Protein expression of metastasis-related genes in human bladder carcinoma

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Background/aim
The discovery of genetic alterations in oncogenes and tumor suppressor genes that accompany tumor formation has encouraged the search for genes that may promote or suppress tumor metastasis. This study aimed to investigate, by immunohistochemical analysis, protein expression of the metastasis-related genes metalloproteinase-2 (MMP-2) and nm-23 in human bladder carcinoma. Their role as prognostic factors against established clinicopathological variables in bladder carcinoma was evaluated.
Materials and methods
A total of 60 specimens of bladder carcinoma were obtained by radical cystectomy with pelvic lymphadenectomy. In addition, 10 tissue samples from normal mucosa adjacent to tumors were examined and served as controls. Immunohistochemical expression of MMP-2 and nm-23 was correlated with histological grade, tumor stage, lymph node metastases, and the presence or absence of bilharziasis.
Results
MMP-2 was expressed in 63% of patients with human bladder carcinoma and was shown to be positively correlated with histological grade, lymph node metastasis, and tumor stage. In contrast, nm-23 was expressed in 61% of patients with carcinoma but with insignificant correlation between its expression and the previous variables. Both proteins showed insignificant correlation with the presence or absence of bilharziasis. The study revealed that nm-23 expression was nonsignificantly correlated with MMP-2 expression and that nm-23 does not behave as a metastasis suppressor gene in bladder carcinoma.
Conclusion
MMP-2 overexpression seems to be related to more aggressive tumors with advanced stages and grades; therefore, it may be used not only as a promoting prognostic marker for bladder carcinoma but also as a novel target for clinical therapy.

Keywords:
bladder carcinoma, immunohistochemistry, metalloproteinase-2, nm-233

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Introduction

Progress in the treatment of urinary bladder carcinoma over the last 20 years and the current diagnosis and treatment strategies for managing the disease have been the subject of many researches [1]. There are more than 57 400 cases of and 12 500 deaths from urinary bladder carcinoma annually in the USA [2].

Egypt has the highest incidence of bladder carcinoma associated with bilharziasis. At the National Cancer Institute (NCI), Cairo, bladder cancer constitutes 30.3% of all cancers, 40.6% of male cancers and 14.3% of female cancers [3].

The prognosis is primarily determined by two independent risk factors, pathological stage and lymph node status [4]. Organ-confined, node-negative tumors are associated with the lowest recurrence rate and longest survival, whereas extravesicular, node-positive tumors have the worst prognosis [5].

As tumor invasion and metastasis are considered to be the major causes of death in bladder carcinoma [6], recent studies have focused on the discovery of molecular markers that can help in identifying patients who have a higher risk of developing an aggressive tumor phenotype or metastases [2].

Matrix metalloproteinases (MMPs) are a family of endopeptidases that play a major role in the extracellular matrix degradation related to cancer cell invasion, metastasis, and angiogenesis [7,8]. A good understanding of the expression pattern of the various MMPs in specific types of cancers may provide a basis for the development of new therapeutic strategies [9].

The nm-23 gene was initially cloned as a metastasis suppressor gene, but the clinical relevance of nm-23 as

a metastasis suppressor or prognostic indicator for human cancers remains controversial [10]. However, it was reported that lower expression of nm-23 could be used as a good indicator in evaluating lymph node metastasis and prognosis in colorectal carcinoma [11]. In contrast, the expression of nm-23 has been shown to be increased in several tumors with lower metastatic potential compared with the corresponding tumors with higher metastatic potential, including breast, hepatocellular, ovarian, and gastric carcinomas, as well as melanoma [12,13].

This study aimed to investigate the expression of nm-23 and MMP-2 proteins in human bladder carcinoma. Their role as prognostic factors against established clinicopathological variables in bladder carcinoma was evaluated.

Materials and methods

A total of 60 specimens of bladder carcinoma were used for this study. They were obtained from the Pathology Department, Cairo University, and from other private laboratories. All the specimens were obtained by radical cystectomy with pelvic lymphadenectomy. In addition, 10 tissue samples from normal mucosa adjacent to tumors were included and served a control. The samples were fixed in 10% formalin, routinely processed, and embedded in paraffin. The pathology reports and the hematoxylin and eosin slides were examined. Bilharziasis was detected in 30 specimens (50%).

The specimens were graded histologically according to the criteria of WHO [14]. None of the specimens were of grade I, 36 were of grade II, and 24 were of grade III.

Immunohistochemical study

Nm-23 and MMP-2 expression was investigated in all tissues using the streptavidin–biotin technique. Two 4-μm-thick sections from each specimen were deparaffinized, hydrated, and incubated in 3% hydrogen peroxide for 30 min to block the internal peroxidase activity. Antigen retrieval was performed by microwave pretreatment for 10 min in 0.01 mol/l citrate buffer. For each specimen, one slide was incubated overnight at 4°C with anti-nm-23-Hl monoclonal antibody at a dilution of 1 : 50 (Lab Vision Corporation, Denmark). The second slide was incubated with the mouse monoclonal antibody against MMP-2 at a dilution of 1 : 50 (Dako Corporation). These steps were followed by a incubation with biotinylated horse anti-mouse antibody for 30 min at room temperature, then with avidin–biotin peroxidase complex for 60 min at room temperature, and finally with diamiobenzidine (DAB) for 3–5 min. The slides were counterstained with hematoxylin, dehydrated, and mounted. Negative control was obtained by omitting the primary antibody.

Results of MMP-2 and nm-23 immunostaining were considered positive if 1% or more of the cells showed positive staining [15,16].

Statistical analysis

The χ^2-test was applied to examine the correlation between the expression of nm-23-Hl and MMP-2 and histological grade, lymph node metastases, tumor stage, histological type, and the presence or absence of bilharziasis. *P*-values less than 0.01 were considered significant.

Results

Routine histopathological examination was performed on 60 specimens of bladder carcinoma, and they were evaluated by immunostaining of metalloproteinase-2 and nm-23. Fourty-five specimens were of transitional cell carcinoma, whereas 15 specimens were of squamous cell carcinoma type. Lymph node metastases were found in 15 specimens, whereas 45 specimens were lymph node negative. Twenty-eight specimens were T2, 24 were T3, and eight were T4. None of the specimens were of grade I, 36 were of grade II, and 24 were of grade III.

Metalloproteinase-2 expression in specimens of bladder carcinoma

MMP-2 was found to be weakly expressed in normal bladder urothelium and was predominantly localized to the cytoplasm.

Immunostaining for MMP-2 was detected in 38 of 60 bladder carcinoma specimens (63%), and the staining was entirely confined to the cytoplasm and showed diffused staining patterns.

Seventeen of 36 specimens of grade II (47%) and 21 of 24 specimens of grade III (88%) were positive for MMP-2, and the correlation was statistically significant.

In addition, significant association was found between MMP-2 overexpression and tumor stage, in which 12 of 28 T2 specimens (43%), 18 of 24 T3 specimens (75%), and all T4 specimens were positive for MMP-2.

With regard to the lymph node status, 26 of 45 nonmetastatic specimens (58%) were positive for MMP-2, whereas 12 of 15 specimens showing metastases (80%) were positive for MMP-2, with significant statistical association.

MMP-2 was overexpressed in 18 of 30 specimens with bilharzial carcinoma (60%) and in 20 of 30 specimens with nonbilharzial carcinoma (67%), revealing a statistically nonsignificant correlation.

Histopathological types showed nonsignificant correlation with MMP-2 expression, in which 28 of 45 (62%) transitional cell carcinoma specimens and 10 of 15 (67%) squamous cell carcinoma specimens were positive for MMP-2.

On studying the correlation between the presence of lymph node metastasis and bilharziasis, there was a significant inverse correlation, in which 80% of the lymph node-positive carcinoma specimens were not associated with bilharziasis and 60% of the lymph node-negative specimens were associated with bilharziasis (Tables 1 and 2).

Expression of nm-23 in specimens of bladder carcinoma

Normal urothelium showed cytoplasmic expression for nm-23, which was marked mostly in the superficial layers.

Table 1 Correlation between MMP-2 and nm-23 expression and clinicopathological parameters in bladder carcinoma

	MMP-2				nm-23			
	Positive	Negative	*T*	*P*	Positive	Negative	*T*	*P*
Grades								
I	0	0	0	<0.01	0	0	0	>0.01
II	17 (47%)	19 (53%)	36		22 (62%)	14 (38%)	36	
III	21 (88%)	3 (12%)	24		15 (63%)	9 (37%)	24	
Total	38	22	60		37	23	60	
T-stage								
T1	0	0	0	<0.01	0	0	0	>0.01
T2	12 (43%)	16 (57%)	28		16 (57%)	12 (43%)	28	
T3	18 (75%)	6 (25%)	24		18 (75%)	6 (25%)	24	
T4	8 (100%)	0	8		3 (38%)	5 (62%)	8	
Total	38	22	60		37	23	60	
Lymph nodes								
+ve	12 (80%)	3 (20%)	15	<0.01	10 (66.7%)	5 (33.3%)	15	<0.01
−ve	26 (58%)	19 (42%)	45		27 (53%)	21 (47%)	45	
Total	38	22	60		37	23	60	
Bilharziasis								
+ve	18 (60%)	12 (40%)	30	>0.01	21 (70%)	9 (30%)	30	>0.01
−ve	20 (67%)	10 (33%)	30		16 (60%)	14 (40%)	30	
Total	38	22	30		37	23	60	
Histologic types								
TCC	28 (62%)	17 (38%)	45	>0.01	27 (60%)	18 (40%)	45	>0.01
SCC	10 (67%)	5 (33%)	15		10 (67%)	5 (33%)	15	
Total	38	22	60		37	23	60	

MMP-2, metalloproteinase-2; SCC, squamous cell carcinoma; TCC, transitional cell carcinoma.

Table 2 Correlation between lymph node status and bilharziasis in bladder carcinoma

	Lymph node metastases			
	Positive	Negative	Total	*P*-value
Bilharziasis				
+ve	3 (20%)	27 (60%)	30	<0.01
−ve	12 (80%)	18 (40%)	30	
Total	15	45	6	

In the studied bladder carcinoma specimens, immunostaining for nm-23 was mainly cytoplasmic; however, foci of nuclear staining were noticed. Thirty-seven specimens (61.7%) showed positive nm-23 expression in the form of brown cytoplasmic staining.

Twenty-two of 36 specimens of grade II (62%) and 15 of 24 specimens of grade III (63%) showed positive immunostaining for nm-23, with no statistically significant correlation between nm-23 and histological grades.

There was nonsignificant association between nm-23 expression and tumor stage, in which 16 of 28 T2 specimens (57%), 18 of 24 T3 specimens (75%), and three of eight T4 specimens (38%) were positive for nm-23.

With regard to lymph node metastases, 10 of 15 specimens showing metastases (66.7%) were positive for nm-23, whereas 27 of 45 nonmetastatic specimens (60%) showed negative staining for nm-23, revealing nonsignificant correlation.

Nm-23 was expressed in 21 of 30 specimens with bilharzial carcinoma (70%), whereas it was positive in 16 of 30 nonbilharzial specimens (53%), with no statistically significant correlations.

With regard to the histological type, nm-23 was expressed in 27 of 45 transitional cell carcinoma specimens (60%) and in 10 of 15 squamous cell carcinoma specimens (67%), revealing nonsignificant statistical correlation between histological type and nm-23 expression.

Correlation between metalloproteinase-2 and nm-23 expression

In this study, 68% of nm-23-positive specimens and 57% of nm-23-negative specimens were MMP-2 positive. In contrast, 32% of nm-23-positive specimens and 43% of nm-23-negative specimens were MMP-2 negative, revealing nonsignificant correlation between expression of both proteins(Fig. 1, Table 3).

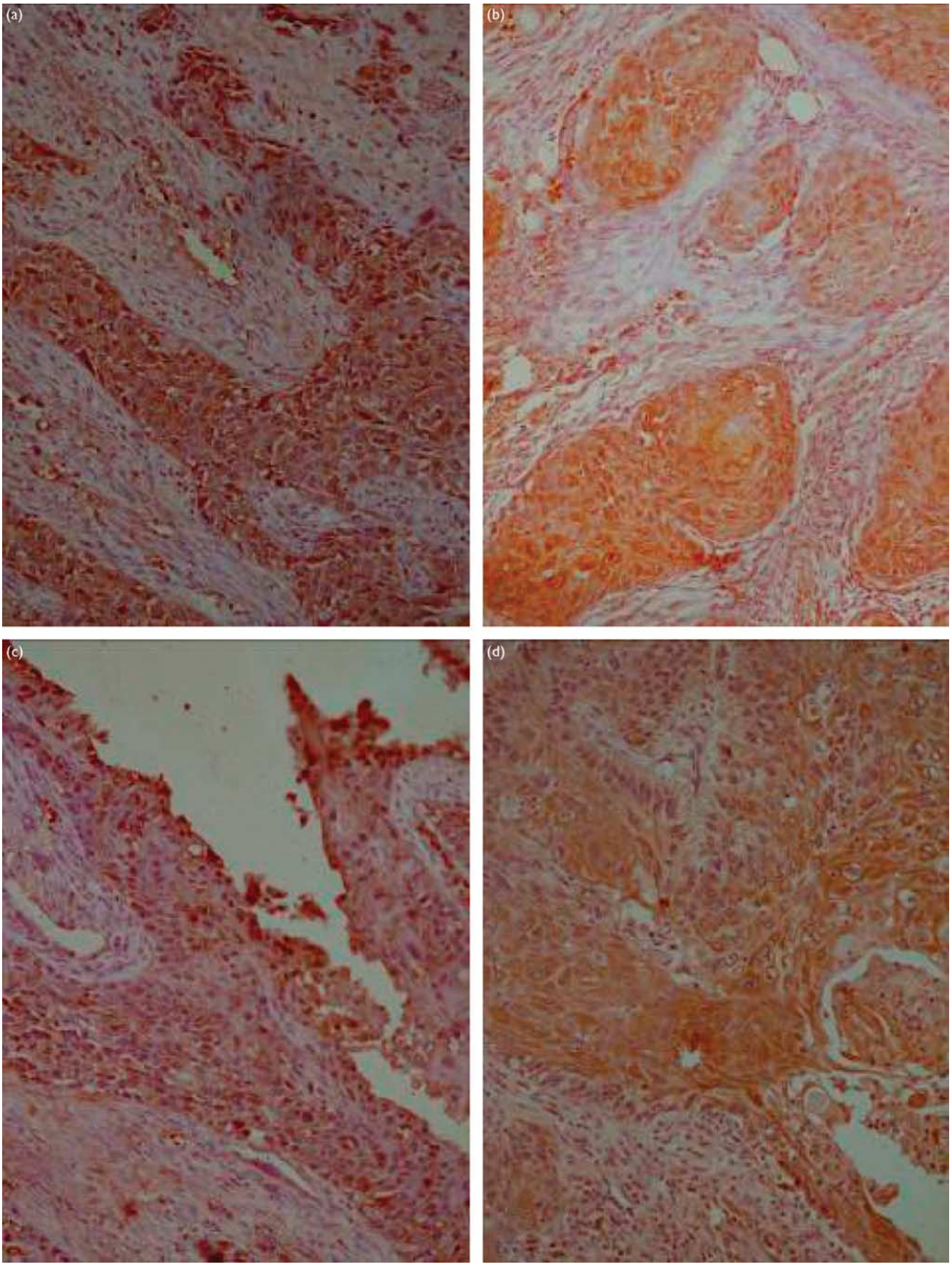
Discussion

Carcinoma of the urinary bladder is the most common malignancy in the Middle East and in parts of Africa where shistosomiasis is a widespread problem [3]. Several studies have attempted to identify the spectrum of genetic changes that occurs during urothelial transformation of bilharzial bladder cancer and elucidate the natural history of tumors with different clinical outcomes [17].

A wealth of information on the molecular pathogenesis of bladder carcinoma has emerged, including cytogenetic analysis, which demonstrates different clinical and pathological features and pathogenic mechanisms of urinary bladder carcinoma [18,19]. Moreover, the discovery of genetic alterations in oncogenes and tumor suppressor genes that accompany tumor formation in a wide variety of human tumor types, has encouraged the search for genes that may promote or suppress tumor spread and metastases [13].

Our study aimed to investigate the expression of MMP-2 and nm-23 proteins in human bladder carcinoma.

Figure 1



(a) Immunostaining for metalloproteinase-2 (MMP-2) in transitional cell carcinoma, (b) immunostaining for MMP-2 in squamous cell carcinoma, (c) transitional cell carcinoma showing positive staining for nm-23, (d) squamous cell carcinoma positively stained for nm-23 (immunoperoxidase, × 200).

Table 3 Correlation between nm-23 and metalloproteinase-2 expression in bladder carcinoma

	nm-23			
	Positive	Negative	Total	*P*-value
MMP-2				
+ve	25 (68)	13 (57)	38	>0.01
−ve	12 (32)	10 (43)	22	
Total	37	23	60	

MMP-2, metalloproteinase-2.

Their value as prognostic factors against established clinicopathological variables was evaluated. Several studies have shown that invasion and metastasis are the most serious problems in most tumors. These events require diverse proteolytic enzymes that are involved in the degradation of type IV collagen, the major component of the basement membrane. Among these enzymes is MMP-2, which plays a significant role in the degradation of this type of collagen and its expression is therefore thought to be mandatory for metastases of the tumors [20,21].

Several studies have reported high levels of expression and activity of MMPs in many kinds of tumors such as carcinoma of the esophagus, lung, stomach, etc. [22]. In the present study, normal bladder urothelium weakly expressed MMP-2 in the cytoplasm. This is consistent with the findings of Zhong *et al.* [23], who showed that MMP-2 protein expression was present in few noncancerous tissues and that the staining was very weak.

In previous studies by Li *et al.* [22] MMP-2 expression was detected in normal colon mucosa, possibly because the MMP-2 gene is known to be a 'house keeper' gene. In addition, they found that MMP-2 expression was much higher in cancerous tissue than in normal tissue.

In the present study, our results showed that MMP-2 was overexpressed in 38 of 60 specimens (63%) of human bladder carcinoma. This overexpression was found in 60% of the bilharzial specimens and in 67% of the nonbilharzial carcinoma specimens, with no statistically significant difference. However, MMP-2 expression was shown to be positively correlated with histological grading ($P<0.01$), lymph node status ($P<0.01$), and tumor stage ($P<0.01$).

These results were in accordance with those of a previous study by Papathoma *et al.* [24], who found a close correlation between MMP-2 expression and higher grade and stage of bladder carcinoma. In addition, Miyata *et al.* [25] showed that tumors positive for MMP-2 exhibited a greater proliferation index than those with negative expression. Further, Mohammad *et al.* [26] reported that MMP-2 expression in bladder carcinoma was statistically associated with high tumor stage ($P = 0.014$).

Zhong *et al.* [23] reported that MMP-2 expression was high in 67.3% of bladder carcinoma specimens and that there was a significant association between MMP-2 overexpression and the tumor stage ($P = 0.006$) and grade ($P = 0.006$). In addition, their findings showed that MMP-2 overexpression was associated with poor outcome and shorter survival in bladder carcinoma. These results were supported by other studies on solid tumors suggesting that MMP-2 protein was a strong independent prognostic factor in such tumors [23,27,28]. In addition, Zhong *et al.* [23] reported that MMP-2 protein has prognostic value in patients with advanced bladder carcinoma after cisplatin-containing chemotherapy.

This initial hypothesis that decreased nm-23 expression in primary tumors correlates with increased frequency of metastases and, thus, decreased patient survival could not be confirmed in all tumor systems [13,29].

In the current study, the normal urothelium showed cytoplasimc expression of nm-23, which was marked mostly at the superficial layers; this was similar to the findings of Khaled *et al.* [2].

Our study showed that expression of nm-23 protein was found in 37 of 60 (61%) specimens of bladder carcinoma. However, the correlation of nm-23 expression with the presence or absence of bilharziasis revealed insignificant association ($P>0.01$).

Khaled *et al.* [2] reported that loss of nm-23 protein was detected in 25 of 59 specimens (42.4%), whereas positive cytoplasmic staining was evident in 57.9% of specimens of transitional cell carcinoma associated with bilharziansis. Their study showed that the reduced expression of nm-23 was significantly associated with positive lymph nodes, differences in histology, advanced disease stage, and reduced overall survival rates. However, some reports showed a positive correlation between nm-23 expression and tumor grading, muscle invasion, and proliferating cell nuclear antigen expression, implying a positive growth regulatory role for nm-23 in bladder carcinogenesis [30,31].

In the present study, the correlation between nm-23 expression and histological grade, tumor stage, lymph node status, and histological type was insignificant. This discordance in the reported results may be partly explained by differential specificity of the antibodies applied. However, Belev *et al.* [32] and Ding and Wu [33] reported that there is correlation between lymph node status and nm-23 expression in patients with invasive breast cancer. They demonstrated that elevated nm-23 expression is related to lower rates of lymph node metastases and long survival. Gohring *et al.* [34] reported that nm-23 expression showed insignificant correlation between node-positive patients and node-negative patients, suggesting that nm-23 expression may be an independent factor but lacks prognostic or predictive value in breast cancer patients.

In our study nm-23 expression was nonsignificantly correlated with MMP-2 expression, and only MMP-2 seems to be related to more aggressive tumors with advanced stages and grades. Zhong *et al.* [23] suggested that although the prognostic value of MMP-2 should be confirmed in a large number of patients, its expression could be a useful marker for selecting patients with a high risk of poor clinical outcome and for proposing better therapy to them.

Indeed we suggested that MMP-2 could be one of the key molecular markers that identifies high-risk patients with progression in bladder carcinoma.

Conclusion

This study revealed that nm-23 does not behave as a metastatic suppressor gene in bladder carcinoma. In contrast, MMP-2 overexpression was correlated with tumor stage, grade, lymph node metastasis, and progression; therefore, it may be used not only as a promising prognostic marker for bladder carcinoma but also as a novel target for clinical therapy.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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