CORRELATION BETWEEN EXPRESSION LEVEL OF MTA2 AND CLINICAL PATHOLOGICAL FEATURES AND PROGNOSIS IN BLADDER CANCER

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ABSTRACT

Objective: *To investigate the expression of tumor metastasis-associated gene 2 (MTA2) in bladder cancer tissues, and to analyze its correlation with clinicopathological features and prognosis.*

Methods: *120 bladder cancer tissue samples of patients who had been accurately diagnosed with bladder canecr and recived urinary surgial operation from Janury 2011 to January 2014, including 80 cases of bladder cancer and 40 cases of paracancerous normal tissues. MTA2 protein was stained and its expression was observed using immunohistochemical staining. The relationship between MTA2 positive expression and negative expression in bladder cancer patients and general clinicopathological features of bladder cancer patients were analzyed. The survival curve of MTA2 protein positive expression and negative expression bladder cancer patients was drew using Kaplan-Meier analysis method. Indicators influencing patient prognosis were analzyed using COX single factor and multivariate risk regression analysis.*

Results: *The positive expression rate of MTA2 in bladder cancer tissues (87.5%) was significantly higher than that in paracancerous tissues (12.5%) (P<0.05); MTA2 expression was associated with histological grade, TNM stage, lymph node metastasis, and lymphatic invasion in patients with bladder cancer (P<0.05 or 0.01), but not related to patient gender, age, tumor diameter (P>0.05). The 5-year survival rate of patients with positive MTA2 protein expression was significantly lower than that of MTA2 negative patients (P<0.05). The 5-year survival curve of patients drew by Kaplan-Meier analysis showed that MTA2 protein expression and patient prognosis were statistically significant (P<0.05). COX single factor regression model analysis showed that the prognosis of patients was related to MTA2 expression, tissue grade, TNM stage, lymph node metastasis, lymphatic invasion (P<0.05 or 0.01); COX multivariate regression model analysis showed that lymphatic invasion and MTA2 expression were independent risk factors for the prognosis of patients with bladder cancer (P<0.05).*

Conclusion: *MTA2 protein is highly expressed in bladder cancer tissues. Its expression is correlated with histological grade, TNM stage, lymph node metastasis and lymphatic invasion. The expression of lymphatic invasion and MTA2 are in dependent factors for the prognosis of bladder cancer patients. MTA2 may be a new target for the treatment of bladder cancer.*

Keywords: *Bladder cancer, MTA2, Clinicopathological features, Prognosis.*

DOI: 10.19193/0393-6384_2019_6_462

Received *November 30, 2018;* ***Accepted*** *February 20, 2019*

Introduction

Bladder cancer is a common urinary malignant tumor in China with the incidence rate in China is around 7.49/100,000, which is significantly higher than the average incidence of bladder cancer in the world (about 4.53/100,000). It can occur in almost any age and even children, and the incidence increases with age[(1)]. In recent years, with the aging of the population and the widespread use of chemical products, and the increase in tobacco consumption, the incidence of bladder cancer is increasing year by year in both rural and urban areas, and it has now ranked the first place in male urological malignancies[(2)]. Surgery and adjuvant therapy are common clinical treatments, which can significantly improve the symptoms of bladder cancer patients, but still have a high recurrence rate after treatment, distant metastasis has become an important cause threatening the prognosis of bladder cancer patients[(3)].

The cause of bladder cancer is complex. Exposure to aromatic amine chemicals and smoking, etc. are currently two clinically clear risk factors, and little is known about some unpredictable biological behaviors and mechanisms within the tumor[(4)]. Therefore, looking for a novel molecular

targeting markers that improve prognosis have become a topic which are widely concerned by clinical scholars. Metastasis-associated gene 2 (MTA2) is a gene closely related to tumor migration and invasion, and its expression level is significantly increased in malignant tumor diseases such as ovarian cancer and hepatocellular carcinoma[5].

However, its expression in bladder cancer and its biological function are still vague. Therefore, this study analyzes the expression of MTA2 in bladder cancer tissues, and analyzes its relationship with clinicopathological features and prognosis, aiming at provding a new direction for early diagnosis and treatment of bladder cancer.

Materials and methods

General data

Eighty (80) patients with pathological paraffin samples confirmed with bladder cancer and urnairy surgical operation from January 2011 to January 2014 in our hospital were enrolled. The subjects included 60 males and 20 females, aged 50-74 years, median age was 57.1 years; 42 cases of grade I in histology, 38 cases of grade II~III; TNM staged from stage I to stage II in 43 cases, stage III to stage IV in 37 cases; lymph node metastasis in 34 cases, and no lymph node metastasis in 46 cases; there were 29 cases of lymphatic infiltration, 51 cases without lymphatic invasion; 50 cases with tumor diameter ≥3cm, 30 cases <3cm. 120 samples included 80 cases of bladder cancer tissue and 40 cases of paracancerous normal tissues.

Inclusion criteria:

- Postoperative pathology confirmed bladder epithelial cancer;
- Patients received any regular radiotherapy or chemotherapy before surgery;
- Patients were excluded from other tumor diseases, serious infectious diseases and immune system dysfunction;
- Subjects have complete clinical and pathological data;
- Patients and their families informed consent and signed informed consent, and the hospital ethics committee approved. Subjects were followed up until January 31, 2019.

Research materials

Mouse anti-human MTA2 monoclonal antibody (purchased from Fujian Maixin Biotechnology Co., Ltd.), 4% paraformaldehyde, PBS solution, SP kit, DAB chromogenic kit (purchased from Zhongshan company), optical microscope, etc.

Immunohistochemical staining

SP staining method, all specimens were fixed with 4% paraformaldehyde, embedded in paraffin, and sectioned. The xylene is dewaxed in turn, and the alcohol gradient is dehydrated etc. The PBS was used instead of the primary antibody as a negative control, and the MTA2 protein-positive bladder cancer tissue was used as a positive control.

Results Identification criteria:

1 according to the degree of staining intensity and browning , they were divided into 0 points, 1 point, 2 points, 3 points; 2 according to the percentage of positive cells, they were divided into 0 points (0.0% of positive cells), 1 point (≤ 25%) 2 points (25% to 50%) and 3 points (>50%).

According to the sum of the two scores, it was divided into negative (0~2 points) and positive (3~6 points).

Statistical methods

Statistical results were expressed as ($\bar{x}\pm s$) or [n (%)], and differences between groups were compared by t test or $\chi 2$ test. The survival rate of bladder cancer patients and Log-rank test were calculated using Kaplan-Meier method. The prognosis was anzlyed using COX single factor and multi-factor regression analysis. And SPSS20.0 statistical software package was used to to record and process the recorded data. $P < 0.05$ it was considered to be statistically significant.

Results

Expression of MTA2 in bladder cancer and paracancerous tissues

The results of immunohistochemical staining showed that MTA2 was expressed both in the nucleus and cytoplasm of bladder cancer tissues, and the coloration was brownish yellow, but not expressed or underexpressed in paracancerous tissues.

Among the 80 specimens, the positive expression of MTA2 in bladder cancer tissues was 35 (87.5%), which was significantly higher than that in paracancerous tissues (12.5%), the difference was statistically significant ($P<0.05$). Seen in Figure 1 and Table 1.

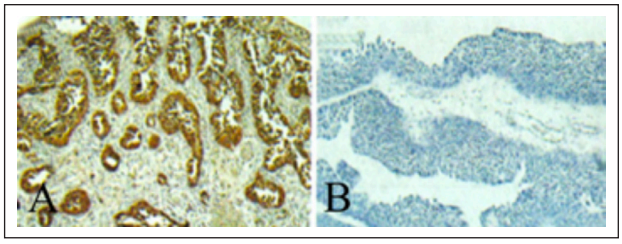

Figure 1: Expression of immunohistochemical staining of MTA2 in bladder cancer and adjacent tissues.
A: Bladder cacner tissues; B: Paracancerous tissues.

Group	n	Positive expression rate of MTA2 protein (%)
Bladder cacner tissues	80	70 (87.5)
Paracancerous tissues	40	5 (12.5)
χ^2		<45.000
P		<0.01

Table 1: Positive expression rate of MTA2 in bladder cancer and paracancerous tissues [n (%)].

Relationship between MTA2 expression and clinicopathological features in cancerous tissue specimens

There was a correlation between MTA2 expression and histological grade, TNM stage, lymph node metastasis and lymphatic invasion in patients with bladder cancer, and the difference was statistically significant (P<0.05 or 0.01), but not related to patient gender, age and tumor diameter. There were no statistical significances (P>0.05). Seen in Table 2.

parameter	n (n=80)	MTA2 positive expression	MTA2 negative expression	χ^2	*P*
Sex				0.267	0.606
Male	60	31	29		
Female	20	9	11		
Age				0.220	.639
>65	52	25	27		
≤65	28	15	13		
Hitological grade				24.261	<0.001
I	42	32	10		
II-III	38	8	30		
TNM stage				36.656	<0.001
I stage- II stage	43	35	8		
III stage- IV stage	37	5	32		
Lmyph node metastasis				16.573	<0.001
Yes	34	26	8		
No	46	14	32		
Lymph tube infiltration				19.537	<0.001
Yes	29	24	5		
No	51	16	35		
Tumor diameter				0.213	0.644
<3cm	30	16	14		
≥3cm	50	24	26		

Table 2: Relationship between protein expression and clinico pathological features of bladder cancer patients.

Relationship between MTA2 protein expression and prognosis of patients with bladder cancer

Eighty (80) patients had 39 deaths within 5 years, 28 patients with positive MTA2 protein expression, and 11 patients with negative MTA2 expression. The 5-year survival rate of MTA2 protein positive patients was significantly lower than that of MTA2 negative patients. The difference was statistically significant. (P<0.05). Patient's 5-year survival curve drew by Kaplan-Meier analysis showed that MTA2 protein expression and patient prognosis were statistically significant (P < 0.05, Figure 2).

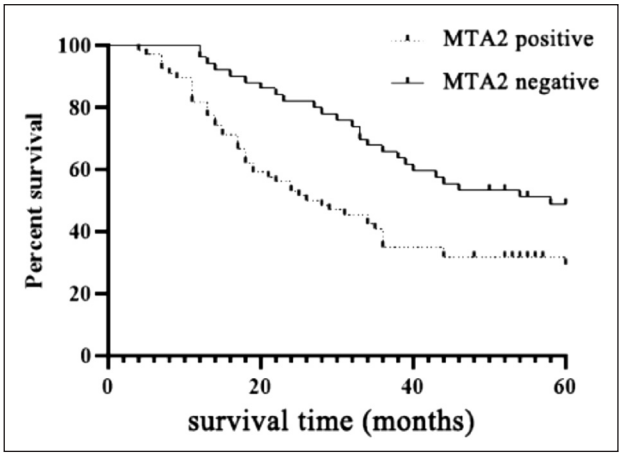

Figure 2: Curve of MTA2 protein expression and 5-year survival rate of bladder cancer patients.

Analysis of independent risk factors for prognosis in patients with bladder cancer

Univariate regression model analysis showed that the prognosis of patients was related to MTA2 expression, tissue grade, TNM stage, lymph node metastasis, lymphatic invasion (P<0.05 or 0.01). Multivariate regression model analysis showed that lymphatic invasion and MTA2 expression were independent risk factors for prognosis in cancer patients (P<0.05). Seen in Table 3.

Parameter	Single factor analysis		Multiplefactor analysis	
	HR (95%CI)	*P vlaue*	HR (95%CI)	*P value*
MTA2 protein	3.40 (1.60~7.31)	0.006	2.40 (1.01~5.90)	0.031
Age	1.80 (0.77~3.52)	0.220	1.24 (0.50~2.99)	0.529
Sex	1.59 (0.83~3.38)	0.139	1.79 (0.96~3.60)	0.101
Tumor diameter	1.89 (0.91~3.42)	0.178	1.38 (0.55~3.10)	0.511
Histological grade	2.62 (0.99~6.68)	0.044	1.76 (0.93~3.48)	0.103
TNM stage	1.84 (0.71~4.53)	0.048	1.72 (0.53~4.79)	0.328
Lymph node metastasis	2.59 (0.92~6.69)	0.046	1.35 (0.53~3.14)	0.542
Lymph tube infiltration	3.58 (1.67~8.10)	0.009	2.61 (1.20~5.42)	0.019

Table 3: Single factor and multifactor survival analysis of COX in 80 patients with bladder cancer.

Discussion

The low survival rate of patients with malignant tumors is result from the distant metastasis of the tumor, not the tumor itself. Tumor metastasis and recurrence are the most important factors affecting the prognosis of patients with malignant tumors, which needs people to solve urgently and is one of the difficulties in understanding tumors at this stage[6]. Bladder cancer, as one of the most common tumors in urinary system in China, its metastasis and recurrence can exert an important effect on the diagnosis and treatment of patients. Therefore, in-depth study of its transfer mechanism and search for appropriate predictive molecules for the diagnosis and treatment of bladder cancer patients are very essential.

MTA2 protein, a complex with nuclear small body plastic activity, contains 68 amino acids, which is closely related to the progress of many tumors. It has been found that MTA2 protein is highly expressed in many malignant tumor tissues or cells, especially in cells with high division, such as colorectal cancer, and is closely related to TNM staging and whether lymph node metastasis or not in tumor tissues[7]. MTA2 is a component of nuclear small-weight plastic and histone deacetylation complex subunit histone deacetylase 1, nucleosome and histone deacetylation complexes involved in histone deacetylation and nuclear small-weight plastic process, and further causes chromatin conformational changes, triggers changes in the biological function of proteins in the downstream. Studies have shown that MTA2 protein regulates histone deacetylation activity in nucleosomes and histone deacetylation complexes, and then inhibits transcription by altering chromatin conformation[8]. In addition, histone deacetylase 1 containing MTA2 can deacetylate and inactivate the p53 tumor suppressor gene[9]. MTA2 is highly expressed in a variety of tumor cells and plays an important role in many processes such as tumor formation, metastasis, invasion, and tumor angiogenesis[10]. In esophageal cancer and cervical cancer, MTA2 is highly expressed and is an independent risk factor for prognosis of patients. MTA2 can promote infiltration and metastasis of non-small cell carcinoma by down-regulating miR-125B[11]. In colorectal cancer, MTA2 is involved in epithelial-mesenchymal transition by increasing the expression of Slug and Snail and inhibiting the expression of E-cadherin protein[12]. MTA2 not only promotes the metastasis of tumor cells, but also is closely related to tumor angiogenesis[13]. It has been shown that MTA2 plays a significant role in the angiogenesis of prostate cancer by regulating the production of angiogenic factors[14], indicating that MTA2 plays an important role in the occurrence and development of bladder cancer.

This study confirmed that the results of immunohistochemistry showed that MTA2 protein was highly expressed in bladder cancer tissues compared with paracancerous tissues ($P<0.05$). It was found that MTA2 expression is closely related to histological grade, TNM stage and lymph node metastasis, and lymphatic infiltration of bladder cancer patients, but it was not statistically significant with the patient's gender, age, and tumor diameter. The expression of MTA2 protein was significantly correlated with the prognosis of patients. The 5-year survival rate of positive patients was significantly lower than that of MTA2-negative patients, and the prognosis was poor. COX multivariate regression model analysis found that lymphatic infiltration and MTA2 expression were independent risk factors for the prognosis of patients with bladder cancer. A large number of studies have confirmed that MTA2 plays a role in malignant tumors mainly by mainly participating in cancer cell metastasis and infiltration[15], and this study just proves that MTA2 expression level is closely related to lymph node metastasis and lymphatic infiltration, suggesting that MTA2 is involved in cancer. However, the specific mechanism is to be further explored by clinical scholars.

In summary, MTA2 is highly expressed in bladder cancer tissues, and its expression is associated with histological grade, TNM staging, lymph node metastasis, and lymphatic infiltration in patients with bladder cancer. Lymphatic infiltration and MTA2 expression are independent risk factors for prognosis. The higher the positive rate of MTA2 expression is not conducive to the prognosis of patients, and its mechanism may be related to bladder cancer metastasis, which may become a new target for the treatment of bladder cancer.

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