MiR-103 Assumes Signifying Capacity in Prostate Cancer Diagnosis

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Abstract

Background: Prostate cancer is one of the common cancers among males with high incidence and mortality. *MiR-103* has been reported to be involved in several human cancers. In our study, we aimed to explore the diagnostic value of *miR-103* in PCa.

Methods: In this study, qRT-PCR was performed to investigate the expression levels of *miR-103* in serum specimens collected from 141 PCa patients and 77 healthy volunteers. MTT and cell invasion assay were performed to detect the effects of *miR-103* on the proliferation and invasion of PCa cells. The diagnostic value of *miR-103* in PCa was evaluated by ROC curve.

Results: The results showed that serum *miR-103* levels were higher in PCa patients than that in healthy group ($P<0.001$). And the expression of *miR-103* was significantly associated with PSA ($P=0.007$), differentiation ($P=0.009$), and lymph node metastasis ($P=0.037$). Cell experiments in vitro demonstrated that *miR-103* could promote PCa cell line proliferation and invasion. In addition, ROC analysis suggested that *miR-103* could discriminate between PCa patients and healthy individuals at the cut-off value of 0.970. The AUC was 0.885, with the sensitivity of 97.2% and specificity of 81.8%.

Conclusions: The increased *miR-103* levels can promote proliferation and invasion of PCa cells. Serum *miR-103* is significantly associated with tumor progression, which may be a novel biomarker for PCa diagnosis.

Background

Prostate cancer (PCa) is one of the common malignant tumors in men worldwide [1, 2]. Although the cancer is not life threatening for most cases, PCa has become one of the leading causes for cancer-related death among male patients, especially in the developed countries [3, 4]. The low survival rate may be caused by high frequency of metastasis and recurrence [5]. Consequently, early detection is crucially important for PCa prognosis. At the present time, serum prostate-specific antigen (PSA) is the commonly used screening biomarker for PCa, which improves the early diagnosis of PCa. However, PSA is with low specificity for PCa, leading to over-diagnosis and over-treatment [6]. Therefore, the effective biomarkers with high sensitivity and specificity for early detection of PCa are urgently needed, which may significantly improve the survival rate of PCa patients.

MicroRNAs (miRNAs) are a group of small, non-coding RNAs which can inhibit translation or induce target mRNAs decay by binding to the 3'-UTR of their target mRNAs [7, 8]. Growing evidences prove that miRNAs take part in various biological processes, including tumorigenesis and diseases development [9, 10]. In the previous studies, a variety of miRNAs are confirmed to be related with cancers, such as *miR-301a* and glioma, *miR-21* and breast cancer, *miR-503* and colorectal cancer [11-13]. *MiR-103*, one of the cancer-related miRNAs, is a member of the *miR-103/107* family which is located on human chromosome 5 [14]. Related studies suggested that abnormal expression of *miR-103* was observed in several cancers, including endometrial cancer, colorectal cancer, gastric cancer and so on [14-16]. The effects of *miR-103*

on PCa were also reported in the previous studies. A study carried out by Singh et al. had indicated that high serum levels of *miR-103* predicted aggressive progression in patients who received radical prostatectomy [17]. However, the diagnostic significance of *miR-103* in PCa has been rarely reported in the previous studies,

In this study, we aimed to investigate the diagnostic value of *miR-103* in PCa patients. Serum level of *miR-103* in PCa patients was detected, as well as its association with clinical characteristics. In addition, receiver operating characteristic (ROC) curve was conducted to evaluate the diagnostic value of PCa patients.

Methods

Patients and specimens collection

Patients pathologically diagnosed with PCa were recruited in the study which was carried out in Zhongnan Hospital of Wuhan University. None of the patients received any chemotherapy or radiotherapy before specimens collection. In addition, healthy volunteers were collected as healthy control. Among the healthy group, no one had been diagnosed with any malignancy. 5mL blood specimens were collected from all the participants on the morning after fasting for 8-10h. The blood specimens were stored in tubes with EDTA and then centrifuged for 10min at 2500rpm. The up-stream was serum sample which was stored at -80°C until used.

Our study was approved by the ethics committee of Zhongnan Hospital of Wuhan University, and the written consents were obtained from all the participants before sampling. The clinicopathological features of the patients were recorded at the beginning of the study, including age, tumor size, PSA, differentiation, TNM stage, and lymph node metastasis.

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

MiRNAs were extracted from serum samples using mirVana™ PARIS™ kit (Invitrogen) according to the manufacturer's protocol. Then the first-strand cDNA was synthesized by TaqMan® MicroRNA Reverse Transcription Kit (Applied biosystems, Carlsbad, CA, USA). Relative expression of *miR-103* expression was measured by qRT-PCR which was performed with a SYBR Premix Ex Taq™ kit (Takara, Dalian, China). *U6* served as internal control. The primer sequences of *miR-103* and *U6* were as followed: *miR-103* forward: 5'-GAGCAGCATTGTACAG-3' and reverse: 5'-GTGCAGGGTCCGAGGT-3', *U6* forward: 5'-CTCGCTTCGGCAGCAC-3', reverse: 5'-AACGCTTCACGAATTTGCGT-3' [14]. The relative expression level of *miR-103* mRNA was normalized to *U6* and calculated by $2^{-\Delta\Delta Ct}$ method.

Cell culture and transfection

Human PCa cell line DU145 was purchased from American Type Culture Collection (ATCC, Manassas, VA). Then the DU145 cells were cultured in RPMI-1640 medium added with 100 IU/ml penicillin, 100

mg/ml streptomycin, 20mM glutamine and 10% heat-inactivated FBS at 37°C in a 5% CO₂ incubator.

MiR-103 mimics, miR-103 inhibitor and its negative control were purchased from Genepharma Co., Ltd. (Shanghai, China). DU145 cells were transfected with miR-103 mimic, miR-103 inhibitor or NC using Lipofectamine 2000 (Invitrogen, Life Technologies, CA) following the manufacturer's instructions. After transfection, qRT-PCR was applied to detect the relative expression of *miR-103* in the cells, to evaluate the effects of transfection. Then cells were harvested for proliferation and invasion analyses after cultured 48 h.

MTT assay

In order to investigate the effects of *miR-103* on DU145 cells proliferation, we performed MTT assay. After transfection, 200µl cell suspension were added into 96-well plates (10²-10³ cells/well) for continual 3-5 days in a 5% CO₂ incubator at 37°C. MTT assay was performed every 24 h. 20µl MTT (Sigma-Aldrich, 0.5 mg/ml) was added to each well, and incubated for 1h continually, and then resolved with 150µl DMSO (Sigma-Aldrich) in each well following low speed oscillation for 10 min. The absorbance values were measured at 570 nm with a spectrophotometer (Bio-Rad Laboratories, Hercules, CA). Every experiment was performed in triplicate.

Cell invasion assay

To measure cell invasion, transwell assay with matrigel was performed. Migration was diluted with serum-free medium (1:3), and then added 25µl migration into the upper well of the Transwell for 30min at 37°C. After digested, the transfected cells were resuspended with serum-free medium. 100µl 5×10⁴ transfected DU145 cells were seeded into Transwell chambers (Corning Inc., Corning, NY) and 500µl FBS were added into the lower chamber. Then the assay plates were incubated at 37°C, 5% CO₂. After 24 h, discard the medium, washed twice with FBS and then fixed with methanal for 30 min. The cells which did not invasive were erased with swab after stained with 0.1% crystal violet for 1 min. The cell number was counted under a microscope. Every experiment was select at least three visions.

Statistical analysis

In this study, all statistical analyses were performed with software of SPSS 19.0 and GraphPad Prism 5. The *miR-103* expression level was expressed as mean ± SD and analyzed by student's t tests. The association between serum *miR-103* expression and various clinicopathological characteristics were assessed using Chi-square tests. To determine the diagnostic performance of serum *miR-103* expression in PCa, the ROC curve was conducted. *P* values less than 0.05 were considered statistically significant.

Results

Demographic characteristics of the study subjects

In the present study, 141 PCa patients and 77 healthy individuals were collected in. The average age of the test group was 60.25 ± 21.03 years, which was similar with the control group. The clinical characteristics of the PCa patients were listed in Table 1.

Higher expression of *miR-103* in PCa patients

Serum levels of *miR-103* expression in PCa patients and healthy individuals were detected by qRT-PCR.. The results showed that *miR-103* expression was significantly higher in PCa patients than that in healthy volunteers ($P < 0.001$) (Figure 1).

Association between *miR-103* expression and clinical features in PCa patients

In this study, chi-square test was used to investigate the association between *miR-103* expression level and the clinicopathological data of PCa patients. The results suggested that *miR-103* expression was markedly associated with PSA ($P = 0.007$), differentiation ($P = 0.009$), and lymph node metastasis ($P = 0.037$). However, there was no significant association between *miR-103* levels and other clinical characteristics, such as age, tumor size, or TNM stage ($P > 0.05$ for all) (Table 1).

Effects of *miR-103* on PCa cells

To investigate the effects of *miR-103* on PCa cells, the transfected cells respectively specific to miR-103 mimic, miR-103 inhibitor and NC were conducted. *MiR-103* levels of transfected cells were detected and the results suggested that compared with NC cells, *miR-103* levels were markedly increased in miR-103 mimic cells, while the levels were significantly decreased in miR-103 inhibitor cell ($P < 0.05$ for both) (Figure 2a). The results revealed that we successfully conducted transfected cells.

MTT assay was carried out to evaluate the effects of *miR-103* on DU145 cells proliferation. The results indicated that after transfection for 48h, the growth rate were significantly increased in miR-103 mimic transfected cells than that in NC group, while the proliferation of miR-103 inhibitor group was markedly inhibited ($P < 0.05$ for all) (Figure 2b).

In addition, invasion analysis suggested that compared with NC cells, knock-down of *miR-103* expression could inhibit DU145 cells invasion. Moreover, over-expression of *miR-103* could promote cell invasion ($P < 0.05$ for both) (Figure 2c).

Diagnostic value of *miR-103* expression in PCa

ROC curve was conducted to evaluate the diagnostic significance of miR-103 in PCa patients. Analysis results indicated that *miR-103* could distinguish the PCa patients from the healthy individuals with the sensitivity of 87.2% and the specificity of 81.8%. The cut-off value was 0.970, and the AUC was 0.885 (Figure 3).

Discussion

PCa is a frequently diagnosed male malignant tumor [18]. Several factors may contribute to its high morbidity, including age, family history, race, and genetic alterations [19]. Although the etiology of PCa remains unknown, a variety of molecules are confirmed to be related with indication and development of PCa, which can be used for diagnostic and prognostic biomarkers for the cancer. A study carried out by Cai et al. demonstrated that dual-specificity phosphatase 5 (*DUSP5*) negatively regulated PCa progression, which could be a prognostic marker for the cancer [20]. A related study scheduled in vitro suggested that knock-down of NIMA-related kinase 2 (*NEK2*) could inhibit PCa cell proliferation, indicating *NEK2* was significantly associated with development of PCa [21]. All of the related studies revealed that genetic factors play important roles in PCa progression and their abnormal expression may serve as non-invasive biomarkers.

MiRNAs are highly conserved in species and act as important roles in various physiological and pathological processes, including developmental abnormalities, autoimmune diseases and cancers [22-24]. In the present study, we investigated the diagnostic value of *miR-103* in PCa. QRT-PCR was applied to detect the serum levels of *miR-103* in PCa patients and healthy individuals. Analysis results indicated that *miR-103* was up-regulated in PCa patients, compared with healthy controls. Moreover, the elevated levels were significantly associated with high PSA levels, poor differentiation, and positive lymph node metastasis. The results might suggest that *miR-103* was an oncogene in PCa, which could promote malignant development of the cancer. However, the mechanisms for *miR-103* regulating PCa were not investigated in the study, which was needed to be identified in the next study.

In the present study, cell experiments were carried out to evaluate the effects of *miR-103* on PCa cell lines in vitro. Our results showed that over-expression of *miR-103* could promote PCa cell proliferation and invasion, while knock-down of its expression would inhibit PCa cell growth. However, a related study carried out by Fu et al. held the different opinions [25]. In their study, *miR-103* was proved to be a tumor suppressor gene, which could inhibit PCa cell proliferation, invasion and promote apoptosis. Several factors might contribute to the differences, such as the cell types, detection tools, and analysis methods. Therefore, lots of studies were needed to investigate the effects of *miR-103* on different PCa cell lines based on the same experiment condition.

In the previous studies, several miRNAs were confirmed to serve as biomarkers for PCa. Wang et al. had reported that serum *miR-410-5p* level was significantly higher in PCa patients than that in the healthy controls and the further analysis suggested that *miR-410-5p* could serve as a diagnostic marker for PCa [26]. A related meta-analysis demonstrated that down-regulated *miR-145* predicted poor outcomes for patients diagnosed with PCa [27]. The study carried out by Sun et al. suggested that the decreased expression levels of *miR-128* in tissues and serum specimens of PCa patients were significantly associated with aggressive tumor progression, which might serve as a marker for PCa diagnosis and prognosis [28]. All of the studies suggested that various miRNAs were involved in the initiation and development of PCa, which could act as biomarkers for the cancer. In the current study, we investigated the diagnostic value of *miR-103* in PCa. Analysis results suggested that *miR-103* could distinguish the PCa patients from the healthy control with high sensitivity and specificity. However, the significance of

miR-103 used for PCa diagnosis in clinical practices was needed to be investigated in the further study with large sample size.

Conclusions

In conclusion, the elevated levels of *miR-103* can promote PCa cell proliferation and invasion, which may be significantly correlated malignant tumor progression. Moreover, serum *miR-103* may be a potential biomarker for PCa diagnosis.

List Of Abbreviations

Prostate cancer (PCa)

prostate-specific antigen (PSA)

MicroRNAs (miRNAs)

receiver operating characteristic (ROC)

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

dual-specificity phosphatase 5 (*DUSP5*)

NIMA-related kinase 2 (*NEK2*)

Declarations

Ethics approval and consent to participate

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

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

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

S.L., T.L. and X.R. conceived and designed the experiments; S.L., T.L. and X.R. conceived and performed the experiments; S.L., T.L. and X.R. prepared figures. S.L., T.L. and X.R. wrote the main manuscript text. All authors reviewed the manuscript.

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Table

Table 1.

Association of *miR-103* expression with clinicopathological features in PCa patients

Characteristics	Case,n=141	MiR-103 expression		χ ²	P values
		Low (n=80)	High (n=61)		
Age (years)					
≥65	72	40	32	0.084	0.772
<65	69	40	29		
Tumor size (cm)					
≥3	77	44	33	0.011	0.915
<3	64	36	28		
PSA (ng/ml)					
≤10	55	39	16	9.797	0.007
10-20	58	31	27		
>20	28	10	18		
Differentiation					
Well	36	27	9	9.393.	0.009
Moderate	61	35	26		
Poor	44	18	26		
TNM stage					
I+II	99	61	38	3.223	0.073
III+IV	42	19	23		
Lymph node metastasis					
negative	72	33	36	4.372	0.037
positive	69	47	25		

Figures

Figure 1

Serum miR-103 expression levels evaluated by qRT-PCR in PCa patients and healthy controls. Analysis results indicated that serum miR-103 levels were significantly higher in PCa patients than that in the healthy controls. ***: suggested $P < 0.001$.

Figure 2

Effects of miR-103 on PCa cells in vitro. a. Expression levels of miR-103 in DU145 cells after transfected with miR-103 inhibitor, miR-103 mimic, or NC. Analysis results suggested that compared with NC, miR-103 levels were significantly higher in miR-103 mimic transfected cells, while the levels were markedly lower in miR-103 inhibitor transfected cells. ***: indicated $P < 0.001$. b. The proliferation of DU145 cell line after transfection. MTT assay suggested that knockdown miR-103 would inhibit DU145 cells proliferation, which over-expression of miR-103 could promote DU145 cells growth. **: compared with NC at the same time point, $P < 0.01$. c. The invasion ability of transfected DU145 cell line. Transwell assay was performed to evaluate the invasion of the DU145 cell lines and the results demonstrated that miR-103 could promote DU145 cell line invasion. Knockdown of miR-103 expression could inhibit PCa cell lines invasion. ***: compared with NC, $P < 0.001$.

Figure 3

ROC analysis for the diagnostic value of serum miR-103 in PCa patients. MiR-103 could distinguish the PCa patients from healthy individuals at the cut-off value of 0.970. The AUC was 0.885, with the sensitivity of 87.2% and the specificity of 81.8%.