

Downregulation of Urinary microRNA-200c acts as a Promising Novel Bio-marker in the Diagnosis of Patients With Bladder Cancer

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Abstract

Background

MicroRNAs (miRNAs) have been reported to be involved in various human cancers. The aim of this study was to explore the diagnostic performance of urine *miR-200c* in bladder cancer.

Methods

Quantitative real-time polymerase chain reaction (qRT-PCR) method was applied to measure the relative expression of urine *miR-200c* in bladder cancer patients. The relationship between urine *miR-200c* level and clinicopathological factors was analyzed using χ^2 test. The diagnostic capacity of urine *miR-200c* was calculated using the receiver operating characteristics (ROC) curve analysis.

Results

Urinary level of *miR-200c* was significantly reduced in bladder cancer patients compared with healthy controls ($P=0.000$). Furthermore, urine *miR-200c* expression was strongly correlated with histologic grade ($P=0.019$), tumor grade ($P=0.003$), and lymph node metastasis ($P=0.001$). ROC curve showed that urine *miR-200c* could distinguish bladder cancer patients from healthy controls with an area under the curve of 0.844. The cutoff value of 1.235, with the sensitivity of 89.0% and the specificity of 70.7% respectively.

Conclusion

Urine *miR-200c* may act as a noninvasive diagnostic biomarker for bladder cancer.

Background

Bladder cancer is one of most common malignancy in urinary system around the world [1]. It is featured by high recurrence, leading to poor outcomes and high mortality [2]. Currently, the standard of diagnostic tool for urinary bladder cancer is cystoscopy, but its clinical values are limited due to the invasive procedure, high cost and low efficacy in flat tumors like carcinoma *in situ* [3]. Non-invasive urine cytology, a convenient test, is used as an adjunct to cystoscopy [4]. It has high diagnostic specificity, but its sensitivity is unsatisfactory, especially for low-grade tumor [5]. Recently, there are numerous urine-based tests for early screening of bladder cancer, such as bladder tumor antigen (BTA), nuclear matrix protein 22 (NMP22) and FISH [6, 7]. However, none of them have been used in clinical guidelines [8]. Thus, it is of great value to identify non-invasive diagnostic markers for the detection of bladder cancer.

MicroRNAs (miRNAs) are small non-coding RNA molecules that play important roles in various biological processes through modulating the activity of specific mRNA [9]. Circulating miRNAs stably exist in a variety of body fluids including serum, plasma, saliva, pleural fluid and urine, and their abnormal expression may lead to human diseases, like cancer [10, 11]. Increasing evidences have showed that miRNAs are implicated in human carcinogenesis by functioning as either oncogenes or tumor

suppressors [12, 13]. The expression patterns of miRNAs show close association with tumor development and progression, which could be employed as predictive biomarkers and therapeutic targets [14]. MicroRNA-200c (*MiR-200c*), belonging to miR-200 family, is located on on chromosome 12p13 [15]. *MiR-200c* is a well-known tumor suppressor, and its downregulation has been observed in various cancers, such as breast cancer, gastric cancer, etc [16, 17]. In bladder cancer, it has been reported that the ectopic expression of *miR-200c* could inhibit bladder cancer cell proliferation and invasion in vitro [18]. *MiR-200c* may be involved in tumorigenesis of bladder cancer, which may be employed as an indicator for early screening of the malignant disease. However, there are few reports to address the issue.

In the present study, we examined the relative expression of urine *miR-200c* in bladder cancer patients, and analyzed the correlation of urine *miR-200c* expression with clinicopathological parameters of bladder cancer patients. We also investigated the diagnostic performance of urine *miR-200c* in bladder cancer patients.

Methods

Patients and urine samples

The study was approved by the Ethic Committee of Huaihe Hospital of Henan University. All the participants had signed the written informed consents.

A total of 109 patients with bladder cancer were obtained at the department of Huaihe Hospital of Henan University. Pathological grading of cases was based on the World Health Organization's classification of urothelialneoplasms. Staging of bladder cancer was according to the classification of Union for International Cancer Control (UICC TNM). Details of the backgrounds and clinicopathological characteristics of the patients with bladder cancer were listed in **Table 1**. In addition, 96 age- and gender-matched healthy volunteers were recruited from the physical examination center of the hospital. All the healthy controls had no evidences for urological disorders or malignant diseases.

Table 1. The relationship of serum *miR-200c* expression with clinicopathological features in bladder cancer patients

Features	Cases (n=109)	MiR-200c expression		χ^2	P-value
		Low (n=56)	High (n=53)		
Age (years)					
<55	44	23	21	0.024	0.878
≥55	65	33	32		
Gender					
Male	62	30	32	0.514	0.473
Female	47	26	21		
Smoking status					
Non-smoker	71	35	36	0.353	0.553
Smoker	38	21	17		
Tumor size (cm)					
<4	75	43	32	3.416	0.065
≥4	34	13	21		
Histologic grade					
Low grade	68	29	39	5.514	0.019
High grade	41	27	14		
Tumor stage					
T1-T2	69	28	41	8.773	0.003
T3-T4	40	28	12		
Tumor types					
NMIBC	81	40	41	0.502	0.479
MIBC	28	16	12		
Lymph node metastasis					
Absent	77	32	45	10.120	0.001
Present	32	24	8		

Notes: NMIBC: non-muscle invasive cancer; MIBC:muscle-invasive bladder cancer

From each subject preoperative urine sample (50ml) was collected in a sterile container and immediately refrigerated at the urology clinic. Within 2 hours urine samples were transported to the laboratory on ice. Each urine sample was assigned a unique identifying number before immediate laboratory processing. The sample was centrifuged at 3000g at 4°C for 10 minutes. The urine supernatant aliquots was decanted, centrifuged at 12000g for 10 minutes at 4°C and stored at -80°C before analysis at a genitourinary tissue bank.

RNA extraction and quantitative real-time transcriptase polymerase chain reaction (qRT-PCR)

Total RNA was extracted from urine samples using mirVana miRNA isolation kit (Ambion, Austin, TX, USA), according to the manufacturer's directions. The concentration and purity of the RNA samples were measured using spectrophotometer, and the RNA samples with the OD_{260/280} value of 1.8-2.0 were used for the subsequent analysis. First strand cDNA was synthesized from the total RNA using a universal cDNA synthesis (Exiqon, Vedbaek, Denmark) in accordance with the manufacturer's instructions. The relative expression of *miR-200c* was estimated using qRT-PCR method which was performed with SYBR Prime Script miRNA RT-PCR kit (Takara, Japan) in ABI 7500 real-time PCR system (Applied Biosystems, USA). RNU6B was used as the reference control. All of the samples were tested in triplicate. The relative level of *miR-200c* was calculated using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

All the statistical analyses were performed using SPSS 18.0 software (SPSS software, Inc, IL, USA), and graphs were plotted using Origin 9.0. The Student's *t* test was performed to compare urine *miR-200c* levels between bladder cancer patients and healthy controls. The relationship between urine *miR-200c* level and clinicopathological features was analyzed by χ^2 test. Receiver operating characteristics (ROC) curves were established to evaluate the diagnostic performance of *miR-200c* in distinguishing bladder cancer patients from the controls. All statistical tests were two-sided and *P* values less than 0.05 were considered as statistical significance.

Results

The relative expression of urine miR-200c in bladder cancer patients

The expression level of *miR-200c* was detected using qRT-PCR in 205 urine samples (109 bladder patients and 96 controls). As shown in **Figure 1**, compared to healthy controls, urine *miR-200c* expression was significantly decreased in bladder cancer patients (*P*=0.000).

The correlation between urine miR-200c expression and clinicopathological characteristics

To identify the potential association between urine *miR-200c* expression and clinicopathological parameters in bladder cancer, the patients were divided into two groups based on the median value (0.82): low *miR-200c* expression group (n=56) and high *miR-200c* expression group (n=53). As indicated

in **Table 1**, urine *miR-200c* expression was dramatically associated with histologic grade ($P=0.019$), tumor grade ($P=0.003$), and lymph node metastasis ($P=0.001$). Nevertheless, there was no relationships between urine *miR-200c* expression and other clinicopathological factors, such as age, gender, smoking status, or tumor types (all $P>0.05$).

The diagnostic efficacy of urine miR-200c in bladder cancer patients

ROC curve analyses revealed that urine *miR-200c* could use as a novel non-invasive biomarker to discriminate bladder cancer patients from healthy controls with an AUC value of 0.844 (95%CI: 0.789-0.898) (**Figure 2**). At a cutoff value of 1.235, the optimal sensitivity and specificity were 89.0% and 70.7% respectively.

Discussion

Bladder cancer is a heterogeneous disease with unpredictable clinical outcome. Several risk factors have been identified for bladder cancer, such as cigarette smoking, exposures to aromatic amines and 4,4'-methylenebis(2-chloroaniline) [19]. However, the molecular mechanisms underlying the bladder cancer development are still unclear. Nowadays, early screening and detection of bladder cancer remain a great challenge in clinic. Therefore, it is important to explore the effective bio-markers for early diagnosis of bladder cancer.

MiRNAs are a group of small endogenous RNAs, and they play regulatory role in gene expression at post-transcriptional level [9]. The expression profile of extracellular cell-free miRNAs are significantly correlated with tumor initiation, development and progression, which have been considered as promising biomarkers for cancer management [20]. The urinary miRNAs are stable at room temperature, and they could not be unaffected by multiple freeze-thaw cycles [21], indicating their properties as molecular biomarkers for cancer diagnosis and prognosis. Urine supernatant is a particularly desirable source of biomarkers for bladder cancer, with greatly convenient, reducing protein interference during RNA extraction and reflecting the status of diseases [22].

MiR-200c belongs to the miR-200 family which consists of *miR-200a*, *miR-200b*, *miR-200c*, *miR-141* and *miR-429* [23]. Accumulating evidences have demonstrated that miR-200 family has the capacity to regulate cancer transformation, growth, metastasis, and therapeutic response through targeting multiple signaling pathways, such as epithelial-mesenchymal transition (EMT), TGF- β signaling, PI3K/Akt signaling, Notch signaling, VEGF signaling, and NF- κ B signaling [15, 24]. The expression pattern of *miR-200c* has been confirmed as a potential diagnostic and prognostic biomarker in a variety of tumors. For instance, Antolín *et al.* reported that circulating *miR-200c* was deregulated in breast cancer and showed close correlation with clinical characteristics of the patients that might serve as an independent prognostic indicator for the patients [25]. Meng *et al.* revealed that *miR-200c* level was involved in tumor progression and could distinguish malignant cases from benign ovarian tumors [26]. However, the clinical significance of *miR-200c* had been rarely reported in bladder cancer.

In the present study, qRT-PCR was applied to detect the relative expression level of *miR-200c* in 205 urine samples collected from 109 bladder cancer patients and 96 healthy controls. Urine *miR-200c* expression was obviously down-regulated in bladder cancer patients compared with healthy controls. Additionally, the decreased expression of *miR-200c* was negatively correlated with histological grade, tumor stage, and lymph node metastasis. All the data suggested that *miR-200c* as a tumor suppressor was involved in the progression and metastasis of bladder cancer patients. ROC analysis illustrated that urine *miR-200c* was an useful biomarker for early detection of bladder cancer. The conclusion was consistent with the previous studies. Wu *et al.* reported that the urinary *miR-200* family levels were decreased in patients with bladder cancer [27]. The study carried out by Liu *et al.* showed that *miR-200c* could inhibit bladder cancer cells proliferation through down-regulating BMI-1 and E2F3 [28]. However, the possible underlying mechanisms for its participation in bladder cancer progression remained not clarify. Additionally, the sample size was relatively small that might influence the accuracy of our results. Therefore, further researches with a extended sample size will be performed to improve our conclusion.

Conclusion

In summary, urine *miR-200c* expression is significantly decreased in bladder cancer patients, and negatively correlated with malignant disease progression. Urine *miR-200c* may be a potential non-invasion biomarker for early diagnosis of bladder cancer.

Abbreviations

MicroRANs (miRNAs)

Quantitative real-time polymerase chain reaction (qRT-PCR)

receiver operating characteristics (ROC)

bladder tumor antigen (BTA)

nuclear matrix protein 22 (NMP22)

MicroRNA-200c (MiR-200c)

Union for International Cancer Control (UICC TNM)

Declarations

Disclosure

The authors report no conflicts of interest in this work.

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Huaihe Hospital of Henan University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

H.Z. design of the work; WL.T. the acquisition, analysis, L.G. interpretation of data; ZH.Y. the creation of new software used in the work; XD.B., XD.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

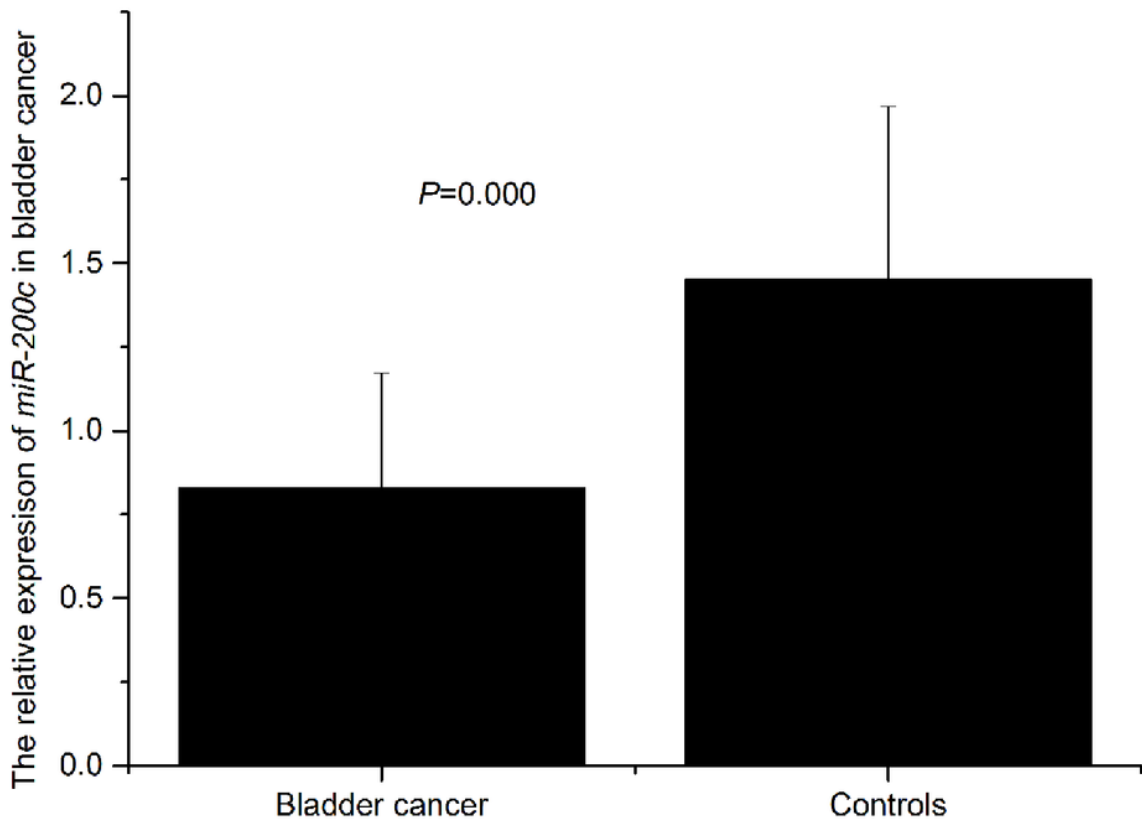


Figure 1

The relative expression level of urine miR-200c in bladder cancer patients was detected by qRT-PCR. Results showed that urine miR-200c expression was significantly reduced in bladder cancer patients compared with healthy controls ($P=0.000$).

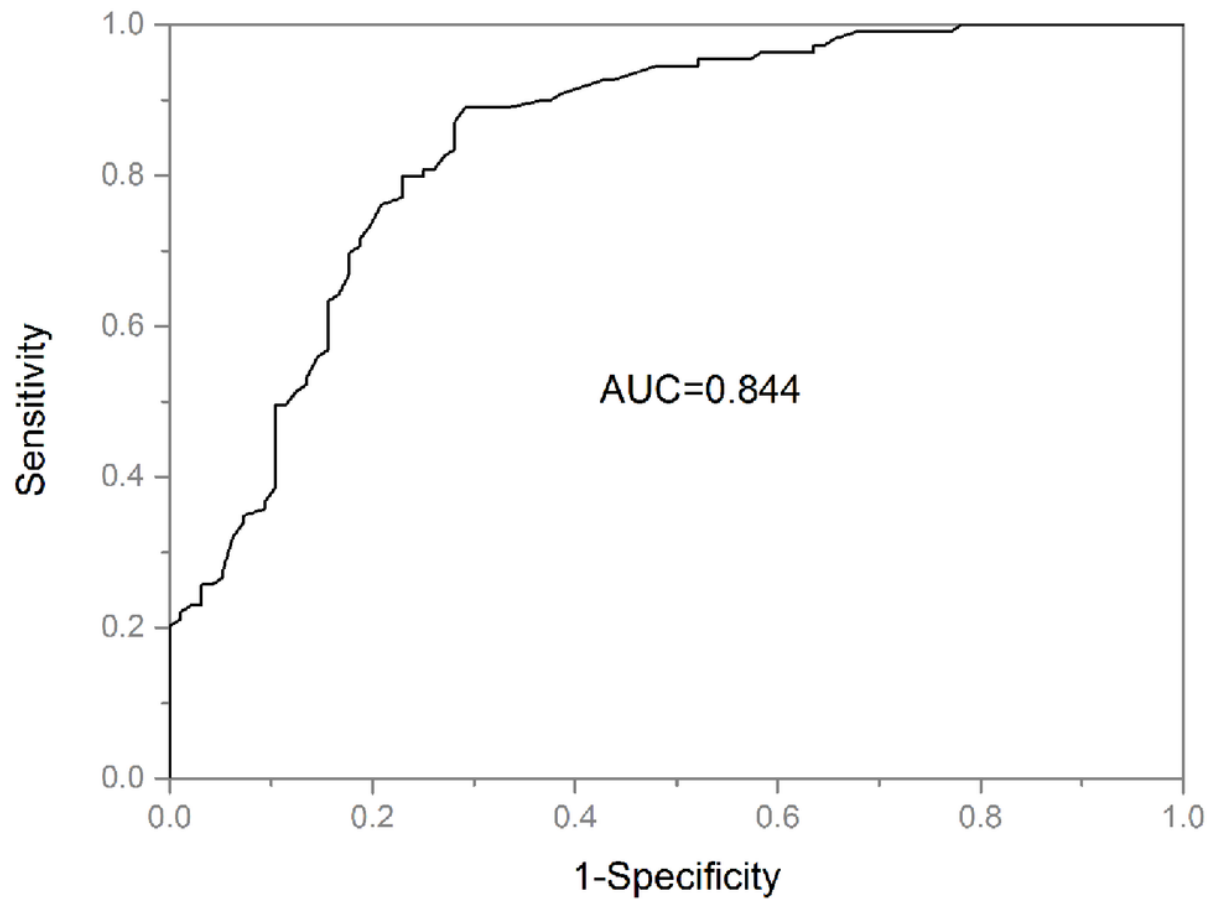


Figure 2

ROC curve analysis for miR-200c to calculate the optimal cutoff point the discriminates bladder cancer patients for healthy controls. Area under the curve (AUC) = 0.844; 95% CI (confidence interval): 0.789-0.898; sensitivity: 89.0%; specificity: 70.7%.