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The role of mta2 expression in bladder cancer cells and its regulation mechanism

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Abstract

Researching the role of the mta2 gene in bladder cancer and its possible therapeutic applications is the goal of this research.Methods: The T24 bladder cancer cells were transfected with a viral vector to induce overexpression of the metastasis-associated gene 2 (mta2), whereas the EJ bladder cancer cells were transfected with si-mta2. Under the two mta2 expression settings, cell lines were tested for their invasiveness and migratory capability in vitro using Matrigel and Transwell methods, respectively.End result: Data from transwell migration experiments showed that T24 bladder cancer cell lines were much more able to migrate when mta2 protein was overexpressed, but EJ cell lines were much less able to migrate when mta2 was knocked down ($p < 0.01$). In the matrigel invasion experiment, it was shown that the invasive capability of the T24 bladder cancer cell line was greatly increased by overexpressing the mta2 protein, whereas the invasive capacity of the EJ bladder cancer cell line was dramatically decreased ($p < 0.01$) by knocking down mta2. In the T24 bladder cancer cell line, which overexpresses mta2, the levels of E-cadherin and N-cadherin were lower than in the cd511b-transfected and untransfected groups, respectively. In addition, compared to cells that were transfected with si-NC or not transfected, the E-cadherin protein expression in the EJ bladder cancer cell line with mta2 knockdown was noticeably greater ($p < 0.01$).In conclusion, mta2 knockdown blocks bladder cancer cell lines' ability to proliferate, migrate, and invade by preventing proteins involved in the epithelial-mesenchymal transition from doing their jobs.Reproductive process, migration, bladder cancer, mta2

INTRODUCTION

The most frequently diagnosed tumor of the urinary tract is bladder carcinoma which is usually located on the mucosal surface and ranks amongst the 10 most-often diagnosed human neoplasms [1]. The age of onset of bladder cancer is relatively broad: it occurs almost at any given age, even in children. However, the occurrence of bladder cancer slowly rises as a function of age. There have been increases in incidence of bladder cancer in recent times, due to factors such as heightened use of sundry chemical products, tobacco use, and aging human populations [2]. At present, radical surgery and adjuvant treatment are used to mitigate the signs of bladder carcinoma, but studies have revealed that postoperative bladder cancer patients still experience high degrees of local recurrence, distant metastasis and poor prognosis [3]. The etiology of bladder cancer is complex. Two clear risk factors are exposure to aromatic amines and smoking, but little is known about some unpredictable biological behaviors within the cancer tissue or signaling routes associated with its progression [4]. Therefore, in order to efficiently carry out timely diagnosis and treatment of bladder tumor patients, there is need to identify new molecular markers for evaluation of its prognosis.

Some investigations have revealed that *mta2*, one of the Metastasis-Associated Genes (MTAs), is up-regulated in ovarian cancer, hepatocellular carcinoma, bladder cancer and other malignant tumors, and is intimately linked to cancer cell migratory and invasive potential[5]. However, the number of reports on relative expression of *mta2* in bladder carcinoma, and its role, is limited. Thus, this research was aimed at determining *mta2* expression in bladder carcinoma cells, as well as the influence of *mta2* overexpression and *mta2* silencing on their multiplication, invasiveness and migratory potential. This was to identify the relevance of *mta2*, and its possible regulatory mechanism in the progression of bladder carcinoma.

EXPERIMENTAL

Materials

Chongqing Youbao Biotechnology Co. Ltd was the supplier of bladder cancer cell lines (24, EJ and J82), *mta2* interference lentivirus and plasmid required.

Handling of cells

The bladder cancer cells were maintained in RPMI-

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1640 medium containing 10 % FBS. The experiments were carried out using logarithmic growth phase cells. The T24 bladder cancer cells were infected with a 1:1 (v:v) mixture of bovine serum-free medium and virus solution, in line with lentivirus transfection kit instructions. The plasmid DNA was incorporated into *Escherichia coli*, followed by 12-h culturing at 37 ^{O}C, after which it was subjected to extraction. Then, transfection of bladder cancer cells with the *mta2* was done in line with the steps indicated in the transfection instructions.

Western blot assay

Total protein was extracted from bladder cancer cells by adding RIPA buffer solution and shaking on ice for lysis for 20 min. The lysed cell samples were centrifuged for 10 min, and the supernatant was collected. The protein contents of the supernatants were determined using the BCA method. Then, each protein sample was subjected to SDS-PAGE, followed by transfer to polyvinylidene difluoride membrane. The membrane was incubated fat-free milk solution to block non-specific binding of the blot. Then, it was incubated with *mta2*-specific primary antibodies overnight at 4 ^{O}C, followed by incubation with a secondary antibody at room temperature for 1 h. Luminescent substrates or staining agents were used for color development to observe the expression level of the target protein.

MTS colorimetry

Bladder cancer cells transfected with mta2 overexpression and low expression were cultured to logarithmic growth stage. The cells were evenly inoculated in 96-well plates, with approximately 5000 cells per well. After incubating the well plates in the incubator for 24 h, MTS reagent was added to each well, and the wells were incubated for 1-4 h. Then, the absorbance of each well was read in a multi- channel ELISA reader and recorded. Using the absorbance values, the proliferation capacity of cells in each group was calculated, and the differences between the mta2 overexpression and low-expression groups were determined.

Matrix invasion and Transwell migration

The upper chamber of Transwell was evenly coated with Matrigel, while the lower chamber contained equivalent amount of cell culture medium. The bladder cancer cells transfected with mta2 overexpression and low expression were made into single-cell suspensions, and the cell suspension was added to the pores covered by Matrigel or Transwell. The well plates were incubated in the incubator for 24 - 48 h. Then, the migrated cells on the upper surface were removed with washing solution or cotton swab. For the Matrigel invasion experiment, cell fixation was performed using repair solution, and then cells were stained with specific staining agents. For the Transwell migration experiment, direct staining was done, and the stained well plates were examined under a microscope. The number of invading or migrating cells was counted, and the differences between different MTA2 expression groups were obtained.

Statistical analysis

The SPSS 22.0 statistical software package was used to perform statistical analysis of the recorded data. The counting data are presented as (n (%)). Groups were compared using χ^2-test. Quantitative data are presented as mean ± standard deviation (SD). Paired data were compared between groups using independent sample *t*-test, while non-normal distribution data were compared using rank sum tests. Statistical significance was considered at $p < 0.05$.

RESULTS

mta2 protein expression in bladder cancercells

There was significantly lower amount of mta2 protein expression in T24 than in EJ bladder cancer cell lines, and the *mta2* expression level in EJ bladder cancer cell line was significantly higher than that in other bladder cancer cell lines ($p < 0.05$). These results are shown in Table 1. Therefore, T24 and EJ bladder cancer cells were chosen for use in follow-up studies.

Table 1: Expression of mta2 protein in bladder cancer cell lines

Group	*mta2* protein
T24	0.31±0.03
EJ	1.65±0.14$^{\#}$
J82	0.87±0.07

Note: $^{\#}P < 0.05$ vs. other two groups

mta2 expressions in selected cell lines transfected with mta2

In the present study, *mta2* was successfully transferred into T24 bladder cancer cell line. The mta2 protein expression level in T24 bladder cancer cells in *mta2* transfection group was significantly higher than those in un-transfected group and cd511b transfection group. However, *mta2* expression was unchanged, irrespective of cd511b transfection status. Moreover, *mta2* of EJ bladder cancer was effectively silenced, resulting in significantly lower *mta2* expression levels in EJ bladder cancer cells than in un-transfected cells. However, *mta2* expression was comparable in si-NC transfected and un- transfected cells. These results are presented in Table 2 and Table 3.

Multiplication of overexpression and low-expression *mta2* cell groups

The absorbance readings of T24 bladder cancer cells in *mta2* transfection group were significantly higher than those in the transfected and cd511b transfection groups at different times ($p < 0.01$). In contrast, absorbance values were not changed by cd511b

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transfection status. However, the absorbance of EJ bladder cancer cell line was significantly reduced in *si-mta2*-transfected cells, when compared with the un-transfected and *si-mta2* transfection groups. The *si-mta2* transfection had no marked effect on absorbance. These results are presented in Table 4.

Table 2: *mta2* protein levels of transfected T24 bladder cancer cell line

Group	mta2 protein
T24 un-transfected	0.81±0.10
T24/*mta2* transfected	1.52±0.22#
T24/CD511B transfected	0.68±0.07

Note: #*P* < 0.05 vs. other 2 groups

Table 3: mta2 protein expression of EJ bladder cancer cell line after transfection

Group	mta2 protein
EJ Un-transfected group	1.41±0.14
EJ/si-MTA2 Transfection group	0.53±0.10#
EJ/si-NC Transfection group	1.38±0.15

Note: #*P* < 0.05 vs. other 2 groups

Table 4: Determination of absorbance values of different groups using MTS colorimetric method

Group	Day 1	Day 2	Day 3
T24 un-transfected	0.62±0.40	1.11±0.09	1.70±0.11
T24/*mta2*-transfected	0.72±0.11	1.38±0.12	2.05±0.10
T24/CD511B transfected	0.58±0.05	0.99±0.12	1.64±0.16
EJ un-transfected	0.60±0.04	1.02±0.02	1.59±0.04
EJ/si-*mta2*-transfected	0.38±0.03	0.83±0.04	1.01±0.07
EJ/si-NC-transfected	0.59±0.02	0.88±0.03	1.45±0.04

Note: **P* < 0.01, absorbance values of EJ/*si-mta2* transfected group decreased significantly, compared to EJ un-transfected and EJ/*si-mta2si-NC* transfected groups.

Migratory potential of overexpression and under-expression *mta2* cell groups

In the *in vitro* migration experiment, the population of T24 bladder cancer cells that crossed the basement in *mta2* transfection group was significantly higher than the corresponding numbers in the un-transfected and cd511b transfection groups. However, cd511b transfection status did not affect *mta2* expression. Significantly lower number of EJ bladder cancer cells crossed the basement membrane in *si-mta2*-transfected cells than in un-transfected and *si-NC* transfection groups.

The *si*-NC transfected and transfected cells did not differ in *mta2* expression. These data are presented in Table 5 and Table 6.

Table 5: Count of cancer cells that traversed the basement at high magnification

Group	T24
T24 un-transfected	0.60±0.37
T24/MTA2 transfected	0.74±0.13#
T24/CD511B transfected	0.57±0.08

Note: #*P* < 0.05 vs. other two groups

Table 6: Population of cancer cells that crossed basement membrane, as seen at high magnification

Group	EJ
EJ un-transfected	61.48±1.20
EJ/si-*mta2* transfected	26.94±3.11#
EJ/si-NC transfected	58.74±1.55

Note: #*P* < 0.05 vs. other two groups

Invasiveness of overexpression and under-expression of mta2 cell groups

In the invasion experiment, the population of T24 bladder cancer cells that crossed the membrane in *mta2* transfection group was significantly higher, relative to the un-transfected and cd511b transfected groups. However, cd511btransfection had no effect on *mta2* expression. The population of EJ bladder cancer cells that crossed membrane was significantly reduced *in si-mta2* transfection cells, relative to un- transfected and si-NC transfection cells ($p < 0.01$). The si-NC transfection had no effect on *mta2* expression. These results are presented in Table 7 and Table 8.

Table 7: Population of cancer cells that crossed the membrane, as seen at high magnification

Group	T24
T24 un-transfected	31.78±5.43
T24/*mta2*-transfected	48.72±3.44#
T24/CD511B-transfected	32.57±3.84

Note: #*P* < 0.05 vs. other two groups

Levels of EMT-associated proteins in overexpression and under-expression mta2 cell groups

E-Cadherin protein level in T24 bladder cancer cells was significantly down-regulated in *mta2* transfection cells, relative to the un-transfected and cd511b transfection groups, but N-cadherin protein was significantly up-regulated in *mta2* transfection cells, relative to un-transfected and cd511b transfection cells. The cd511b transfection had no marked effect on the expressions of the two proteins. These data are presented in Table 9.

Table 8: Population of cancer cells that crossed the membrane, as seen at high magnification

Group	EJ
EJ un-transfected	60.27±4.34
EJ/si-*mta2*-transfected	24.16±2.65#
EJ/si-NC-transfected	58.33±3.86

Note: #*P* < 0.05 vs. other two groups

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Table 9: EMT-related protein expression in overexpressing MTA2 cell group

Group	E-cadherin	N-cadherin
T24 un-transfected	1.04±0.07	1.03±0.08
T24/*mta2*-transfected	0.62±0.06#	1.55±0.12#
T24/CD511B-transfected	1.01±0.08	0.98±0.06

Note: #$P < 0.05$ vs. other two groups

There was significantly higher E-cadherin protein expression level in *si-mta2* transfection EJ bladder cancer cells than in un-transfected and si-NC transfection cells, but N-cadherin protein was expressed significantly lowly in *si- mta2* transfection cells, relative to the corresponding levels in un-transfected and si- NC transfection cells. However, si-NC transfection did not affect the expression levels of the two proteins. These data are presented in Table 10.

Table 10: EMT-associated protein levels in *mta2*-knockdown cells

Group	E-cadherin	N-cadherin
EJ Un-transfected	0.95±0.08	0.97±0.07
EJ/si-*mta2*-transfected	1.46±0.14#	0.64±0.11#
EJ/si-NC -transfected	0.92±0.09	0.92±0.09

Note: #$P < 0.05$ vs. other two groups

DISCUSSION

Malignant tumor is one of the most common diseases threatening people's lives at present. The incidence and case fatality rate of malignant tumors are rising all over the world, and they have also become one of the main causes of death among the Chinese population [6]. The metastatic and invasive nature of malignant tumors is the main cause of poor prognosis in the vast majority of patients. Therefore, studying the mechanisms underlying the metastatic and invasive potential of cancer cells has become an urgent issue that needs to be addressed. Bladder carcinoma is one of the most frequently seen malignant diseases in urinary canal, with high metastasis and invasion, but its specific regulatory mechanism is still unclear [8].The mta2 protein is a 68-amino acid polypeptide with nucleosome remodeling activity, and it belongs to one of the mta family members associated with tumor metastasis. Studies have revealed that mta2 protein is well-expressed in certain cancers such as cancers of the bladder, colon and esophagus, with close association with Tumor Node Metastasis stage, metastasis to lymph nodes, and differentiation [9]. Thus, *mta2* gene is likely to be involved in the occurrence and progression of bladder carcinoma. In order to investigate the link between *mta2* and the occurrence of bladder carcinoma, lentivirus-overexpressing vectors were employed to establish *mta2* overexpression plasmids so as to generate *mta2*-overexpressing T24 cells. In addition, *si-mta2* and knockdown *mta2* were used to transfect EJ cancer cells.

Through Matrigel, MTS and Transwell assays, it was found that T24 cancer cells overexpressing mta2 protein had increased proliferation and invasion potential. The migratory potential was significantly raised in *mta2*-silenced T24 cells, relative to cd511b-transfected and un- transfected cells, but the invasive, proliferative and migratory potential of *mta2*-knockdown EJ cells were suppressed. Thus, *mta2* may participate in the bladder carcinoma development via its effect on bladder cancerous cell invasiveness and proliferation. Therefore, it is important to unravel the regulatory process through which *mta2* influences the above biological characteristics of bladder carcinoma cells.

The reduction in adhesion between cells and epithelial-mesenchymal transition (EMT) is an important reason for the metastatic potential of cancer cells [10]. Therefore, the regulation of mta2 may be related to the invasiveness and migratory capacity of bladder carcinoma cells. It is known that E-cadherin is a Ca^{2+}-reliant adhesion protein which regulates the structural intactness of cells of the epithelium, maintains tight connections between cells, and prevents cell proliferation and invasion [11]. In contrast, N-cadherin mainly exists in interstitial cells and is loosely connected. Therefore, E-cadherin upregulation and N-cadherin downregulation were used as crucial indices for evaluating EMT transformation in this study. The experimental results showed that knocking down *mta2* upregulated the expression of E-cadherin and downregulated the expression of N-cadherin. Overexpression of *mta2* had the opposite effect, with downregulation of E-cadherin expression and upregulation of N-cadherin expression. This suggests that *mta2* may promote the invasiveness and spread of bladder carcinoma cells by regulating EMT transformation. Tumor metastasis-associated gene 2 (*mta2*) plays an important role in bladder cancer [12,13]. This gene plays a key role in regulating the multiplication, invasiveness and migration of bladder carcinoma cells. By regulating the expression levels of E-cadherin, N-cadherin and other related proteins, *mta2* affects the metastatic potential of bladder carcinoma[14,15].

CONCLUSION

Overexpression of *mta2* enhances the multiplication, invasiveness and migration of bladder carcinoma cells, but silencing *mta2* significantly reduces their multiplication, migratory potential and invasiveness. *Mta2* exhibits these properties through a mechanism that is related to the suppression of EMT. Therefore, targeting *mta2* may be a treatment approach in developing a suitable therapy against bladder carcinoma. Further studies on the regulatory mechanism of *mta2* may reveal its exact mechanism in the pathogenesis of this disease.

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