

# Expression of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer and their effects on the biological characteristics of bladder cancer cells

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**Abstract.** Changes in the expression of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer tissues were studied. A total of 30 patients with bladder cancer who had surgical resection in the Hunan Provincial People's Hospital (Changsha, China) from April 2015 to August 2016 were selected. RT-qPCR was used to detect the expression levels of miR-490-5p, miR-148a-3p and miR-608. The expression vectors of miR-490-5p, miR-148a-3p and miR-608 were respectively transfected and divided into three groups: blank cell group, gene transfection group (groups A-C) and negative transfection group (NC group). CCK8 was used to detect cell proliferation and flow cytometry was used to detect the condition of apoptosis of each group, and the Transwell chamber was used to detect the invasion ability of the cells. After the transfection, the expression level of miR-490-5p in group A was significantly higher than that in the NC and blank groups, and the expression level of miR-148a-3p in group B was significantly higher than that in the NC and blank groups. The expression level of miR-608 in group C was significantly higher than that in the NC and blank groups ( $P<0.001$ ). The survival rates of the cells in groups A-C were significantly lower than those in the NC and blank groups at 48 and 72 h ( $P<0.001$ ). After the transfection, the number of invasive cells and the apoptosis rates in groups A-C were significantly higher than those in the NC and blank groups ( $P<0.05$ ). miR-490-5p, miR-148a-3p and miR-608 promoted proliferation of bladder cancer T24 cells and inhibited apoptosis of the cells and showed potential to become a new target for the future treatment of bladder cancer.

## Introduction

Bladder cancer is the most common malignant tumor in the bladder mucosa in the urinary system (1). The morbidity and mortality of bladder cancer head the list in urogenital tumors and rank in ninth place in all malignant tumors worldwide. The number of patients who survive for 5 years is less than 62%, and with the changes in lifestyle and environmental effects, the morbidity and detection rate of bladder cancer globally have shown a rapid upward trend (2,3). Treatment strategy for bladder cancer is mainly surgical resection in clinic. Although surgery can excise bladder cancer partially, there is still no effective control method for the high recurrence and high metastasis rate of bladder cancer following surgery (4,5). Bladder cancer has the characteristics of easy recurrence and easy metastasis, clarifying that the pathogenic mechanism of bladder cancer has important clinical significance for treating bladder cancer and improving the survival time of patients (6,7). MicroRNAs (miRNAs/miRs) are an endogenous and non-coding RNA, which has regulatory functions and is always involved in the proliferation, differentiation and apoptosis of cells and some other processes. An increasing number of studies have shown that the abnormal expression of miRNA is associated with the development and metastasis of bladder cancer, and researchers have found abnormal profiles of miRNA in a variety of tumors, including bladder cancer. An in-depth study of its mechanism is expected to provide new ideas and targets for the diagnosis and treatment of bladder cancer (8,9).

miR-490-5p is abnormally expressed in many types of cancer such as colorectal and bladder cancer, and there is an obvious downward trend in its expression level in tumor tissues. We speculated that it plays a similar role as a tumor suppressor gene in bladder cancer (10). It is currently believed that miR-148a-3p and miR-608 mainly play a role as anti-cancer miRNA in solid tumors and inhibit the growth and progression of tumors (11). In addition, the overexpression of miR-608 can significantly inhibit the proliferation, cell cycle progression and migration ability of cancer cells in colon cancer (12). However, the expression features of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer and their specific effects on the biological characteristics of bladder cancer cells are still unclear. Therefore, this experimental study was carried out

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on the expression features of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer and their effects on the biological characteristics of bladder cancer cells in order to provide a new theoretical basis for the diagnosis and treatment of bladder cancer in molecular biology.

## Materials and methods

**Collection of the data.** A total of 30 patients with bladder cancer who had surgical resection in the Hunan Provincial People's Hospital (Changsha, China) from April 2015 to August 2016 were selected. There were 20 males and 10 females, aged 35-83 years, with an average age of  $59.01 \pm 8.93$  years. During the operation, 30 samples of bladder cancer tissues and 30 of normal adjacent tissues were excised and collected after obtaining patient consent. Inclusion criteria for the study were: the patient tissue sections were diagnosed as bladder cancer tissues or normal adjacent tissues by the Pathology Department of the hospital; and all the specimens were immediately placed and reserved in liquid nitrogen at  $-180^{\circ}\text{C}$ . Exclusion criteria were: patients who received chemotherapy, immunotherapy, radiotherapy and any other treatments before the surgery.

Patients and their families were informed before the study was carried out and they signed the informed consent. The study was approved by the Ethics Committee of the Hunan Provincial People's Hospital.

**Main reagents and instruments.** Human bladder cancer T24 cells (Cell Bank of Chinese Academy of Science, Shanghai, China), TRIzol reagent (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA), RT-qPCR kit and minScript reverse transcription kit (Takara Biotechnology Co., Ltd., Dalian, China), HBS-1096A enzyme analyzer (Nanjing Detie Laboratory Equipment Co., Ltd., Nanjing, China), qPCR instrument (Bio-Rad Laboratories, Inc., Hercules, CA, USA), DMEM medium (Gibco; Thermo Fisher Scientific, Inc.), fetal bovine serum (FBS) and trypsin (HyClone; GE Healthcare Life Sciences, Logan, UT, USA), CCK8 kit (Beijing Zhijie Fangyuan Technology Co., Ltd., Beijing, China), Transwell Chamber (BD Biosciences, Franklin Lakes, NJ, USA), and CyFlow Cube 8 flow cytometer (Sysmex Partec GmbH, Görlitz, Germany) were used in the study. Primer sequences of miR-490-5p, miR-148a-3p, miR-608 and internal reference U6 and miRNA negative control were produced and designed by Shanghai Jima Industrial Co., Ltd., Shanghai, China (Table I).

**Detection of miR-490-5p, miR-148a-3p and miR-608.** RT-qPCR technology was used to detect the expression of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer tissues and normal adjacent tissues. Total RNA in the tissues was extracted and dissolved in 20  $\mu\text{l}$  of DEPC water according to the instructions of TRIzol reagent. The reverse transcription kit was used to reverse transcribe total RNA, reaction system: 1  $\mu\text{l}$  of M-MLV, 1  $\mu\text{l}$  of Olig (dT), 0.5  $\mu\text{l}$  of RNA enzyme inhibitor, 1  $\mu\text{l}$  of dNTPs. RNase free water was replenished to 15  $\mu\text{l}$ . The mixture was incubated for 60 min at  $38^{\circ}\text{C}$ . Subsequently, 1  $\mu\text{l}$  of cDNA was taken, at  $85^{\circ}\text{C}$  for 5 sec and the synthesized cDNA was used as a template for the amplification of RT-qPCR. The prepared PCR reaction system comprised: 2.5  $\mu\text{l}$  of 10X PCR buffer solution, 1  $\mu\text{l}$  of dNTPs,

1  $\mu\text{l}$  of the upstream and downstream primers for each, 0.25  $\mu\text{l}$  of Taq DNA Polymerase, and ddH<sub>2</sub>O which was replenished in 25  $\mu\text{l}$ . The reaction procedure was:  $94^{\circ}\text{C}$  for 1 min;  $94^{\circ}\text{C}$  for 15 sec,  $60^{\circ}\text{C}$  for 20 sec, a total of 39 cycles. Three replicate wells were set in each sample and the experiment was repeated 3 times. miR-490-5p, miR-148a-3p and miR-608 all used U6 as an internal reference. After the reaction was finished, the amplification curve and the solubility curve of qPCR were confirmed, and the relative amount of the target gene was calculated according to the result parameters.

**Culture and cell transfection.** The human bladder cancer T24 cells were placed in DMEM medium containing 10% PBS and cultured at  $37^{\circ}\text{C}$  in 5% CO<sub>2</sub>. When the adherent growth and fusion of the cells reached 85%, 25% trypsin was added for digestion. After the digestion was finished, the cell line was placed in the medium and cultured further. The cells in log phase were selected and transfected, and grouped prior to the transfection. Cells that were not transfected were divided into 5 groups: blank group, negative RNA control (NC group), miR-148a-3p mimics (group B), miR-490-5p mimics (group A), and miR-608 mimics (group C). Lipofectamine 2000 and DNA were diluted and mixed according to the protocol of the Lipofectamine 2000 manufacturer kit, and liposome Lipofectamine 2000 was used to, respectively, transfect NC, miR-490-5p mimics, miR-148a-3p mimics, miR-608 mimics into the bladder cancer cells, then it was incubated for 5 min at room temperature, and finally the mixed solution was mixed with the cells, prior to transfection at  $37^{\circ}\text{C}$  in 5% CO<sub>2</sub>. After transfection for 48 h, RT-qPCR technology was used to detect the condition of the expression of miR-490-5p, miR-148a-3p and miR-608 of T24 cells in which miR-490-5p, miR-148a-3p, miR-608 and miR-NC were transfected.

**Cell proliferation detected by CCK8.** Bladder cancer T24 cells transfected for 48 h in each group were inoculated into a 96-well plate, and there was 100  $\mu\text{l}$  of bladder cancer T24 cells in each well, which was diluted at  $4 \times 10^3$  cells/ml after digestion by trypsin, and then the culture plate was placed in a cell culture incubator for 24 h. Next, the culture plate was taken out and the original culture solution was discarded, prior to the addition of NC, miR-490-5p mimics, miR-148a-3p mimics, and miR-608 mimics onto the culture plate. After culturing for 48 and 72 h, the culture plate was taken out and the original culture solution was discarded, followed by the addition of 100  $\mu\text{l}$  of CCK8 solution into each well. Subsequently, the culture plate was incubated for 4 h at  $37^{\circ}\text{C}$ , and the microplate reader (Thermo Fisher Scientific, Inc.) was used to measure the absorbance at 450 nm to detect the condition of cell proliferation. The experiment was repeated 3 times.

**Apoptosis detected by flow cytometry in each group.** After digestion by trypsin, the cells treated with miR-490-5p, miR-148a-3p, miR-608 and NC for 48 h were collected, and a concentration of 75% ethanol was used to fix them for 24 h at  $20^{\circ}\text{C}$ . The cells were centrifuged at  $3,000 \times g$  for 5 min at a constant temperature of  $4^{\circ}\text{C}$ , the ethanol was discarded and PBS was used to rinse them once. They were again centrifuged at  $3,000 \times g$  for 5 min at a constant temperature of  $4^{\circ}\text{C}$  and the supernatant was discarded. DNA Staining Solution (500  $\mu\text{l}$ )

Table I. Primer sequences of miR-490-5p, miR-148a-3p, miR-608 and internal reference U6.

Group	Sequences of upstream primer	Sequences of downstream primer
miR-490-5p	5'-CATGGATCTCCAGGTGG-3'	5'-TGGTGTCTCGTGGAGTCG-3'
miR-148a-3p	5'-TCAGTGCACCTACAGAACTTTGT-3'	5'-GTCACCCCTGTTTCTGGCAC-3'
miR-608	5'-ATTTTATTTTAAAGTTGGGTTAGG	5'-CTAACCTCAATCTCTACTACTACAAC-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

Table II. The expression of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer and normal adjacent tissues.

Group	Bladder cancer tissues (n=30)	Normal adjacent tissues (n=30)	t	P-value
miR-490-5p	1.19±0.71	8.42±2.01	18.580	<0.001
miR-148a-3p	0.12±0.06	1.01±0.12	36.330	<0.001
miR-608	0.07±0.03	0.31±0.02	36.460	<0.001

was added into the samples and the samples were mixed adequately. Finally, the prepared solution was transferred to the flow tube. After incubation for 30 min in the dark, CyFlow Cube 8 flow cytometer was used for detection.

*Extracorporeal invasive ability of the cells detected by Transwell chambers.* Firstly, trypsin was used to digest the cells, and the culture solution was discarded after centrifugation for 10,000 x g at 4°C for 5 min. PBS was used to rinse the solution twice, and then in serum-free medium containing BsA resuspend, and the cell density was adjusted to 5x10<sup>4</sup>/ml, after which 1 ml of medium containing FBS was added to the lower chamber plate of 6 wells and 2 ml of the cell suspension was added into the Transwell chamber. After 24 h of routine culture, the cells in the Transwell chamber were wiped off with a cotton swab. After the Transwell chamber was dried, the film was made and the slice was sealed, and microscope (Olympus; Tokyo, Japan) was used for observation.

*Statistical analysis.* Statistical analysis was carried out using the SPSS 17.0 (Beijing Sichuang Weida Information Technology Co., Ltd., Beijing, China) software system; [n(%)] was used to express the enumeration data, and  $\chi^2$  test was applied in the comparison between two groups. Mean  $\pm$  standard deviation was used to express the measurement data, t-test or F-test was used for comparisons between the groups. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Expression of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer tissues and normal adjacent tissues.* The expression level of miR-490-5p in bladder cancer tissues and normal adjacent tissues was 1.19±0.71 and 8.42±2.01, respectively. When the two groups were compared with each other, the expression level of miR-490-5p in bladder cancer tissues was significantly lower than that in normal adjacent tissues, and the difference was statistically significant (P<0.001). The expression level of miR-148a-3p in bladder cancer tissues and normal adjacent tissues was 0.12±0.06 and

1.01±0.12, respectively; when the two groups were compared with each other, the expression level of miR-148a-3p in bladder cancer tissues was significantly lower than that in normal adjacent tissues, and the difference was statistically significant (P<0.001). The expression level of miR-608 in bladder cancer tissues and normal adjacent tissues was 0.07±0.03 and 0.31±0.02, respectively; when the two groups were compared with each other, the expression level of miR-608 in bladder cancer tissues was significantly lower than that in normal adjacent tissues, and the difference was statistically significant (P<0.001; Table II).

*Relative expression level of miR-490-5p, miR-148a-3p and miR-608 in the cells in each group after transfection.* The expression level of miR-490-5p in group A, group NC and blank group was 8.50±3.49, 1.17±0.43 and 1.21±0.35, respectively; the expression level of miR-490-5p in group A was significantly higher than that in group NC and blank group; the difference of the expression level was statistically significant (P<0.001), and there was no significant difference in the expression level of miR-490-5p in group NC and blank group (P>0.05). The expression level of miR-148a-3p in group B, group NC and blank group was 0.96±0.14, 0.13±0.02 and 0.11±0.02, respectively; the expression level of miR-148a-3p in group B was significantly higher than that in group NC and blank group; the difference of the expression level was statistically significant (P<0.001). No significant difference in the expression level of miR-148a-3p in NC and blank groups was found (P>0.05). The expression level of miR-608 in group C, group NC and blank group was 0.36±0.04, 0.07±0.04 and 0.07±0.02, respectively. The expression level of miR-608 in group C was significantly higher than that in the NC and blank groups, and the difference was statistically significant (P<0.001), but there was no significant difference in the expression of miR-608 in the NC and blank groups (P>0.05; Fig. 1A-C).

*Comparison of the proliferation ability of T24 cells in each group after transfection.* The results of the comparison between the groups showed that the differences were not

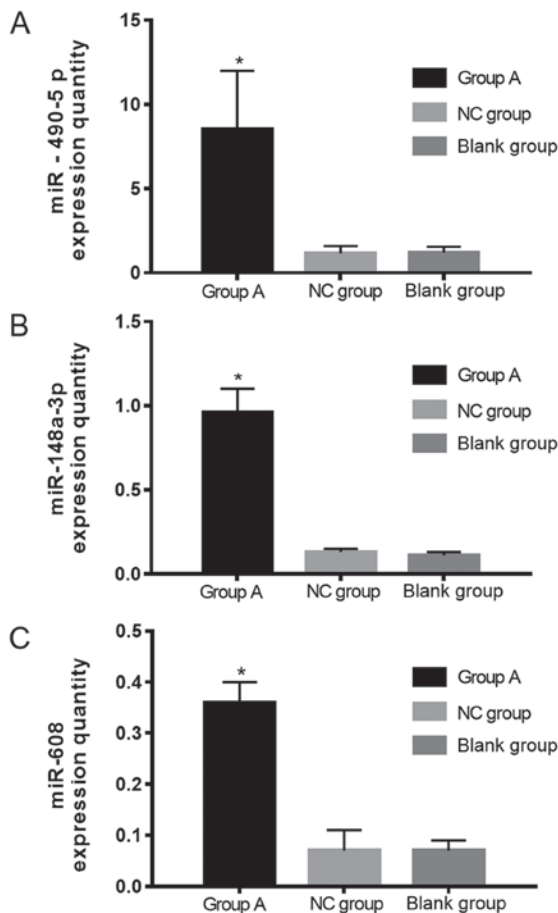


Figure 1. The relative expression level of miR-490-5p, miR-148a-3p and miR-608 in the cells in each group after transfection. The expression level of (A) miR-490-5p in group A was significantly higher than that in the NC and blank groups, and the difference of the expression level was statistically significant ( $P < 0.001$ ). There was no significant difference in the expression level of miR-490-5p in the NC and blank groups ( $P > 0.05$ ). The expression level of (B) miR-148a-3p in group B was significantly higher than that in NC and blank groups, the difference of the expression level was statistically significant ( $P < 0.001$ ). There was no significant difference in the expression level of miR-148a-3p in the NC and blank groups ( $P > 0.05$ ). The expression level of (C) miR-608 in group C was significantly higher than that in the NC and blank groups, and the difference was statistically significant ( $P < 0.001$ ). There was no significant difference in the expression of miR-608 in group the NC and blank groups ( $P > 0.05$ ). The expression level of this group is significantly higher than that in the other two groups, the differences are statistically significant ( $P < 0.001$ ).

statistically significant when the survival rate of the cells at 24 h among groups A-C, group NC and blank group was compared ( $P > 0.05$ ); there was no significant difference in the survival rate of the cells among groups A-C ( $P > 0.05$ ); at 48 and 72 h, there was no significant difference in the survival rate of the cells among groups A-C ( $P > 0.05$ ), but the survival rate of the cells in these groups was significantly lower than that in group NC and blank group ( $P < 0.001$ ), and there was no significant difference in the survival rate of the cells between group NC and blank group at 48 and 72 h ( $P > 0.05$ ). The results of the comparison within the groups showed that the survival rate of the cells within groups A-C showed a gradual downward trend from 24 to 72 h, and the differences were statistically significant when compared at different time points within the three groups ( $P < 0.001$ ); the survival rate of the cells within group NC and blank group also showed a

gradual downward trend from 24 to 72 h, but the differences were not statistically significant ( $P > 0.05$ ; Table III).

*Comparison of the condition of the invasion of T24 cells in each group.* The number of the invasive cells in groups A-C was  $200.56 \pm 21.78$ ,  $202.32 \pm 20.41$ , and  $199.56 \pm 23.53$ , respectively, and these numbers were significantly lower than that in blank group ( $498.38 \pm 50.25$ ) and that in group NC ( $500.47 \pm 46.91$ ). The differences were statistically significant ( $P < 0.05$ ). However, there was no significant difference among groups A-C, and there was no significant difference among blank group and group NC also ( $P > 0.05$ ; Table IV).

*Comparison of apoptosis ability of T24 cells in each group after the transfection.* Apoptosis rate in groups A-C was  $20.14 \pm 3.32\%$ ,  $19.92 \pm 4.47\%$  and  $19.73 \pm 4.01\%$ , respectively, and there was no significant difference in the apoptosis rate of the cells among groups A-C ( $P > 0.05$ ); but the apoptosis rates were significantly higher than that in group NC ( $3.75 \pm 0.65\%$ ) and blank group ( $3.67 \pm 0.91\%$ ) ( $P < 0.05$ ), and there was no significant difference in the apoptosis rate between group NC and blank group ( $P > 0.05$ ; Table V).

## Discussion

The development of bladder cancer is affected by the biological characteristics of bladder cancer cells, such as proliferation, invasion and apoptosis, and is closely related to the regulation of miRNA (13). A large number of studies have shown that both proliferation and apoptosis of tumor cells determine the growth and decline of tumor to control the growth rate of tumors (14). Cell proliferation is the basis of the growth, development and reproduction of organism and is an important life feature of organisms (15). The proliferation of normal cells will stop naturally when it reaches a certain extent, but the proliferation of cancer cells is generally not affected by neurohumoral and environmental factors, which can divide and proliferate unlimitedly and has the characteristics of being out of control (16). The unceasing proliferation of cancer cells will also carry out the invasion and metastasis of cancer cells through blood or lymph, and the invasion and metastasis of cancer cell are often the main reasons of death of patients who have malignant tumors (17). miRNA plays an important role in the process of carcinogenesis and tumor progression, and the expression of miRNA affects the invasiveness of tumor cells (18). Studies have demonstrated that as members of miRNA, the expression changes of miR-490-5p, miR-148a-3p and miR-608 have been confirmed to be associated with the occurrence and development of malignant tumors (11,19,20); but the specific effect and mechanism of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer cells are unclear; therefore, this experimental study on the expression features of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer and their effects on the biological characteristics of bladder cancer cells was carried out in order to provide a new theoretical basis for the diagnosis and treatment of bladder cancer in molecular biology.

In this study, we first used RT-qPCR technology to detect the expression of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer tissues and normal adjacent tissues. The results showed that the expression levels of miR-490-5p, miR-148a-3p



Table III. Comparison of the survival rate of the cells (%) in each group.

Group	Group A	Group B	Group C	Group NC	Blank group	F	P-value
24 h	97.42±3.01	97.87±3.68	96.99±3.54	97.12±1.04	97.78±3.02	0.501	0.735
48 h	85.24±2.03	84.92±3.58	85.16±2.57	97.01±1.56	96.89±2.91	183.300	<0.001
72 h	79.82±2.48	80.08±2.73	80.27±2.58	96.25±2.22	96.33±3.58	312.100	<0.001
F	378.300	225.200	257.900	2.392	1.583		
P-value	<0.001	<0.001	<0.001	0.052	0.211		

Table IV. Comparison of the condition of the invasion of T24 cells in each group.

Group	Group A	Group B	Group C	Group NC	Blank group	F	P-value
Number of the invasive cells	200.56±21.78	201.32±20.41	199.56±23.53	498.38±50.25	500.47±46.91	651.800	<0.001

Table V. Comparison of the apoptosis rate (%) in each group.

Group	Group A	Group B	Group C	Group NC	Blank group	F	P-value
Apoptosis rate (%)	20.14±3.32	19.92±4.47	19.73±4.01	3.75±0.65	3.67±0.91	651.800	<0.001

and miR-608 in bladder cancer tissues were significantly lower than those in normal adjacent tissues, and the differences were statistically significant ( $P<0.001$ ); there are now a large number of similar studies that have confirm the expression levels of miR-490-5p, miR-148a-3p and miR-608 in bladder cancer tissues were significantly lower than those in normal adjacent tissues and they have a low expression in bladder cancer tissues (10,12,21). Next, we investigated the condition of the proliferation, invasion and apoptosis of bladder cancer cells affected by miR-490-5p, miR-148a-3p and miR-608, and the results showed that after the transfection, the expression level of miR-490-5p in group A was significantly higher than that in group NC and blank group, the expression level of miR-148a-3p in group B was significantly higher than that in group NC and blank group, and the difference of the expression level was statistically significant; the expression level of miR-608 in group C was significantly higher than that in group NC and blank group, and the difference was statistically significant ( $P<0.001$ ). After the transfection, miR-490-5p, miR-148a-3p and miR-608 were overexpressed in T24 cells. Then we continued to analyze the effects of miR-490-5p, miR-148a-3p and miR-608 on the ability of proliferation, invasion and apoptosis of bladder cancer T24 cells, and the results showed that at 48 and 72 h, the survival rate of the cells in groups A-C was significantly lower than that in group NC and blank group ( $P<0.001$ ); the results of the comparison within the groups showed that the survival rate of the cells within groups A-C showed a gradual downward trend from 24 to 72 h, and the differences were statistically significant when compared at different time points within the three groups ( $P<0.001$ ). After the transfection, the number of invasive cells in groups A-C was significantly lower than that in blank group and group NC, and the difference was statistically significant

( $P<0.05$ ). However, there was no significant difference among groups A-C, and there was no significant difference among blank group and group NC ( $P>0.05$ ). After the transfection, the apoptosis rate in groups A-C was significantly higher than that in group NC and blank group ( $P<0.05$ ). Based on the above results, we considered that the overexpression of miR-490-5p, miR-148a-3p and miR-608 could reduce the survival rate of bladder cancer T24 cells and inhibit the ability of proliferation and invasion of bladder cancer cells to some extent as well as promoting apoptosis of bladder cancer cells. He *et al* (22) used CCK8 method for detection after they transfected miR-608 Inhibitor, found that the proliferation ability of tumor cells was enhanced to a certain extent after miR608 was inhibited, which reversely proved that the expression of miR608 could inhibit the proliferation ability of tumor cells. Regarding the effects of miR-490-5p and miR-148a-3p on bladder cancer cells, there have been related studies which carried out experimental observation on the ability of proliferation, invasion and apoptosis of miR-490-5p or miR-148a-3p which have been transfected, and the results of the related studies show that the transfected miR-490-5p and miR-148a-3p are overexpressed in bladder cancer T24 cells, and the ability of the proliferation and invasion of T24 cells was inhibited at this moment and the apoptosis rate increased (23,24); this is similar to the results of our study.

This study has some shortcomings, for example, the number of patients included were small, which was not satisfactory for statistical data. Increasing the number of subjects at a later date is anticipated.

In summary, miR-490-5p, miR-148a-3p and miR-608 are lowly expressed in bladder cancer T24 cells, and the overexpression of miR-490-5p, miR-148a-3p and miR-608 has inhibitory effect on the proliferation and invasion of cancer

T24 cells and promotes apoptosis of bladder cancer T24 cells. This experiment showed that miR-490-5p, miR-148a-3p and miR-608 are involved in the biological process of bladder cancer cells and they show potential to be used as a diagnostic marker and therapeutic target of bladder cancer.

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## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

MX and WY conceived and designed the study. WY collected the patients' data. WZ analyzed and interpreted the data. JH and MX performed the experiment. MX was a major contributor in writing the manuscript. JH reviewed the manuscript and helped with cell culture and transfection. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was approved by the Ethics Committee of Hunan Provincial People's Hospital (Changsha, China). Each patient who participated in this research had complete clinical data. Signed informed consents were obtained from the patients or guardians.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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