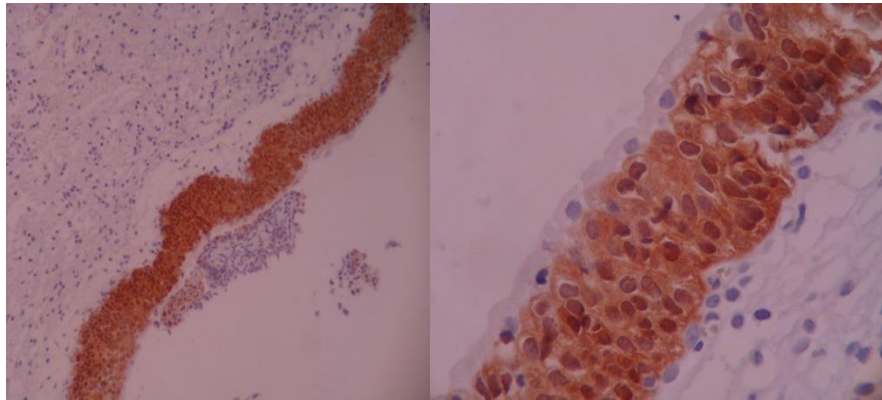
Samples were classified into five categories, from 0 to 4 based on Maspin expression in ordinal measurement, and the intensity which increased by increasing the Maspin staining. Among these, 8 patients (16.3%) had zero Maspin, 12 patients (24.5%) in group 1+, 13 patients (26.5%) in group 2+, 13 patients (26.5%) in group 3+ and three patients (6.11%) in group 4+ (Table 2). There was no significant association between age and Maspin index (p=0. 425). The association between stage of the disease and Maspin expression was statistically significant (p=0. 000). Also, the association between grade of the disease and Maspin expression was statistically significant (p=0.02).

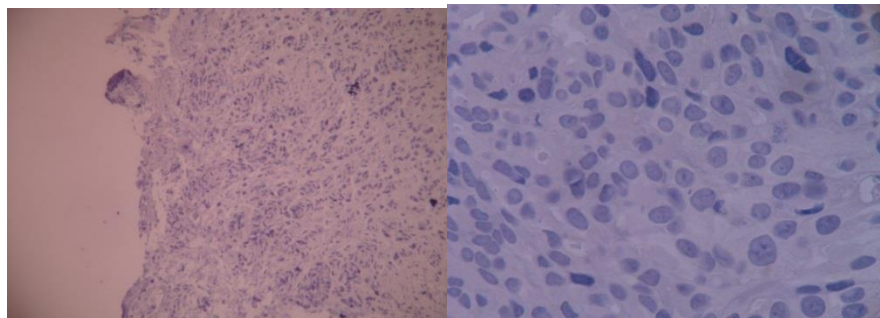**Figures 3-4: Negative immunoreactivity for Maspin in a high grade invasive transitional cell carcinoma**

Table-1: Maspin expression based on tumor stage

Tumor stage	Maspin staining				
	0	1	2	3	4
PTa	2(25)	1(8)	0(0)	0(0)	0(0)
PT1	0(0)	0(0)	3(23)	2(15)	0(0)
PT2a	2(25)	8(67)	5(38.5)	5(39)	0(0)
PT2b	3(37.5)	2(17)	5(38.5)	4(31)	0(0)
PT3	1(12.5)	1(8)	0(0)	0(0)	0(0)
Unremarkable	0(0)	0(0)	0(0)	2(15)	3(100)
Total	8(100)	12(100)	13(100)	13(100)	3(100)

Table 2. Maspin expression based on tumor grade

Tumor grade	Maspin staining									
	0		1		2		3		4	
	TCC*	Normal	TCC	Normal	TCC	Normal	TCC	Normal	TCC	Normal
Low grade TCC	1(13)	0(0)	2(17)	0(0)	4(33)	0(0)	8(74)	0(0)	1(100)	0(0)
High grade TCC	7(87)	0(0)	10(83)	0(0)	8(67)	0(0)	3(36)	0(0)	0(0)	0(0)
Unremarkable	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	2(100)	0(0)	2(100)
Total	8(100)	0(0)	12(100)	0(0)	12(100)	1(100)	11(100)	2(100)	1(100)	2(100)
TCC=Transitional Cell Carcinoma.										

DISCUSSION

The expression of Maspin, a 42 kDa protein, is seen in wide spectrum of tissues including normal epithelial cells cells of mammary ducts, placental parenchyma, prostate gland, thymus, testis, oral cavity, small intestinal mucosa, epidermis and cornea [14]. It has been established that Maspin is a product of a tumor suppressor gene [15].

The aim of this study was to investigate the Maspin expression in normal tissue and TCC and determine its association with the status of disease; the findings showed a statistically significant association between stage and grade of the disease and Maspin expression, intensity of Maspin expression was reduced as both the grade and stage were increased (Figures 1-4). Like us, Beecken and colleagues found an affirmative correlation between intensity of Maspin expression and the disease progression. They found that absence of Maspin was associated with tumor growth, whereas in normal tissues, it was found significantly [4]. In Abd, El-Maqsoud and Tawfiek's study in 2010, Maspin expression was high in low grade TCCs compare to the high grade TCCs and they found a better prognosis in patients with higher Maspin expression [5] Juengel and colleagues stated that Maspin protein as a modulator resulting in adhesion of bladder carcinoma cells to endothelium of the vessels.

They found a positive association between superficial Maspin in cancer cells for binding to vascular endothelial cells. Thereby, blocking this process was presented as a method to treat bladder cancer [9,16]. found that expression of Maspin does not statistically correlate with the disease-free interval or recurrence of non-invasive bladder carcinomas [16].

In a study [17], Maspin expression was evaluated in a series of muscular invasive and non-muscular invasive urothelial carcinomas and they found that Maspin expression was restricted to the muscular- invasive carcinomas, so having a worse prognostic meaning, in contrast with other studied human carcinomas [17,18] established the important role of Maspin in homeostasis of epithelial cells and that a sub-cellular locality of Maspin protein seems to be a sign of a distinct tumor progression pathway.

[19] showed that Maspin leading to alterations in proteins involving in the action of network and a subsequent reduction in the motility of the cells, and has an additional pro-apoptotic effect. Additionally, Maspin is involved in

protein degradation of ubiquitin proteasome pathway; a p53-dependent regulatory pathway of Maspin in human malignancy has been also reported [19,20].

Maspin has specific *in vitro* direct effect in apoptosis of endothelial cell. Recently, study of [21] showed that neo-vessels of tumors got leaky after treatment with maspin, whereas non tumoral mature vasculature was unaffected following Maspin treatment. The tumoral vascular endothelium and be targeted, serving an effectual therapy against tumor angiogenesis and metastasis [21,22].

In this study, specific clones of the primary antibodies were used. Although these clones are made by the most prestigious producers of antibodies, however, their sensitivity and specificity were not 100% and replacing them with antibodies from another source, may have different affinity and avidity and change the rate of positive and negative reactions, resulting in false positive reactions. To ensure the quality of the Immunohistochemistry process, positive control samples with the same process were used. Since the histopathologic diagnosis was made by expert pathologists and performed twice, the possibility of false histopathology specimens was negligible. To respect the principles of internal quality control in Immunohistochemistry, all reagents used in this project, including the primary antibodies, detection system, and the chromogen were provided by one company. Due to the frequency of the bladder transitional cell carcinoma and because the diagnosis alters the type of treatment, accurate diagnostic method is important. To achieve this goal, Immunohistochemistry staining for Maspin is helpful. Also, because down-regulation of Maspin expression is important in development and deterioration of metastatic bladder cancer, so Maspin re-expression by pharmacological methods can help the development of new therapies for the treatment of malignant, recurrent and metastatic bladder transitional cell carcinoma.

CONCLUSION

The evaluation of Maspin can be diagnostic in TCC of urinary bladder. According to the results, Maspin gene expression is reduced in TCCs of the bladder, as shown by Immunohistochemistry method, also Maspin staining intensity is decreased, associated with increasing in tumor grade and stage.

REFERENCES

1. Siegel R, et al. Cancer statistics. *CA Cancer J Clin*, **2012**, 62,10-29.

2. Ploeg M, et al. The present and future burden of urinary bladder cancer in the world. *World J Urol,* **2009**,27, 289-293.

3. Abedi Gh. et al., Assessment of quality of life in hepatitis B patients compared with healthy people, *Life Science Journal*, **2012**, 9:5339-5343.

4. Beecken WD, et al. Clinical relevance of Maspin expression in bladder cancer, *World J Urol,* **2006**, 24(3), 338-44.

5. Abd El-Maqsoud N M, Tawfiek E R, Loss of Maspin Expression in Bladder Cancer: It's Relationship with p53 and Clinicopathological Parameters, *J Egypt Natl Canc Inst*, **2010**,22, 1-12.

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6. Shariat S F, et al. Urine detection of survivin is a sensitive marker for the noninvasive diagnosis of bladder cancer, *J Urol,* **2004**, 171, 626-630.

7. Wang J, et al. Livin, Survivin and Caspase 3 as early recurrence markers in non-muscle-invasive bladder cancer, *World J Urol*, **2014**.

8. Zou Z, et al. Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. *Science* (Washington DC), **1994**,263, 526-529.

9. Juengel E, et al. Maspin modulates adhesion of bladder carcinoma cells to vascular endothelium, *World J Urol,* **2010**,28, 465-471.

10. Bass R, et al. Maspin inhibits cell migration in the absence of protease inhibitory activity, *J Biol Chem*, **2002**,277,46845-46848.

11. Chim S S, et al. Detection of the placental epigenetic signature of the Maspin gene in maternal plasma, *Proc Natl Acad Sci USA,* **2005**,102, 14753-14758.

12. Woenckhaus M, et al. uclear and cytoplasmic Maspin expression in primary non-small cell lung cancer, *J Clin Pathol*, **2007**,60, 483-486.

13. Blandamura S, Expression of Maspin in papillary Ta/T1 bladder neoplasms, *Anticancer Res,* **2008**,28, 471-478.

14. Ngamkitidechakul C, et al. Sufficiency of the reactive site loop of Maspin for induction of cell matrix adhesion and inhibition of cell invasion, *J Biol Chem*, **2003**,78, 31796-31806.

15. Kim D H, et al. Association of Maspin expression with the high histological grade and lymphocyte-rich stroma in early-stage breast cancer, *Histopathology,* **2003**,42, 37-42.

16. Friedrich M G, et al. Expression of Maspin in non-muscle invasive bladder carcinoma: Correlation with tumor angiogenesis and prognosis, *Eur Urol,* **2004**,45,737-743.

17. Sugimoto S, et al. xpression and regulation of tumor suppressor gene Maspin in human bladder cancer, *Cancer Lett*, **2004**,203,209-215.

18. Lockett J, et al. Tumor suppressive Maspin and epithelial homeostasis, *J Cell Biochem*, **2006**, 97, 651-660.

19. Chen E I, et al. Maspin alters the carcinoma proteome, *FASEB J,* **2005**,19, 1123-1124.

20. Oshiro M M, et al. Mutant p53 and aberrant cytosine methylation cooperate to silence gene expression, *Oncogene*, **2003,** 22, 3624-3634.

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

22. Abedi Gh, et al. the study of waiting line of receiving intensive care unit services in the hospitals: *HealthMED*, **2012**,6, 126-130.