MiR-10a-5p: A Promising Biomarker for Early Diagnosis and Prognosis Evaluation of Bladder Cancer

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Introduction: MiRNAs play a critical role in carcinogenesis, among which miR-10a-5p has been reported in several types of human cancer. Nevertheless, the role of miR-10a-5p remain uncovered in bladder cancer (BCa).

Methods: We recruited 88 BCa patients and 36 healthy controls (HC) to form the training cohort, and other 120 BCa patients to form the validation cohort. The clinical samples were collected for analysis. The expression level of miR-10a-5p was evaluated using RT-qPCR. Receiver operating characteristic (ROC) curves were utilized to calculate diagnostic accuracy. Survival curves were generated to analyze survival outcomes. CCK-8 and transwell assays were conducted to test the cell proliferation, migration, and invasion capacities.

Results: MiR-10a-5p was upregulated in human BCa tissues and closely associated with advanced clinicopathological features, including advanced tumor grade, histological grade, and T stage. High expression of miR-10a-5p was associated with worse survival outcomes in BCa patients. Circulating plasma miR-10a-5p expression had the great performance power to discriminate BCa patients form HC patients before surgery, and to differentiate muscle invasive bladder cancer (MIBC) from non-muscle invasive bladder cancer (NMIBC). In addition, over-expression of miR-10a-5p could promote BCa cell proliferation, migration, and invasion.

Conclusion: This study indicates that miR-10a-5p is a crucial diagnostic and prognostic biomarker for BCa patients, and miR-10a-5p exerted a tumor promoting role during BCa cell progression.

Keywords: bladder cancer, miR-10a-5p, diagnostic, prognosis, biomarker

Introduction
Bladder cancer (BCa) is one of the most frequently occurring malignancies worldwide, with about 380,000 new cases and 15,000 deaths each year. The incidence of BCa in males is three times that in females. BCa incidence and mortality rates vary across countries due to differences in risk factors, diagnostic practices, and availability of treatments. BCa has different histological types including urothelial carcinoma, squamous cell carcinoma and adenocarcinoma, and urothelial carcinoma is the most frequent histological type, accounting for more than 95% of all BCa. Recently, despite the obvious progress in diagnosis and treatment, the long-term prognosis remains poor for BCa patients, with the 5-year overall survival remaining only 50%-60%. The high tumor recurrence rate and a high migratory and invasive ability of BCa have contributed greatly to the unsatisfactory prognosis. With the increasing understanding of molecular abnormality, molecular diagnosis and targeted therapy...
have developed into a crucial part for the diagnosis and treatment of many cancers. Moreover, some personalized approaches have grown rapidly for BCa treatment over the past several years, including novel targeted small-molecule and biological treatments, as well as immunotherapies.

Several immune checkpoint inhibitors that target programmed cell death protein 1 (PD1), its ligand PDL1, and cytotoxic T lymphocyte-associated protein 4 (CTLA4) have already been approved for use in bladder cancer. Hence, it is urgent to seek effective and efficient biomarkers for the diagnosis and treatment of BCa patients.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs at a length of 19 to 25 nucleotide. Recently, researches have elucidated the important roles of miRNAs in carcinogenesis, including cell differentiation, proliferation, apoptosis, and cell metastasis. Moreover, miRNAs can act as either oncogene or tumor suppressors depending on the genes it targets in various types of cancers. Recently, the role of miRNAs has been increasingly determined for the early diagnosis and targeted treatment for tumors of urinary system, including prostate cancer and BCa patients. MiR-10a-5p, which has been widely reported in recent years, plays critical roles in different kinds of cancers including pancreatic ductal adenocarcinoma, acute myeloid leukemia, ovarian cancer, lung cancer, renal cell carcinoma, and cervical cancer. Nevertheless, the role of miR-10a-5p still remain uncovered in BCa.

In this study, we determined that miR-10a-5p was upregulated in human BCa tissues, and its high expression could be an indicator for worse survival. Moreover, circulating plasma miR-10a-5p expression employed outstanding diagnostic value for BCa patients. In addition, enhanced expression of miR-10a-5p promoted proliferation, migration, and invasion of BCa cells.

Materials and Methods

Clinical Samples

A total of 88 BCa patients and 36 healthy controls (HC) were recruited from the Wuxi No.2 Chinese Medicine Hospital between January 2009 and December 2014 to constitute the training cohort. Meanwhile, other 120 BCa patients were enrolled at the same institution between January 2012 and May 2016 to form the validation cohort. Out of 88 bladder cancer patients in training cohort, 32 were diagnosed with non-muscle invasive bladder cancer (NMIBC); while the other 56 were diagnosed with muscle invasive bladder cancer (MIBC). Out of 120 bladder cancer patients in validation cohort, 40 were diagnosed with NMIBC; while the other 80 were diagnosed with MIBC. In the training cohort, plasma samples were extracted on the day of admission (BCa patients and HC patients) and 1 month after surgery (BCa patients); while in the validation cohort, plasma samples were extracted from BCa patients on the day of admission. The tumor tissues and paired adjacent normal tissues were obtained from BCa patients after surgery both in training and validation cohort. The inclusion criteria for BCa patients were: all patients were histologically confirmed as BCa; no other associated malignancies; all patients underwent primary section of bladder cancer (TURBt) or radical cystectomy (RC) without other pre-surgical anti-cancer treatments; patients had complete follow-up and clinicopathological information. The peripheral blood samples were collected from all participants in EDTA gel tubes. Each sample was centrifuged at 2000 g for ten minutes to separate plasma and then stored at −80°C until tested. The collected tumor tissues and paired normal tissues were immediately frozen in liquid nitrogen and frozen at −80°C before tested. Some clinical data were collected including age, gender, tumor grade, histological grade, T stage, lymph nodes metastasis, and multiplicity. Written informed consent was obtained from all the patients. This study was approved by the ethics committee of Wuxi No.2 Chinese Medicine Hospital and was conducted in accordance with the Declaration of Helsinki.

Cell Culture

Human BCa cell line 253j and J82 was obtained from Shanghai Chinese Academy of Sciences cell bank (China). 253j and J82 cells were cultured in DMEM medium (Life Technologies, Carlsbad, CA, US) supplemented with 10% fetal bovine serum (FBS, Life Technologies), 100 U/mL penicillin and streptomycin under a humidified incubator at 37 °C with 5% CO₂.

RNA Extraction and Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from tissues or cells using the RNA Isolation Kit (Qiagen, USA). Complementary DNA (cDNA) was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, USA) in accordance with the manufacturer’s protocol. RT-qPCR reactions were then conducted on the ABI prism 7500 sequence detection
system (Applied Biosystems) using the TaqMan miR assay system (Applied Biosystems, CA, USA). The thermal cycling conditions were: 95°C for 20 sec, followed by 40 cycles of 94°C for 20 sec, 60°C for 40 sec and 72°C for 10 sec. Relative expression of miR-10a-5p were normalized to that of U6 using 2^{-ΔΔCt} method. The sequences of oligonucleotides (GenePharma Co., Ltd, Shanghai, China) used in this study are as follows: miR-10a-5p: forward: 5ʹ-CGCTAGAAGCTTTGGTTA-3ʹ, reverse: 5ʹ-GCCCTAGACCATGGATT-3ʹ; U6: forward: 5ʹ-CGCTTCGTCGTGATCTA-3ʹ, reverse: 5ʹ-TTCACGAATTTGCAAGACCATGGATTT-3ʹ.

Cell Transfection

BCa cells were transfected with miR-10a-5p mimics, miR-10a-5p inhibitors, or their corresponding negative controls (NC mimics or NC inhibitors) (GenePharma Co., Ltd, Shanghai, China). Briefly, BCa cells were seeded in 6-well plates at a density of 1×10^5 cells per well for 24h. Cell transfection was conducted using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) based on the manufacturer’s instructions. Transfected cells were further cultured for an additional 48 h at 37°C before being used in downstream experiments.

Cell Counting Kit-8 (CCK-8) Assay

Three replicates of transfected BCa cells were seeded into 96-well plates at a density of 1×10^4 cells per well, following which 10 µL of CCK-8 solution (Dojindo, Kumamoto, Japan) was added to the medium at 1, 2, 3, 4 day before the cells were further incubated at 37 °C for 2 h. Optical density (OD) values were measured at 450 nm using an automatic microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Transwell Assay

The polyethylene membranes (24-well inserts; 8.0 µm; Corning, Inc.) were utilized to detect the migration and invasion abilities of BCa cells. Chambers precoated with 50 µL Matrigel (BD Biosciences) at 37°C for 1 h were used for invasion assays, while uncoated chambers were utilized for migration assays. Cell suspensions containing 1×10^5 cells in 100 µL FBS-free DMEM were seeded in the upper chamber. Meanwhile, the lower chamber was covered with 500 µL DMEM supplemented with 10% FBS. Cells were cultured at 5% CO₂ and 37°C for 48 hours. After 48 hours, cells that have remained in the upper membranes were gently removed using a cotton swab; while cells that have migrated or invaded the bottom of the membrane were fixed with polyoxymethylene at room temperature for 20 min and then stained with 0.5% crystal violet at room temperature for 20 min. Cells were counted in 5 randomly selected fields under a light microscope (Leica Microsystems GmbH, Wetzlar, Germany).

Statistical Analysis

All data are expressed as mean ± standard deviation of at least three experiments. Statistical evaluations were performed using SPSS 20.0 (IBM SPSS Inc., Chicago, IL, USA). Differences between two groups were analyzed using unpaired Student’s t-test, while the expression of miR-10a-5p in tumor tissues and matched normal tissues was compared using paired Student’s t-test. Comparisons of multiple groups were analyzed using the ANOVA followed by Dunnett’s test. Categorical data were compared using chi-square test. Receiver operating characteristic (ROC) curves were utilized to calculate diagnostic accuracy. Overall survival (OS) and recurrence-free (RFS) of patients with BCa were evaluated using Kaplan-Meier curves and compared using Log rank test. Prognostic factors were analyzed by Cox regression proportional hazards analysis. Differences were considered to be significant when P< 0.05.

Results

MiR-10a-5p is Upregulated in BCa Tissues

We firstly detected the expression profiles of miR-10a-5p in BCa tissues in the training cohort, and a total of 88 BCa tissues and paired normal tissues were collected for RT-qPCR analysis. Our results showed that miR-10a-5p expression was significantly upregulated in primary BCa tissues compared to that in paired normal tissues (P< 0.001, Figure 1A). Moreover, RT-qPCR analysis showed a significant upregulation of miR-10a-5p in patient with MIBC compared to those with NMIBC (P< 0.001, Figure 1B). Subsequently, the relative expression levels of miR-10a-5p were evaluated in plasma samples of the 32 patients with NMIBC, 56 patients with MIBC and 36 HC patients. RT-qPCR analysis results showed that miR-10a-5p expression was significantly higher in the MIBC group compared to the NMIBC or HC group (P< 0.001, Figure 1C). The plasma levels of miR-10a-5p were also measured by RT-qPCR at 1 month after surgery, and the results showed that plasma levels of miR-10a-5p were significantly downregulated at 1 month after treatment when compared to the pre-surgery levels of miR-10a-5p (P< 0.001, Figure 1D). We analyzed the
relationship between miR-10a-5p expression in BCa tissues and the clinicopathological features of these BCa patients. We firstly separated the 88 BCa patients into miR-10a-5p low expression group (n=44) and miR-10a-5p high expression group (n=44) based on the median values of miR-10a-5p expression in BCa tissues. MiR-10a-5p expression in tumor tissues was significantly correlated with tumor grade, histological grade, and T stage (Table 1). Moreover, we further confirmed the above findings with miR-10a-5p expression in validation cohort and GEPIA online database. As shown in Figure S1A–C, significant upregulation of miR-10a-5p was also observed in primary BCa tissues and patients with MIBC. Plasma miR-10a-5p expression level was significantly higher in the MIBC group compared to the NMIBC or HC group (P<0.001, Figure S1D). The clinicopathological analysis showed that miR-10a-5p expression was significantly correlated with tumor grade, histological grade, and T stage in validation cohort (Table S1).

The Diagnostic Value of miR-10a-5p in BCa Patients

To evaluate the diagnostic value of miR-10a-5p in BCa patients, we analyzed the performance of plasma miR-10a-5p in distinguishing BCa patients from HC patients using ROC analysis in the training cohort. As shown in Figure 2A and Table 2, the optimal diagnostic cut-off value for miR-10a-5p was 1.09, and the AUC value for miR-10a-5p was 0.815 (95% confidence interval [CI], 0.734–0.896), with a sensitivity and specificity of 79.5% and 65.6%, respectively, in distinguishing the BCa patients from HC patients. Next, the ROC curve was used to explore the potential of utilizing miR-18a as a biomarker for differentiating MIBC from NMIBC. As
shown in Figure 2B and Table 2, the optimal diagnostic cut-off value for miR-10a-5p was 1.28, and the AUC value for miR-10a-5p was 0.785 (95% CI, 0.688–0.883), with a sensitivity and specificity of 75.0% and 64.2%, respectively, in distinguishing the MIBC patients from NMIBC patients. Subsequently, the diagnostic power of miR-10a-5p was confirmed in the validation cohort. As demonstrated in Figure S2, miR-10a-5p still had the great value to distinguish BCa patients form HC patients with the AUC of 0.954 (0.688–0.883), and further to differentiate MIBC patients from NMIBC patients with the AUC of 0.796 (0.688–0.883).

The Prognostic Value of miR-10a-5p in BCa Patients

Next, by generating Kaplan-Meier curves of OS, our results showed that BCa patients with a high level of miR-10a-5p was associated with worse OS (Figure 3A; P= 0.005) and RFS (Figure 3B; P= 0.002) compared with patients with a low level of miR-10a-5p in the training cohort. By using univariate and multivariate COX regression analysis (Tables 3 and 4), we found that tumor grade (HR: 2.02, 95% CI: 1.32–2.85, P= 0.028), T stage (HR: 1.81, 95% CI: 1.51–2.13, P= 0.001), and high miR-10a-5p expression (HR: 1.74, 95% CI: 1.31–2.01, P= 0.002) were independent indicators of poor OS in BCa patients; and tumor grade (HR: 2.52, 95% CI: 1.16–3.31, P= 0.035), T stage (HR: 2.11, 95% CI: 1.21–3.05, P= 0.001), and high miR-10a-5p expression (HR: 1.95, 95% CI: 1.20–2.64, P= 0.001) were independent indicators of poor RFS in BCa patients. Additionally, upregulation of miR-10a-5p correlated with suboptimal OS (Figure S3A; P= 0.008) and RFS (Figure S3B; P= 0.016) in the validation cohort. The COX regression analysis (Tables S2 and S3) further verified the prognostic role miR-10a-5p for BCa in the validation cohort. Taken together, our data indicated that miR-10a-5p is an independent favorable prognostic factor in BCa patients.

Relationship Between miR-10a-5p and the Proliferation, Migration, and Invasion Ability of BCa Cells

Next, we further investigated the proliferation, migration, and invasion abilities of miR-10a-5p in BCa tumor progression using CCK-8 and Transwell assays. Firstly, we overexpressed and knocked down the expression level of miR-10a-5p in 253j cells using miR-10a-5p mimics and inhibitors (P< 0.001, Figure 4A). The CCK-8 assay results showed that the proliferation of the 253j cell lines transfected with the miR-10a-5p mimics was significantly increased compared with that of the cells transfected with the negative control, while the proliferation of the 253j cell lines transfected with the miR-10a-5p inhibitors was significantly inhibited (Figure 4B). Transwell assays demonstrated that miR-10a-5p mimics led to a significant increase in the migratory and invasive capability of 253j cells (P< 0.001, Figure 4C), whereas miR-10a-5p inhibitors markedly suppressed the migration and invasion of 253j cells compared to that in respective controls (P< 0.001, Figure 4D). In addition, as revealed in Figure 5, the promoting role of miR-10a-5p overexpression as regards cell proliferation, migration, and invasion was confirmed in another BCa cell lines J82.
Currently, a great number of studies have shown that miRNAs are abnormally expressed and may play crucial roles in diagnostic, prognostic, or biological functions in BCa progression. For instance, Wataru et al reported a miRNA panel, including miR-6087, miR-6724-5p, miR-3960, miR-1343-5p, miR-1185-1-3p, miR-6831-5p and miR-4695-5p, which could discriminate bladder cancer from healthy volunteers. Moreover, Lin et al established a predictive model to predict the overall survival of bladder cancer based on 7 miRNAs including miR-185-5p, miR-663a, miR-30c-5p, miR-3648, miR-1270, miR-200c-3p, and miR-29c-5p. Various studies have implicated miR-10a-5p in tumor progression. For example, Takayuki et al revealed the anti-tumor role of miR-10a-5p in renal cell carcinoma that low expression of miR-10a-5p was correlated to worse overall survival and overexpression of miR-10a-5p could inhibit cancer cell migration and invasion. Moreover, Zhai et al reported that downregulation of miR-10-5p inhibited the cell viability and promoted cell cycle arrest of cervical cancer cells. In addition, Xiong et al determined that miR-10a-5p was an independent adverse prognostic factor in patients with pancreatic ductal adenocarcinoma (PDAC) and promoted progression of PDAC cells in vitro and vivo. In this study, through analyzing the clinical samples, we found that miR-10a-3p was upregulated in BCa tissues and further increased in patient with MIBC compared to those with NMIBC both in tumor samples and plasma samples. Our study also revealed the close relationship between miR-10a-5p expression and advanced tumor grade, histological grade, and T stage. High expression of miR-10a-5p closely correlated with advanced clinicopathological characteristics and poor survival outcomes. Up to date, cystectomy is still the most reliable and accessible method for the detection of bladder cancer, which is expensive, invasive, time-consuming, and cannot be widely used especially for developing countries. With the development of microarray technology, a great number

**Figure 2** Diagnostic value assessment of miR-10a-5p in bladder cancer. (A) ROC curves indicate the ability of plasma miR-10a-5p to distinguish BCa patients from HC patients. (B) ROC curves indicate the ability of plasma miR-10a-5p to distinguish MIBC patients from NMIBC patients.

**Table 2** ROC Curves Analysis for the Diagnostic Power of Plasma miR-10a-5p in BCa

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC (95% Confidence Interval)</th>
<th>P value</th>
<th>Cut-Off Value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCa vs HC</td>
<td>0.815 (0.734–0.896)</td>
<td>&lt;0.001</td>
<td>1.09</td>
<td>79.5</td>
<td>65.6</td>
</tr>
<tr>
<td>MIBC vs NMIBC</td>
<td>0.785 (0.688–0.883)</td>
<td>&lt;0.001</td>
<td>1.28</td>
<td>75.0</td>
<td>64.2</td>
</tr>
</tbody>
</table>

**Abbreviations:** BCa, bladder cancer; MIBC, muscle invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer.
of biomarkers, including miRNAs, have been developed for the early diagnosis of multiple cancers. Specific serum miRNAs are very stable in blood plasma and serum, which is more convenient and noninvasive to predict the initiation and progression of BCa. As reported in acute myeloid leukemia (AML), miR-10a-5p had

Table 3 Univariate and Multivariate Analyses of Prognostic Factors Associated with Overall Survival

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th></th>
<th>Multivariate</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Age (≥60 vs &lt;60)</td>
<td>1.09 (0.74–1.63)</td>
<td>0.154</td>
<td>2.02 (1.32–2.85)</td>
<td>0.028*</td>
</tr>
<tr>
<td>Gender (male vs female)</td>
<td>1.04 (0.71–1.72)</td>
<td>0.395</td>
<td>1.81 (1.51–2.13)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Tumor grade (MIBC vs NMIBC)</td>
<td>2.13 (1.54–2.96)</td>
<td>0.003*</td>
<td>2.52 (1.16–3.31)</td>
<td>0.035*</td>
</tr>
<tr>
<td>Histological grade (high vs low)</td>
<td>1.56 (0.89–2.35)</td>
<td>0.097</td>
<td>2.11 (1.21–3.05)</td>
<td>0.001*</td>
</tr>
<tr>
<td>T stage (T2-T4 vs Tis-T1)</td>
<td>1.98 (1.64–2.32)</td>
<td>&lt;0.001*</td>
<td>1.74 (1.31–2.01)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Lymph nodes metastasis (yes vs no)</td>
<td>1.15 (0.72–1.86)</td>
<td>0.111</td>
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<tr>
<td>Multiplicity (high vs low)</td>
<td>1.29 (0.81–1.84)</td>
<td>0.136</td>
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<tr>
<td>miR-10a-5p expression (high vs low)</td>
<td>1.85 (1.46–2.15)</td>
<td>0.001*</td>
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</tbody>
</table>

Note: *P <0.05.

Abbreviations: MIBC, muscle invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; HR, hazard ratio; CI, confidence interval.

Table 4 Univariate and Multivariate Analyses of Prognostic Factors Associated with Recurrence-Free Survival

<table>
<thead>
<tr>
<th>Variables</th>
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<th></th>
<th>Multivariate</th>
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<tbody>
<tr>
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<td>HR (95% CI)</td>
<td>P-value</td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Age (≥60 vs &lt;60)</td>
<td>1.16 (0.81–1.75)</td>
<td>0.263</td>
<td>2.52 (1.16–3.31)</td>
<td>0.035*</td>
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<tr>
<td>Gender (male vs female)</td>
<td>1.11 (0.68–1.65)</td>
<td>0.185</td>
<td>2.11 (1.21–3.05)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Tumor grade (MIBC vs NMIBC)</td>
<td>2.85 (1.76–3.94)</td>
<td>0.001*</td>
<td>1.94</td>
<td></td>
</tr>
<tr>
<td>Histological grade (high vs low)</td>
<td>1.31 (0.76–2.12)</td>
<td>0.194</td>
<td>0.351</td>
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</tr>
<tr>
<td>T stage (T2-T4 vs Tis-T1)</td>
<td>2.64 (1.32–3.42)</td>
<td>&lt;0.001*</td>
<td>2.11 (1.21–3.05)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Lymph nodes metastasis (yes vs no)</td>
<td>1.32 (0.65–1.98)</td>
<td>0.656</td>
<td>1.95 (1.20–2.64)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Multiplicity (high vs low)</td>
<td>1.19 (0.91–1.45)</td>
<td></td>
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<tr>
<td>miR-10a-5p expression (high vs low)</td>
<td>2.08 (1.35–2.95)</td>
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</table>

Note: *P <0.05.

Abbreviations: MIBC, muscle invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; HR, hazard ratio; CI, confidence interval.
important diagnostic value in differentiating AML from normal subjects. In addition, Bao et al showed that miR-10a-5p was upregulated in non-small cell lung cancer (NSCLC) and also had great value for the clinical diagnosis of patients with NSCLC. However, the diagnostic and biological role of miR-10a-5p have not been elucidated in BCa. In this study, our results indicated that circulating plasma miR-10a-5p expression had the great performance power to discriminate BCa patients form HC patients before surgery, and further to differentiate MIBC from NMIBC. In addition, our biological function assays determined the promoting role of miR-10a-5p in cell proliferation, migration, and invasion of BCa cells. However, this study had its limitation that the underlying mechanism of miR-10a-5p biological function is unclear.

In conclusion, our results demonstrate that miR-10a-5p was upregulated in BCa patients, and miR-10a-5p could act as a promising diagnostic and prognostic biomarker for BCa patients. Moreover, miR-10a-5p exerted a tumor promoting role during the BCa cell progression.

Figure 4 MiR-10a-5p promotes BCa cell proliferation, migration, and invasion. (A) RT-qPCR analysis of miR-10a-5p expression levels in 253j cells after transfection with miR-10a-5p mimics/inhibitors and respective negative controls. (B) CCK8 assays were performed to test the effect of miR-10a-5p mimics or inhibitors on cell proliferation of 253j cells. (C) Transwell assays were performed to test the effect of miR-10a-5p mimics on cell migration and invasion of 253j cells (magnification: 200×). (D) Transwell assays were performed to test the effect of miR-10a-5p inhibitors on cell migration and invasion of 253j cells (magnification: 200×). **P < 0.01, ***P < 0.001.

Abbreviation: BCa, bladder cancer.
Data Sharing Statement
All data generated in this study will be made available on reasonable request.

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Disclosure
The authors report no conflicts of interest in this work.

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