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CORRELATION BETWEEN TRIP13 EXPRESSION AND CLINICOPATHOLOGICAL CHARACTERISTICS OF BLADDER CANCER AND ITS EFFECT ON THE BIOLOGICAL BEHAVIOR OF BLADDER CANCER CELLS

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ABSTRACT

Objective: *To investigate the correlation between the expression of thyroid hormone receptor interactor 13 (TRIP13) and the clinicopathological characteristics of bladder cancer, along with the influence of TRIP13 on the biological behavior of bladder cancer cells.*

Methods: *From January 2013 to February 2015, 38 samples of bladder cancer tissue were selected for inclusion in this study from those that were frozen in our hospital, along with 18 samples of normal bladder mucosa from patients in our hospital who had undergone surgery for benign diseases during the same time period. An immunohistochemical method was employed to detect the expression of TRIP13 in the bladder cancer tissues and the normal bladder mucosa tissues, and to analyze the correlation between its expression level and the clinicopathological characteristics and prognosis of the bladder cancer patients. At the same time, the human bladder cancer cell line T24 was collected for routine cultivation; its logarithmic growth phase was randomly divided into a blank control group, a negative control group, and a siRNA group. Small interfering RNA against TRIP13 were added to the cells in the siRNA group; they were then transfected with liposomes and introduced into T24 cells. The negative control group was transfected with irrelevant siRNA. All three groups of T24 cells were implanted in 6-well plates, at a density of 5×105/well, one day before transfection. The next day, the cells were transfected when the degree of cell fusion reached 70%; the serum and antibiotics were replaced 5 hours later. The three groups of T24 cells' clone formation rates, migration distances, and cell proliferation inhibition rates were compared at 24 h, 48 h, 72 h, and 96 h.*

Results: *The positive expression rate of TRIP13 in the bladder cancer tissues was found to be 60.53% (23/38), significantly higher than that of TRIP13 in the normal bladder mucosa tissues, at 27.78% (5/18; P<0.05). The expression level of TRIP13 was related to the depth of invasion, lymph node metastasis, and TNM stage in patients with bladder cancer (P<0.05), while it was unrelated to the age, sex, and pathological grade of the patients (P>0.05). The 5-year survival rate of TRIP13-positive-expression patients was found to be 30.43% (7/23), which was significantly lower than that of the TRIP13-negative-expression patients, at 60% (9/15, P<0.05). At 24 h, 48 h, 72 h, and 96 h, the proliferation inhibition rate of T24 cells in the siRNA group was significantly higher than that in the blank control group, while the inhibition rate of T24 cell proliferation in the blank control group was not statistically different to that in the negative control group (P>0.05). The cloning rate of T24 cells in the siRNA group was significantly lower than that in the blank control group, while there was no statistically significant difference between the blank control group and the negative control group in this regard (P>0.05). The migration distance of T24 cells in the siRNA group was significantly lower than that in the blank control group, while the blank control group was not different from the negative control group to a statistically significant degree (P>0.05).*

Conclusion: *The expression level of TRIP13 in bladder cancer tissue is elevated, and its expression level is closely related to the depth of infiltration, lymph node metastasis, and TNM stage of the patient. TRIP13 is expected to become an important reference index for use to evaluate the prognosis of bladder cancer patients. In addition, TRIP13 has been found to play an important role in the behavior of bladder cancer cells; reducing its expression, therefore, holds the potential to significantly inhibit the proliferation, migration, and clonal formation of bladder cancer cells.*

Keywords: *TRIP13, bladder cancer, clonal formation, migration, proliferation.*

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Introduction

Bladder cancer is a common tumor of the urinary male reproductive system that is associated with high morbidity and mortality, and the current incidence rate in China is increasing year by year(1). Although the development of medical technology continues to progress and the clinical symptoms of patients have been improved to a certain extent, the problems of invasion and metastasis are still difficult when faced in clinical practice. Over 30% of bladder cancer patients present invasion and metastasis after treatment, which seriously threatens the patient's prognosis and can directly lead to the failure of the

treatment and the patient's death(2). Therefore, it is particularly important that we explore and clarify the molecular mechanism of bladder cancer invasion and metastasis, so as to improve the prognosis of patients. In recent years, there have been many reports on a large number of tumor markers that function to predict the prognosis of bladder cancer patients. Examining a combination of these markers, the bladder cancer's onset, and the prognosis-related protein molecular diagnosis can play a role in evaluating a patient's prognosis.

On this premise, new targeted therapy drugs are in development for multiple diseases(3). Research has demonstrated that thyroid hormone receptor-interacting factor 13 (TRIP13) is a transcriptional regulator, playing an important role in the processes of mitosis and meiosis. With the continuous advancement of gene sequencing and analysis technologies, studies have found that TRIP13 can be highly expressed in many cancer tissues, such as multiple myeloma, head and neck squamous cell carcinoma, etc.(4). One study has suggested that TRIP13 can exist as an oncogene in bladder cancer; this oncogene is expected to become a new target for the treatment of bladder cancer(5-6). However, the correlation of TRIP13 expression with the clinicopathological characteristics of patients with bladder cancer is not yet clear, nor is its mechanism of action on the biological behavior of bladder cancer cells, or its resulting influence; this study intends to elaborate on these three areas of research.

Materials and methods

Subjects

• 38 samples of bladder cancer tissue were chosen for inclusion in this study, which were frozen in our hospital from January 2013 to February 2015.

The inclusion criteria were as follows:

• All patients must have been diagnosed with bladder cancer through relevant pathological and imaging examinations, the records for all patients were required to include detailed clinical-pathological data and follow-up information, the patient or their family must have signed with informed consent for us to collect the tissue specimen, and the ethics committee of our hospital were required to approve all specimens for inclusion in the study prior to their collection.

The exclusion criteria were as follows:

• All patients undergoing preoperative radiotherapy or chemotherapy were excluded from the study, along with patients with severe heart disease, hypertension, diabetes, recurrence of the bladder cancer, or distant metastasis. In addition, 18 samples of normal bladder mucosa tissue were included in the study, collected from patients who underwent benign disease-related surgery in our hospital in the same time period.

• The human bladder cancer cell line T24 was purchased from Nanjing Cobioer Biological Technology Co., Ltd.

Method

• All samples were fixed with 4% formaldehyde for 1 hour, embedded in conventional paraffin, and then examined with an immunohistochemistry kit to measure the expression of TRIP13 in both the bladder cancer tissue and the normal bladder mucosa tissue. The correlation between TRIP13 expression and the clinicopathological characteristics of the patients with bladder cancer was then analyzed. All patients with bladder cancer were then followed up for 5 years, mainly by telephone.

• The human bladder cancer cell line T24, in the logarithmic growth phase, was randomly divided into a blank control group, a negative control group, and a siRNA group. The cells in the blank control group were not subjected to any treatment; small interfering RNA against TRIP13 were applied to the cells in the siRNA group, before the cells were transfected with liposomes and introduced into T24 cells; the negative control group was transfected with irrelevant siRNA. The three groups of T24 cells were placed in 6-well plates, at a density of 5×10^5/well, 1 d before transfection. The next day, when cell fusion reached 70%, transfection was performed. After 5 h, the serum and antibiotics were replaced to continue the culture. Cells were collected according to the needs of the research.

• At set 24 h, 48 h, 72 h, and 96 h time points, cells in the logarithmic growth phase were collected. The MTT colorimetric method was employed to analyze the proliferation of each group of cells over the period between each time point.

• After transient transfection of the cells in each group had been carried out for 48 h, the clone cloning experiment and the cell scratch experiment were utilized to analyze the cloning rate and migration ability of the cells, respectively.

Immunohistochemical scoring criteria

The samples for each group were placed under the microscope and pictures were taken for the

purpose of parallel immunohistochemical scoring. The scoring criteria were as follows: Staining intensity was divided into negative, weak positive, moderate positive, and strong positive, which were recorded as 0 points, 1 point, 2 points, 3 points, respectively; dyeing area was divided into below 5%, 5%-25%, 26%-50%, 51%-75%, and more than 75%, which were recorded as 0 points, 1 point, 2 points, 3 points, and 4 points, respectively.

The two scores were then multiplied together. A score ≥1 was classified as a positive expression, while a score of 0 was classified as a negative expression.

Statistical methods

All data were analyzed using SAS 8.2 software. The expression of TRIP13 in bladder cancer tissues and normal bladder mucosa tissues was expressed as a rate, and a comparison of rates between the groups was tested using χ^2. The correlation between TRIP13 expression and the clinicopathological characteristics of patients with bladder cancer was analyzed using Spearman's correlation coefficient. The Kaplan-Meier method was employed to enable survival curves to be drawn for the bladder cancer patients, while the log-rank method was utilized to compare the groups. The three groups of T24 cell clone formation rates, and other measurement data, were applied (P>0.05). A comparison between two groups was conducted using a t test, while a comparison between multiple groups was carried out through a multivariate analysis of variance. P<0.05 was regarded to be statistically significant.

Results

TRIP13 expression in bladder cancer and normal bladder mucosa

The positive expression rate of TRIP13 in the bladder cancer tissues was found to be 60.53% (23/38), which was significantly higher than that of TRIP13 in the normal bladder mucosa tissues, at 27.78% (5/18, P<0.05). See Figure 1.

Correlation between TRIP13 expression level and the clinicopathological characteristics of patients with bladder cancer

The expression level of TRIP13 was related to the depth of invasion, lymph node metastasis, and TNM stage in patients with bladder cancer (P<0.05), though it was not related to the age, sex, or pathological grade of the patients (P>0.05). See Table 1.

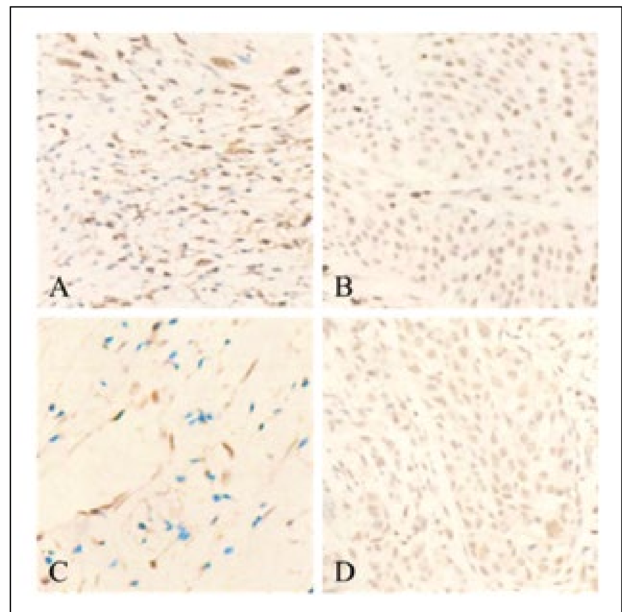

Figure 1: TRIP13 expression in bladder cancer tissue and normal bladder mucosa tissue.

Note: Figure A. TRIP13 was negatively expressed in bladder cancer tissue; Figure B. TRIP13 was positively expressed in bladder cancer tissue; Figure C. TRIP13 was negatively expressed in normal bladder mucosa; Figure D. TRIP13 was positively expressed in normal bladder mucosa.

Clinicopathological characteristics	*n*	TRIP13 expression		*P*
		Negative expression (*n*=15)	Positive expression (*n*=23)	
Age				0.898
<65 years old	30	12	18	
≥65 years old	8	3	5	
Gender				0.599
Male	20	11	15	
Female	18	4	8	
Pathological grade				0.569
G1	11	4	7	
G2	17	6	11	
G3G	10	5	5	
Depth of invasion				0.020
Muscularis and intramuscularis	19	4	15	
Extramuscular	19	11	8	
Lymph node metastasis				0.021
Yes	28	8	20	
No	10	7	3	
TNM stage				0.039
I~II	26	11	9	
III~IV	12	4	14	

Table 1: Correlation between TRIP13 expression level and the clinicopathological characteristics of patients with bladder cancer.

Correlation between the expression level of TRIP13 and the prognosis of bladder cancer patients

The 5-year survival rate of TRIP13-positive-expression patients was found to be 30.43% (7/23), which was significantly lower than that of the TRIP13-negative-expression patients, at 60% (9/15, P<0.05). See Figure 2.

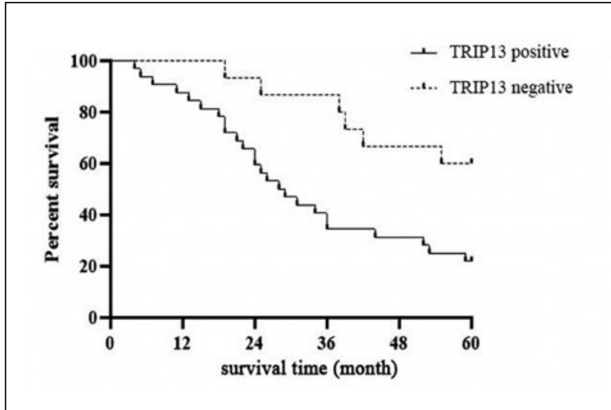

Figure 2: The correlation between TRIP13 expression level and the prognosis of bladder cancer patients.

Effect of interference on the proliferation of T24 cells after downregulation of TRIP13

At 24 h, 48 h, 72 h, and 96 h, the proliferation inhibition rate of the T24 cells in the siRNA group was found to be significantly higher than that of the T24 cells in the blank control group, while the rate in the blank control group was not found to be different to a statistically significant degree to that in the negative control group (P>0.05). See Table 2.

Group	24 h (%)	48 h (%)	72 h (%)	96 h (%)
Blank control group	0.83±0.02	0.84±0.01	0.95±0.05	0.79±0.01
Negative control group	0.79±0.01	0.81±0.03	0.89±0.02	0.82±0.03
siRNA group	6.02±0.09[a]	22.93±1.55[a]	38.61±0.12[a]	41.38±0.19[a]
Female	18	4	8	8

Table 2: Effect of interference on the inhibition rate of T24 cell proliferation migration after downregulation of TRIP13 ($\bar{x}$±s).
Note: a = Compared with the blank control group, P<0.05.

Effect of interference on the formation of T24 cell clones after downregulation of TRIP13

The cloning rate of the T24 cells in the siRNA group was found to be significantly lower than that of the T24 cells in the blank control group, while there was no statistically significant difference found in the rates in the blank control group and the negative control group (P>0.05). See Table 3.

Group	Cloning rate (%)
Blank control group	23.72±0.47
Negative control group	22.79±0.37
siRNA group	8.31±0.09[a]

Table 3: Effect of interference on the formation rate of T24 cells after downregulation of TRIP13 ($\bar{x}$±s).
Note: a = Compared with the blank control group, P<0.05.

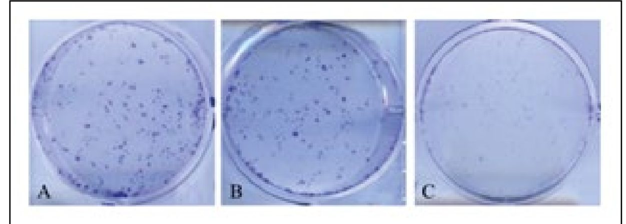

Figure 3: Effect of interference on the formation of T24 cell clones after downregulation of TRIP13.

Effect of interference on the migration ability of T24 cells after downregulation of TRIP13

The migration distance of the T24 cells in the siRNA group was found to be significantly lower than that of the T24 cells in the blank control group, while the distance in the blank control group was not found to be different to a statistically significant degree to that in the negative control group (P>0.05). See Table 4.

Group	Migration distance (mm)
Blank control group	594.70±1.84
Negative control group	590.17±5.97
siRNA group	435.67±8.50[a]

Table 4: Effect of interference on the migration ability of T24 cells after downregulation of TRIP13 ($\bar{x}$±s).
Note: a = Compared with the blank control group, P<0.05.

Discussion

Bladder cancer is characterized by slow growth, easy recurrence, invasion, and strong metastasis, and its incidence is increasing worldwide[7]. Though surgical treatment can prolong the survival times of patients and improve clinical efficacy to a certain extent, there are still many patients with distant metastases, which seriously threaten their lives and health[8]. Therefore, it is of utmost importance to explore new treatment methods for bladder cancer that enhance clinical efficacy and prolong patients' survival time. Tolppanen et al.[9] observed that the prognosis of bladder cancer patients is closely related to the depth of tumor invasion and the risk of metastases, further clarifying the correlation

between the depth of invasion and the prognosis of bladder cancer patients. Retrospective studies of bladder cancer have also shown that among depth of invasion, tumor size, age, gender, and other clinical-pathological features, depth of invasion is the key factor that affects the prognosis of patients(10).

TRIP13 is a hormone-dependent transcription factor; its overexpression can promote cell proliferation. If is transfected with siRNA or shRNA, TRIP13 expression is significantly inhibited and cell proliferation and death are induced(11). Head and neck-related studies have shown that TRIP13's expression level in diseased tissue is significantly higher than in normal tissue(12). One study used an enzyme-linked immunosorbent assay to detect the expression of TRIP13 in the peripheral blood of 351 patients diagnosed with untreated multiple myeloma, 44 patients with multiple myeloma treated with monoclonal gamma globulin, and 22 healthy volunteers, finding that the level of TRIP13 expression in the peripheral blood of patients with untreated multiple myeloma was significantly higher than that of healthy volunteers. There was no significant difference between the expression level of TRIP13 in the serum of patients with multiple myeloma treated with monoclonal gamma globulin and that of the healthy volunteers.

The study also tested the expression level of TRIP13 in the peripheral blood of patients with new multiple myeloma, as well as relapsed patients. It was found that the expression level of TRIP13 in the peripheral blood of the relapsed patients was significantly higher than that of the new patients, while the prognosis of the relapsed patients was worse than that of the new patients(13). In another study, clinical reports demonstrate that CD19 + B lymphocytes were isolated from the peripheral blood of patients with chronic lymphocytic leukemia, as well as from healthy people. Real-time fluorescence quantitative PCR was employed to detect the expression level of TRIP13 mRNA in lymphocytes, finding that the TRIP13 mRNA expression level was significantly higher in patients with chronic lymphocytic leukemia, compared with the healthy people(14). A different study found an expression level of TRIP13 mRNA in the cancer tissue of colorectal cancer patients that was also significantly higher than that of the non-cancerous colorectal epithelial tissue of other patients(15).

This study has found that a positive expression rate of TRIP13 in bladder cancer tissues of 60.53% (23/38), which is significantly higher than the rate of TRIP13 in normal bladder mucosa tissues, at 27.78% (5/18, $P<0.05$). The expression level of TRIP13 has been found to relate to depth of invasion, lymph node metastasis, and TNM stage in the patients with bladder cancer ($P<0.05$). The 5-year survival rate for TRIP13-positive-expression patients has been found to be 30.43% (7/23), which is significantly lower than that for the TRIP13-negative-expression patients, at 60% (9/15, $P<0.05$). The proliferation inhibition rate, migration distance, and clone formation rate of the T24 cells in the siRNA group have been found to be significantly higher than those of the T24 cells in the blank control group. To conclude, the expression level of TRIP13 in bladder cancer tissues is elevated, and its expression level is closely related to the depth of infiltration, lymph node metastasis, and TNM staging of patients. In addition, TRIP13 plays an important role in the behavior of bladder cancer cells. Reducing its expression can significantly inhibit the proliferation, migration, and clonal formation of bladder cancer cells.

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