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# *MiR-101* acts as a novel bio-marker in the diagnosis of bladder carcinoma

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**Abstract**

**Backgrounds:** *MiR-101* plays an important role in tumorigenesis. The aim of this study was to estimate diagnostic potential of serum *miR-101* in bladder cancer.

**Methods:** Serum level of *miR-101* in 122 bladder cancer patients and 110 healthy volunteers was detected using quantitative real-time polymerase chain reaction method. The association between *miR-101* expression and clinicopathological characteristic was analyzed via $\chi^2$ test. Then receiver operating characteristic (ROC) curve was plotted to evaluate diagnostic value of serum *miR-101* in bladder cancer.

**Results:** *MiR-101* expression was statistically down-regulated in bladder cancer patients compared to healthy controls. *MiR-101* expression was significantly associated with TNM stage ($P = .019$), pathological grade ($P = .006$) and lymph node metastasis ($P = .010$). ROC analysis suggested that *miR-101* had high value in discriminating between bladder cancer patients and healthy individuals with an AUC value of 0.884. The cut-off value for serum *miR-101* in bladder cancer diagnosis was 1.645, with a sensitivity of 82.0% and a specificity of 80.9%.

**Conclusion:** *MiR-101* is decreased in bladder cancer patients, and shows negative association with aggressive clinical characteristics. *MiR-101* may serve as a bio-marker in diagnosing bladder cancer.

**Abbreviations:** AUC = The area under the ROC curve, BTCC = Bladder transitional cell carcinoma, GOLPH3 = Golgi phosphoprotein 3, HBV-HCC = hBV-associated hepatocellular carcinoma, HBV-LC = hBV-associated liver cirrhosis, MiRNAs = MicroRNAs, qRT-PCR = Quantitative real-time polymerase chain reaction, ROC = Receiver-operating characteristic, SD = Standard deviation, VEGF-C = Vascular endothelial growth factor C.

**Keywords:** bladder cancer, diagnosis, *MiR-101*, serum

## 1. Introduction

Bladder cancer is one of the most common malignancies in urinary system, posing great threat to human healthy in the world.[1] In the United States alone, bladder cancer was estimated to see 74,000 new cases and 16,000 deaths in 2015.[2] The cancer is characterized by high recurrent rate. Reportedly, more than half of the patents would undergo recurrence within 5 years after operation.[3,4] Early detection and monitoring are pivotal for clinical outcomes of bladder cancer patients. At present, cystoscopy and cytology are commonly used in early screening of bladder cancer.[5] However, these methods are invasive and costly, and frequently cause infections.[6] Thus, it is in urgent need to explore novel and effective biomarkers for non-invasive diagnosis of bladder cancer.

MicroRNAs (MiRNAs) are a class of small, endogenous, non-coding RNA that regulate gene expression by affecting mRNA translation and stability or by modulating promoter activity of their target genes.[7,8] MiRNAs were originally identified in *Caenorhabditis elegans*.[9] They are involved in diverse biological processes, including cell growth, apoptosis, and differentiation.[10,11] Cumulated evidences have suggested that the dysregulation of miRNAs may play oncogenic or suppressive roles in cancer development.[12,13] Given their functional roles in tumorigenesis, miRNAs are considered as promising biomarkers for early diagnosis, prognosis evaluation and therapeutic response prediction in oncology.[11]

*MiR-101*, a common member of miRNAs family, is reported to be a suppressor gene in various human malignancies, such as gastric cancer, prostate cancer, osteosarcoma, and bladder cancer.[14–17] The study carried out by Zhang et al reported that down-regulated *miR-101* in bladder transitional cell carcinoma (BTCC) showed obvious association with aggressive clinical characteristics, which might be a promising prognostic biomarker for the disease.[17] However, whether serum *miR-101* could serve as a diagnostic biomarker for bladder cancer is still unclear.

In the present study, we aimed to investigate serum level of *miR-101* in bladder cancer. Furthermore, we analyzed the association of *miR-101* expression with clinicopathological characteristics of the patients with bladder cancer. Additionally, diagnostic value of serum *miR-101* in bladder cancer was also investigated in the current study.

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## 2. Materials and methods

### 2.1. Patients and specimens

A total of 122 patients with bladder cancer and 110 healthy control were recruited in this study from Zhongnan Hospital. The patients were diagnosed with bladder cancer by 2 independent experienced pathologists based on the 1973 diagnosis criteria. TNM staging was performed for the patients according to the American Joint Committee on Cancer staging system (7th edition, 2010). No patients had received preoperative treatment. Detailed clinicopathologic characteristics of the patients with bladder cancer were obtained from their medical records, and summarized in Table 1. In addition, none of the healthy individuals had bladder diseases or malignancy history. The bladder cancer patients and healthy individuals were matched in gender and age. Our study was approved by the Ethics Committee of Zhongnan Hospital. All participants signed written informed consents prior to sampling.

The 5 ml whole blood was collected from every participant on the morning after fasting for 8 to 10 hours. Serum was separated from whole blood through centrifugation, and then stored at −80°C until RNA extraction.

### 2.2. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from serum specimens using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. First-strand cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. Relative expression level of *miR-101* mRNA was estimated by qRT-PCR method which was performed using SYBR Green PCR Master Mix (Applied Biosystems) in the 7900 Real-Time PCR System (Applied Biosystems). PCR primers used to amplify *miR-101* were as follows: 5'-CGGGTACCGGTAGTCCTTCACTTCATGGGGAG-3' (forward) and 5'-CGGAATTCAAAAAACCCAGCCACCTGTTTCAC-3' (reverse).[18] *U6* was used as reference gene and its primers were as follows: 5'-CTCGCTTCGGCAGCACA-3' (forward) and reverse 5'-AACGCTTCACGAATTTGCGT-3' (reverse). Ct values of the samples were recorded, and relative levels of *miR-101* were calculated through the $2^{-\triangle\triangle Ct}$ method. Every sample was tested three times.

### 2.3. Statistical analysis

All statistical analyses were conducted using SPSS 21.0 software (SPSS, Inc., Chicago, IL) and GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA). Data for gene expression were presented as mean ± standard deviation (SD). Comparisons on *miR-101* expression between tumor and normal control groups were performed with 2-tailed paired Student's t test. The relationship between *miR-101* expression and clinicopathologic characteristics was analyzed by $\chi^2$ test. Receiver-operating characteristic (ROC) curve was constituted to assess diagnostic value of serum *miR-101* in bladder cancer, and the results were estimated through calculating the area under the ROC curve (AUC), sensitivity and specificity according to standard formulas. *P* values less than .05 were considered as statically significant. * $P < .05$, ** $P < .01$ indicated significant difference.

## 3. Results

### 3.1. The expression of miR-101 was decreased in bladder cancer

A total of 88 male and 34 female bladder cancer patients were collected in our study, with an average age of 55.62 ± 15.48 years. There were 77 men and 33 women in the control group, and their average age was 53.12 ± 12.22 years. The distributions of gender and age were similar between bladder cancer patients and healthy controls ($P > .05$ for both). QRT-PCR analysis was

**Table 1**

**The clinicopathological characteristics of 122 patients and the association with *miR-101* expression.**

| Characteristics | No. (n = 122) | *MiR-101* expression | | $X^2$ | *P* values |
|---|---|---|---|---|---|
| | | Low (n = 65) | High (n = 57) | | |
| Age (yr) | | | | 1.212 | .271 |
| <60 | 60 | 35 | 25 | | |
| ≥60 | 62 | 30 | 32 | | |
| Gender | | | | 0.002 | .963 |
| Male | 88 | 47 | 41 | | |
| Female | 34 | 18 | 16 | | |
| Tumor size | | | | 0.296 | .586 |
| <3cm | 61 | 34 | 27 | | |
| ≥3cm | 61 | 31 | 30 | | |
| Tumor stage | | | | 2.525 | .112 |
| T1-T2 | 57 | 26 | 31 | | |
| T3-T4 | 65 | 39 | 26 | | |
| TNM stage | | | | 5.459 | .019 |
| I-II | 59 | 25 | 34 | | |
| III-IV | 63 | 40 | 23 | | |
| Pathological grade | | | | 7.547 | .006 |
| G1,G2 | 63 | 26 | 37 | | |
| G3 | 59 | 39 | 20 | | |
| Lymph node metastasis | | | | 6.653 | .010 |
| Negative | 64 | 27 | 37 | | |
| Positive | 58 | 38 | 20 | | |

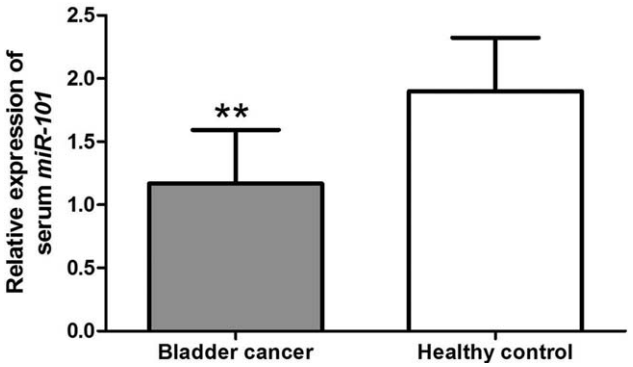


**Figure 1.** The expression of *miR-101* in bladder cancer cases and healthy controls. The result showed that serum *miR-101* level was downregulated in bladder cancer patients, compared to healthy individuals (** $P < .01$).

used to detect relative expression levels of *miR-101* in bladder cancer patients and healthy individuals. As shown in Figure 1, expression level of *miR-101* was decreased in bladder cancer patients compared with the normal controls ($P < .01$).

### 3.2. Relationship between miR-101 and clinicopathological parameters

To investigate the relationship between *miR-101* expression and clinicopathlogic characteristics of bladder cancer patients, we divided the patients into high- (> mean value) and low-(≤ mean value) expression groups based on their mean expression level of *miR-101*. Statistical analysis results were summarized in Table 1. The expression of *miR-101* was found to be significantly associated with TNM stage ($P = .019$), pathological grade ($P = .006$) and lymph node metastasis ($P = .010$), but not with age, gender, tumor size or tumor stage (all $P > .05$).

### 3.3. Diagnostic value of miR-101 in bladder cancer

To investigate diagnostic performance of serum *miR-101* in bladder cancer, ROC curve analysis was performed. As shown in Figure 2, *miR-101* possessed relatively high accuracy in differentiating bladder cancer patients from healthy individuals with an AUC of 0.884 (95%CI: 0.842–0.927). The optimal cut-off point was 1.645, with a sensitivity of 82.0% and a specificity of 80.9%.

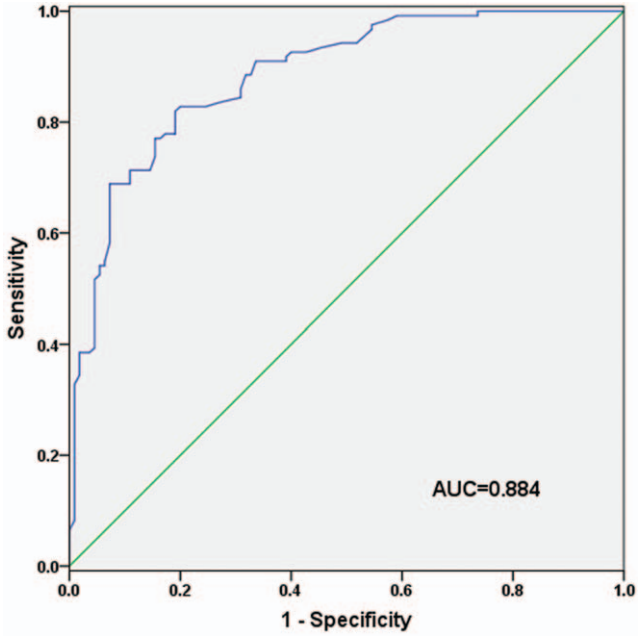


**Figure 2.** ROC curve was established to evaluate diagnostic value of serum *miR-101* in bladder cancer. The AUC value for the curve was 0.884, suggesting that serum *miR-101* could discriminate between bladder cancer patients and healthy individuals. The cut-off value of serum *miR-101* for bladder cancer diagnosis was 1.645, with the corresponding sensitivity of 82.0% and the specificity of 80.9%.

## 4. Discussion

Bladder cancer is one of the most common genitourinary malignancies with high morbidity and mortality. Despite great advances in surgery, radiotherapy and chemotherapy, overall survival of bladder cancer patients has not been significantly improved.[19] Early diagnosis is key for clinical outcomes of bladder cancer patients. Currently, standard measures for the diagnosis of bladder cancer contain conventional cystoscopy and biopsy, but cystoscopy is invasive, uncomfortable and costly while urine cytology always shows low sensitivity.[20] Therefore, novel reliable biomarkers with high sensitivity and specificity are urgently needed for non-invasive diagnosis of bladder cancer.

Recent years, more and more researches explored molecular biomarkers for the prediction of tumor development and progression. For instances, Zhang et al reported that DCAMKL1 (doublecortin and CaM kinase-like 1) was up-regulated in bladder cancer tissues and cell lines, moreover, its elevated expression showed positive association with malignant status of the disease. DCAMKL1 might be a potential prognostic biomarker for bladder cancer.[21] A meta-analysis including 6 studies indicated that urine *UCA1* exhibited high diagnostic accuracy for bladder cancer.[22] The study carried out by Zhang et al reported that Golgi phosphoprotein 3 (GOLPH3) was involved in the progression of bladder cancer via modulating AKT/mTOR signaling, and that it was a novel prognostic biomarker for the malignancy.[23] Molecular biomarkers may provide new insights in the etiology of bladder cancer, thus contributing to improvements in the disease management.

MiRNAs expression profiles show obvious association with tumor development, progression and therapy response, revealing their potential as biomarkers for early screening and monitoring in oncology.[24,25]*MiR-101* is widely accepted as a tumor suppressor in several malignancies. Growing evidences have suggested that *miR-101* hold the capacity to serve as a biomarker for cancers. Xie et al suggested that serum *miR-101* level could be employed as a non-invasive biomarker to distinguish hBV-associated hepatocellular carcinoma (HBV-HCC) from hBV-associated liver cirrhosis (HBV-LC).[26] The expression of *miR-101* also exhibited significant differences between cervical cancer patients and non-cancer patients, which might be a potential biomarker for early diagnosis of the disease.[27] Luo et al reported that the down-regulation of *miR-101* predicted malignant clinical characteristics in non-small cell lung cancer, suggesting its potential as a biomarker for prognosis and therapeutic response in this disease.[28] However, predictive function of *miR-101* in bladder cancer remains poorly known.

In the present study, we investigated serum level of *miR-101* in bladder cancer patients using qRT-PCR analysis. The result proved that the expression of *miR-101* was down-regulated in bladder cancer patients compared to the healthy individuals. Then we further analyzed the association of *miR-101* expression with clinicopathological characteristics of the bladder cancer patients. The down-regulation of *miR-101* was found to be significantly associated with advanced TNM stage, high pathological grade and positive lymph node metastasis, indicating *miR-101* as a tumor suppressor gene was participated in the development and progression of bladder cancer. In addition, diagnostic value of serum *miR-101* in bladder cancer was also investigated using ROC curve analysis. Analysis results suggested that *miR-101* might be employed as a biomarker for bladder cancer detection with high sensitivity and specificity. Serum *miR-101* might be a potential biomarker for non-invasive detection of bladder cancer. In previous studies, *miR-101* was also reported to be associated with the prognosis of multiple cancers. In the study of He, low serum level of *miR-101* was obviously correlated with poor prognosis of colorectal cancer.[29] However, Lv et al reported high expression of *miR-101* was an independent prognostic factor for HCC.[30] Therefore, *miR-101* may be closely associated with bladder cancer prognosis. In next step, we will explore prognostic value of *miR-101* in bladder cancer and underlying mechanism of *miR-101* functioning in bladder cancer progression.

However, there were still several limitations in the current study. Firstly, the sample size was relatively small. Secondly, the mechanisms underlying anti-tumor action of *miR-101* in bladder cancer was not investigated in the current study. In bladder cancer, there were several targets of *miR-101* confirmed in previous studies, including c-Met,[31] VEGF-C (vascular endothelial growth factor C),[18] and c-FOS.[32] Relevant researches might provide ideas for our further investigations.

In conclusion, the expression of serum *miR-101* is lower in bladder cancer patients than in healthy individuals. Serum *miR-101* may serve as a noninvasive biomarker for the diagnosis of bladder cancer. Further studies are needed to enhance our findings on *miR-101* value in the detection of bladder cancer.

## Author contributions

**Conceptualization:** Xiaoyan Chen.
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**Writing – original draft:** Xiaoyan Chen.
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