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ORIGINAL ARTICLE

Expression of mir-143 in serum of bladder cancer patients and its correlation with clinical features and prognosis

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Summary

Purpose: *To explore the expression of mir-143 in the serum of bladder cancer patients and its correlation with clinical pathological features and prognosis.*

Methods: *A retrospective study was performed on 68 patients (observation group) diagnosed with bladder cancer and treated in the Qilu Hospital of Shandong University from June 2013 to January 2014 and another 40 healthy individuals (control group) in the physical examination center at the same period. Real-time PCR (RT-PCR) was used to detect the expression levels of mir-143 in the serum of bladder cancer patients and healthy subjects. The expression of mir-143 in the serum of bladder cancer patients and its correlation with clinical pathological indicators and prognosis were explored.*

Results: *The expression level of mir-143 in the serum of bladder cancer patients was significantly lower than that of healthy people ($p<0.05$). There was no statistically significant difference in age and sex ($p>0.05$). There was a correlation between the expression level of mir-143 and differentiation grade, lymph node metastasis, distant metastasis and clinical stage ($p<0.05$). The median expression level of mir-143 in the serum of bladder cancer patients was 0.652. Thirty four patients with lower expression than the median of mir-143 were in the low expression group, and 34 patients with higher expression than the median of mir-143 were in the high expression group. The mean survival time of bladder cancer patients with the low expression of mir-143 was 37.32±1.26 months, significantly lower than that with the high expression of mir-143 (46.17±1.54;$p<0.05$).*

Conclusion: *mir-143 is lowly expressed in the serum of bladder cancer patients. Its expression level is correlated with clinical stage, lymph node metastasis, distant metastasis and prognosis.*

Key words: *mir-143, bladder cancer, clinical pathological feature, prognosis*

Introduction

Bladder cancer, the most common malignant tumor in the urinary system and one of the top ten common tumors in humans, is a malignancy that originates from the bladder mucosa [1]. According to the 2012 National Cancer Registration, its incidence reached 6.61/100,000, ranking 9th in the incidence of malignant tumors [2]. Bladder cancer can develop at any age, even in children. Its incidence increases with age, and its top incidence is at 50-70 years (men vs women 3-4:1) [3]. At present, the usual examinations include cystoscopy CT, B-ultrasound and cystography. About 80% of bladder cancer patients can be diagnosed at an initial stage and can be actively treated by bladder surgery and intravesical therapy, but the 5-year recurrence rate reaches 50-70%. Among them, 10-30% of patients further develop muscular invasive urothelial carcinoma [4]. Bladder cancer has a strong capability to invade, and the prognosis of patients is very poor, with an extremely low 5-year survival rate [5].

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Therefore, it is crucial for improving the prognosis and survival of bladder cancer patients to find more reliable biomarkers to diagnose the disease earlier and in time, and to find more effective therapeutic targets.

Recent studies [6,7] have found that about 50% of miRNAs have genomic localization associated with the occurrence of tumors, which are abnormally expressed in many human tumor cells. More studies [8-12] have shown that miRNAs play an important role in the development, metastasis and prognosis of tumors. Dysregulation of cell miRNA expression can affect the occurrence, development and post-treatment outcome of patients [13]. This is because regulating genes encoded by various proteins, miRNAs can inhibit or promote protein synthesis, and regulate the expression level of genes in cell apoptosis, differentiation and development [14]. Inducing the expression of oncogenes to promote tumor formation, they can also inhibit the expression of cancer genes to inhibit tumor formation [15].

According to some authors, mir-143 is lowly expressed in various tumors such as cervical cancer and colon cancer [15]. Studies have also shown that it differs in the expression profiling chip technology of bladder cancer tissues and adjacent cancer tissues, which may play an important role in the development of bladder cancer [16].

In this study, the expressions of mir-143 in the serum of bladder cancer patients and normal subjects were evaluated. The expression level and clinical significance of mir-143 in the serum of bladder cancer patients, and the relationship between the expression level of mir-143 and the prognosis of patients were explored.

Methods

Patient data

A retrospective study was performed on bladder cancer patients (observation group) treated in the Qilu Hospital of Shandong University from June 2013 to January 2014, and healthy subjects (control group) in the physical examination center. Patients were followed up for 48 months. A total of 68 patients in the observation group were diagnosed with bladder cancer according to the postoperative biopsy. None of them were treated before the test, including 51 males and 17 females, with the average age of 54.5±6.7 years. There were 40 cases in the healthy control group, including 32 males and 8 females, with the average age of 54.8±8.6 years. The pathological staging of bladder cancer was based on the eighth edition (2017) of UICC [18]. CT and ultrasound were performed in both groups. All patients or their family members and healthy subjects signed informed consent.

Inclusion and exclusion criteria

Inclusion criteria: Patients pathologically diagnosed with bladder cancer and patients with complete clinical data.

Exclusion criteria: Patients with urinary tract infection or severely impaired liver and kidney function; patients having undergone radiotherapy and chemotherapy and biological targeting therapy; patients having recently used a large number of hormones or immune inhibitors; patients with tumors in other sites or familial hereditary malignancies; patients with severe heart, brain and lung diseases.

Main reagents and instruments

The miRcute miRNA extraction and isolation kit and cDNA first-strand synthesis kit were purchased from Tiangen Technology Co., Ltd.; real-time PCR instrument (ABI 7500) from American ABI Corporation; Trizol reagent from Shanghai Huiying Biotechnology Co., Ltd.; micro-UV-visible spectrophotometer (K5600) from Beijing Kaiao Technology Development Co., Ltd. mir-143 and primers for internal reference U6 were designed and synthesized by Shanghai Shenggong Biotechnology Co., Ltd (Table 1).

Detection methods

Collection and storage of serum samples

4 mL of fresh blood were taken aseptically from the experimental and control group. After centrifugation, the serum was placed in an EP tube and stored at -70°C until use.

Extraction of serum total RNA

Serum samples taken from the refrigerator were immediately thawed in a water bath at 37°C for 2 min. Another 300 μL of serum was taken and processed according to the miRcute miRNA extraction and isolation kit instruction.

Reverse transcription

Reverse transcription was performed according to the instruction of the miRcute miRNA cDNA first-strand synthesis kit. Reaction conditions were: 16°C for 30 min, 42°C for 30 min and 85°C for 5 min.

Table 1. Primer sequences

Genes	*Upstream primer*	*Downstream primer*
mir-143	5′-ACACTCCAGCTGGGTGAGATGAAGCACT-3′	5′-TGGTGTCGTGGAGTCG-3′
U6	5′-CTCGCTTCGGCAGCACA-3′	5’-AACGCTTCACGAATTTGCGT-3’

Real-time PCR

The cDNA amplification reaction system was a total of 20 μL: pre-denaturation at 95°C for 10 min, denaturation at 95°C for 10 s, annealing at 60°C for 20 s, extension at 72°C for 10 s, for a total of 40 cycles. After the cycles were completed, they were extended at 72°C for 5 min. U6 was used as a reaction internal reference. All of the procedures were performed in triplicate and the results were analyzed by 2-ΔCt method.

Follow-up

The patients were followed up by telephone and outpatient follow-up visits for 48 months. The relationship between the expression level of mir-143 and the patient's survival was analyzed.

Statistics

SPSS19.0 statistical software (purchased from Beijing Think & Wealth Information Technology Co., Ltd.) was used. The quantitative data were expressed as mean±SD, and tested by two independent t-samples. The qualitative data were expressed as percents. Kaplan-Meier method was used for survival analysis and Log Rank for comparison of survival rates. P<0.05 denoted statistical significance.

Results

Comparison of patient clinical basic data

There was no statistically significant difference in the clinical basic data such as sex, age and place of residence between patients and healthy subjects (p>0.05;Table 2).

Comparison of expression level of mir-143 in serum between experimental and healthy control group

The expression level of mir-143 in the serum of bladder cancer patients was lower than that of healthy people, with a statistically significant difference (p<0.05;Table 3).

Relationship between expression level of mir-143 in serum and clinical pathological features in bladder cancer patients

According to the pathological staging, bladder cancer patients were divided into 4 stages. The results showed that there was no statistically significant difference of the serum miR-143 expression level concerning age and sex (p>0.05), but there was a correlation between the expression level of mir-143 and grade of differentiation, lymph node metastasis, distant metastasis and clinical staging (p<0.05;Table 4).

Relationship between expression level of mir-143 and prognosis of bladder cancer patients

As of the end of follow-up date, the median expression level of mir-143 in the serum of bladder cancer patients was 0.652. Divided by the median, 34 patients with lower expression than the median

Table 2. Clinical patient data

Groups	*Observation group (n=68) n (%)*	*Control group (n=40) n (%)*	t/x^2	*P*
Gender			0.35	0.55
Male	51 (75.00)	32 (80.00)		
Female	17 (25.00)	8 (20.00)		
Age (years)			0.01	0.96
≥50	49 (72.06)	29 (72.50)		
<50	19 (27.94)	11 (27.50)		
Smoking			1.69	0.69
Yes	32 (47.06)	24 (60.00)		
No	36 (52.94)	16 (40.00)		
Alcoholism			0.63	0.43
Yes	41 (60.29)	21 (52.50)		
No	27 (39.71)	19 (47.50)		
BMI index (kg/m^3)	22.18±2.16	22.39±2.14	0.49	0.63

BMI: body mass index

Table 3. Comparison of expression level of mir-143 in serum between two groups

Indicators	*Observation group (n=68)*	*Control group (n=40)*	*t*	*p*
mir-143	0.68±0.32	0.87±0.26	3.186	0.002

of mir-143 were in the low expression group, and 34 patients with higher expression than the median of mir-143 were in the high expression group. The mean survival time of bladder cancer patients with low expression of mir-143 was 37.32±1.26 months, significantly lower than that with the high expression of mir-143 (46.17±1.54). The difference in survival rate was statistically significant ($p<0.05$). The results of survival curve analysis are shown in Figure 1.

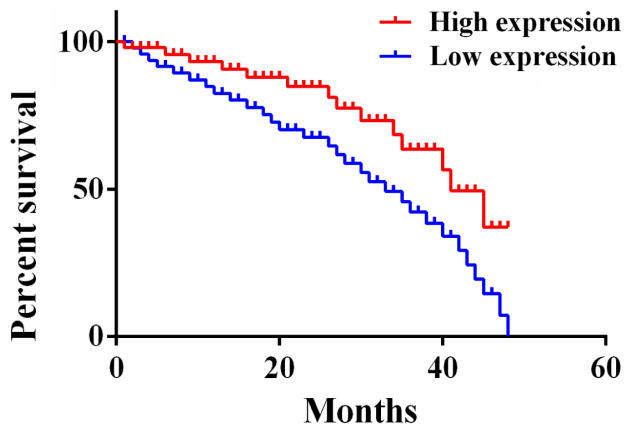

Figure 1. Survival analysis of relationship between expression level of mir-143 and prognosis of bladder cancer patients. The results showed that the overall survival of bladder cancer patients with the low expression of mir-143 was significantly lower compared with the high expression of mir-143 ($p<0.05$).

Discussion

So far, the main treatment of bladder cancer is surgery, supplemented by intravesical bladder therapy. However, the patient prognosis is still poor, with a high postoperative recurrence rate and poor quality of life [18].

miRNAs can be used as potential markers for the diagnosis and prognosis of tumors. According to some authors [19], a variety of tumor markers for bladder cancer have been reported as useful for disease diagnosis or monitoring, but they have not yet been applied clinically. Some authors [20] have pointed out that approximately 50% of human cancers are related to miRNAs. Since the first global report on the change in miRNA expression in bladder cancer published in 2007 [21], the role of miRNAs in the occurrence and disease development has become a hot topic. Results of some studies [22-24] have shown that mir-143 displays a number of functions such as inhibiting the proliferation of vascular smooth muscle cells and maintaining the balance between smooth muscle cells and blood vessel. mir-143 has been shown to be highly expressed in myometrium, vascular smooth muscle cells, osteoclasts and adipocytes. In recent years, it has been found that mir-143 is important for the occurrence and development of malignant tumors. For example, in the study of He et al. [26], it was found that mir-143-3p played a tumor suppressor role in oral squamous cell car-

Table 4. Relationship between expression level of miR-143 in serum and clinicopathological features in bladder cancer patients

Variables	*n (%)*	*mir-143 (mean±SD)*	*t*	*p*
Age, years			0.394	0.694
≥50	49 (72.06)	0.65±0.32		
<50	19 (27.94)	0.67±0.27		
Sex			0.166	0.868
Male	51 (75.00)	0.64±0.32		
Female	17 (25.00)	0.65±0.38		
Differentiation grade			2.718	0.007
Low and moderate	43 (63.24)	0.57±0.22		
High	25 (36.76)	0.69±0.29		
Lymph node metastasis			2.207	0.029
Yes	36 (52.94)	0.58±0.27		
No	32 (47.06)	0.69±0.31		
Distant metastasis			2.314	0.022
Yes	24 (35.29)	0.59±0.24		
No	44 (64.71)	0.70±0.31		
Clinical staging			2.410	0.017
I+II	41 (60.29)	0.69±0.24		
III+IV	27 (39.71)	0.58±0.29		

cinoma, acting on the proliferation, apoptosis and epithelial-mesenchymal transition of cancer cells. In this study, real-time PCR was used to detect the expression level of mir-143 in the serum of bladder cancer patients. The results showed that the serum mir-143 level in bladder cancer patients was lower compared with healthy subjects. It is consistent with some research results showing the correlation of the expression level of mir-143 with many digestive system malignant tumors, including esophageal cancer [27], gastric cancer [29,30], pancreatic cancer [30] and colon cancer [31,32]. Based on this, it can be deduced that mir-143 may also inhibit bladder cancer. At the same time, from the results of this study, it can be seen that the expression level of mir-143 progressively decreases from clinical stage I-IV of bladder cancer. These results suggest that the dysregulation of mir-143 expression may be closely related to the occurrence and development of bladder cancer. According to the follow-up results and survival analysis, the median expression level of mir-143 in the serum of bladder cancer patients was 0.652. Divided by the median, 34 patients with lower expression than the median of mir-143 were in the low expression group, and 34 patients with higher expression than the median of mir-143 were in the high expression group. The mean survival time of bladder cancer patients with low expression of mir-143 was 37.32±1.26 months, significantly lower than that with the high expression of mir-143 (46.17±1.54). The difference in survival was statistically significant ($p<0.05$), suggesting that patients with low expression of mir-143 had a poor prognosis and we suggest that it can be used as a potential indicator for prediction of prognosis evaluation of bladder cancer patients.

This study was retrospective. Investigation of basic situation data, clinical examination, special examinations and other data of patients were not complete. Because of the small size of specimens, there was no enough data to perform statistical comparisons. To overcome such deficiencies, relevant data were collected, sample sizes were expanded, and experimental researches and statistical methods were further designed. More clinical data were collected to conduct research and obtain more accurate experimental results.

In summary, the serum mir-143 level in bladder cancer patients is lower compared with normal subjects, in relation to clinical staging, differentiation grade, lymph node metastasis, distant metastasis and prognosis. Among them, patients with high expression of mir-143 have a better prognosis. mir-143 is expected to be a potential marker for the diagnosis of bladder cancer and predictor of prognosis.

Conflict of interests

The authors declare no conflict of interests.

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