Clinical competence of serum miR-372 expression in diagnosing prostate cancer

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Abstract

Background: Prostate cancer (PCa) is considered as a serious healthy burden among males around the world. The limited diagnosis leads to poor outcomes of this malignancy. Aberrant expression of microRNAs (miRNAs) have been found in human cancers and play crucial roles in these malignant diseases. *MicroRNA-372* (*miR-372*), as a type of miRNAs, has ever been investigated in various cancers. The purpose of the present study was to assess the expression patterns and diagnostic value of *miR-372* in patients with PCa.

Methods: 134 PCa patients and 68 healthy individuals were included in this study. The serum expression of *miR-372* was examined by quantitative real-time PCR (qRT-PCR). To verify the diagnostic significance of *miR-372*, receiver operating characteristics (ROC) analysis was conducted for PCa patients.

Results: Downregulated *miR-372* expression was detected in serum samples collected from PCa patients compared with the healthy controls (P<0.001). The decreased expression of *miR-372* was influenced by the positive lymph node metastasis (P=0.016), high prostate-specific antigen (PSA) concentration (P=0.002) and advanced TNM stage (P=0.013). The ROC curve was obtained with an AUC value of 0.896. At the cutoff value of 2.855, the sensitivity and specificity were 82.8% and 87.3%, respectively, suggesting the high diagnostic accuracy of *miR-372*.

Conclusion: In summary, serum downregulated expression of *miR-372* could serve as an efficient diagnostic biomarker for patients with PCa.

Background

Prostate cancer (PCa) ranks the second leading cause of deaths due to malignancy for males in Western countries and United State [1]. It is considered as the first common type among all the tumors diagnosed for men with an increasing incidence and mortality in recent decades [2]. The abundant new diagnosed PCa individuals have poor quality of life [3]. Given the pain from PCa, various strategies have been conducted for the cancer treatment. Prostate-specific antigen (PSA) testing is considered as the most useful method to diagnose PCa till now [4]. However, the limited sensitivity and specificity for this method leads to controversy for the nonmalignant prostate diseases also perform high PSA concentration [5]. Besides, the inappropriate indication on aggressiveness usually causes the over treatment for some patients with PCa [6]. Considering total of the challenges in the diagnosis and treatment, focused efforts should been taken out on the identification of novel and more sensitive and specific biomarkers to meet the clinical requirements for PCa treatment.

Accumulated evidences demonstrate that various processes are involved in the tumorigenesis and progression of human cancers, including abundant genetic mutations, abnormal expression of genes and aberrant microRNAs (miRNAs) expression [7]. MiRNAs, widespread in eukaryotic cells, represent a series of endogenous single stranded small RNA molecules with approximately 22 nucleotides in length. A wide of clinical processes are influenced by miRNAs, such as differentiation, cell cycle, proliferation and

apoptosis [8]. Aberrant miRNAs expression levels are detected in lots of cancers including PCa, which have been proved to be involved in the tumor progression by regulating the expression of tumor oncogenes and suppressors [9–12]. As a member of the miRNAs, *microRNA-372* (*miR-372*) expression dysregulation has also been found in some malignancies, such as colorectal carcinoma and testicular germ cell cancer [13, 14]. The downregulated *miR-372* was detected in PCa serum samples, and was demonstrated to inhibit the migration and invasion of cancer cells in the study by Kong and his colleagues [15]. However, whether *miR-372* could perform any clinical significance for diagnosis of PCa has never been discussed.

In the present study, the diagnostic value of *miR-372* was evaluated and its serum patterns was also investigated in patients with PCa.

Methods

Patients and serum specimens

Total of the protocols in our study were approved by the Ethics Committee of Zhongnan Hospital of Wuhan University, and the written informed consents were all obtained from the participators and their families. This study include 134 PCa patients and 68 age and gender matched healthy individuals. The patients were all diagnosed with PCa in Zhongnan Hospital of Wuhan University with no history of other malignancies and had never received any therapy prior to sample collection. 68 healthy volunteers who received the routine physical examination in the hospital without any history of cancers were recruited as controls in this study. The venous blood samples were collected from the patients and healthy controls and stored in EDTA tubes. Serum specimens were obtained by using centrifugation from the blood samples and kept at -80 ℃ for subsequent utilization. The clinical data of patients were recorded in Table 1. The clinicopathological features included age, prostate size, Gleason score, lymph node metastasis, pathological stage, PSA concentration and TNM stage.

Table 1
Relationship between *miR-372* expression and clinicopathological features of PCa patients

Features	No.	*miR-372* expression		*P* values
	n = 134	Low (n = 75)	High (n = 59)	
Age (years)				0.431
≤ 65	34	21	13	
> 65	100	54	46	
Prostate size				0.667
Normal	79	43	36	
Hyperplastic	55	32	23	
Gleason score				0.326
≤ 7	64	33	31	
> 7	70	42	28	
Lymph node metastasis				**0.016**
Negative	66	30	36	
Positive	68	45	23	
Pathological stage				0.061
pT1-pT2	65	31	34	
pT3-pT4	69	44	25	
PSA concentration (ng/mL)				**0.002**
≤ 10	71	31	40	
> 10	63	44	19	
TNM stage				**0.013**
I-II	61	27	34	
III-IV	73	48	25	

PSA, prostate-specific antigen.

RNA extraction

Total RNAs including miRNAs were isolated from the serum specimens using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as per the instruction. To confirm the purity of RNA, NanoDrop ND-1000 (NanoDrop,

Wilmington, DE) was used to measure the absorbance at 260 nm and 280 nm, and the ratio of OD A260/A280 was calculated. Only the RNA with the ratio of close to 2.0 was permitted to be used in the subsequent experiments.

Quantitative real-time PCR (qRT-PCR)

The revers transcription was carried out to obtain the single stranded cDNA from RNA samples with the application of Transcriptor First Strand cDNA Symthesis Kit (Roche, Vilvoord, Brussel, Belgium). Expression of *miR-372* was examined by qRT-PCR, which was conducted with the SYBR green I Master Mix kit (Invitrogen). The obtained cDNA was amplified in these reactions in 7300 Real-Time PCR System (Applied Biosystems, USA). *U6* was adopted to act as the endogenous control gene to normalize the relative expression of *miR-372*. The final *miR-372* expression levels were computed with method of $2^{-\Delta\Delta Ct}$.

Statistical analysis

The comparison of *miR-372* expression levels between PCa patients and healthy controls was performed by Student's t-test. Chi-square test was adopted to analyze the relationship between *miR-372* expression and clinicopathological characteristics of PCa patients. The diagnostic performance of *miR-372* expression in patients with PCa was assessed with receiver operating characteristics (ROC) analysis. All these statistical analyses above were carried out by SPSS 18.0 software. Results were considered as statistically significance when the *P* value was less than 0.05.

Results

Serum expression patterns of *miR-372*

The expression of *miR-372* levels in 134 PCa patients and 68 healthy volunteers were examined by qRT-PCR. The results showed that *miR-372* expression was significantly downregulated in PCa serum samples than that in the healthy controls ($P < 0.05$, Fig. 1). This result suggested the potential tumor suppressor role of *miR-372* in PCa patients.

Association of *miR-372* expression with clinicopathological features of PCa patients

To verity whether there was involvement for *miR-372* with PCa progression, the relationship between *miR-372* expression and clinicopathological characteristics of cancer patients was assessed by Chi-square test. The mean *miR-372* expression value was used to classify the patients into low *miR-372* expression group (n = 75) and high expression group (n = 59). The clinicopathological data shown in Table 1 revealed that there were closely correlation between *miR-372* expression and lymph node metastasis ($P = 0.016$), PSA concentration ($P = 0.002$) and TNM stage ($P = 0.013$). However, the other parameters, including age, prostate size, Gleason score and pathological stage, were not found associated with *miR-372* expression (all $P > 0.05$).

Diagnostic performance of *miR-372* in patients with PCa

Considering the aberrant expression of *miR-372* in PCa serum specimens, the clinical significance of *miR-372* was also analyzed for cancer patients. In the current study, ROC curve was constructed to confirm the diagnostic value of *miR-372.* The results shown in Fig. 2 revealed that the AUC value was 0.896 and the sensitivity and specificity were 82.8% and 87.3% at the cutoff value of 2.855. These data indicated that *miR-372* could be used as a high sensitive and specific diagnostic biomarker of PCa patients.

Discussion

PCa represents a serious healthy problem among men, which ranks the most common malignancy diagnosed in males around the world [16]. It is characterized by its high rates of mortality and complex biological heterogeneity [17]. Since the specific characteristics of PCa, clinical challenges are presented to identify the cancer patients from healthy individuals. Despite the PSA has been widely used in diagnosis and prognosis of PCa, the outcomes of cancer patients remain dismal mainly due to the limitations of the available diagnostic methods [18]. Thus, it is an urgent need to find novel and efficient diagnostic biomarkers for patients suffering from PCa. Current studies have highlighted the utilization of tumor related molecules for their diagnostic significance to improve the diagnosis of diverse cancers, and results in these studies demonstrated the pivotal role of diagnostic biomarker for cancer treatment [19, 20].

Among these diagnostic factors, miRNAs are considered as a class of crucial members, which have been investigated in a great deal of malignancies [21]. Previous data revealed that various biological processes, such as proliferation, metastasis, invasion and apoptosis, can be regulated by miRNAs, indicating their potential function in cancer progression [22]. The aberrant expression patterns of miRNAs have been found in lots of cancers, which suggested their role of tumor oncogene or suppressor [23, 24]. As a member of miRNAs, *miR-372* has ever been assessed in different human cancers [25, 26]. For example, *miR-372* was demonstrated to be correlated with proliferation and metastasis of hepatocellular carcinoma [27]. Study by Chen and his colleagues revealed that *miR-372* could regulate cancer cell invasion and proliferation of glioma according to target *PHLPP2* [28]. Given the results of previous studies, rarely data has been reported about clinical significance of *miR-372* for patients with PCa. Therefore, we aimed at to explore the diagnostic performance of *miR-372* via measuring its serum expression levels and various statistical analyses in PCa patients.

In the present study, the expression of *miR-372* in the serum specimens collected from the PCa patients were examined by qRT-PCR. Student's t test was adopted to compare the different *miR-372* expression between the two groups. According these analyses, the downregulated *miR-372* expression was detected in PCa serum specimens than that in the healthy controls. Furthermore, the relationship of *miR-372* expression with clinicopathological characteristics of PCa patients was assessed with Chi-square test. The results revealed that there were closely correlation between *miR-372* expression and lymph node metastasis, PSA concentration and TNM stage. Conversely, no influence was found for other parameters.

The similar data has also been found in the previous study by Kong et al., which also found the downregulated expression of *miR-372* in PCa serum samples [15]. Our results might suggested that *miR-372* represented a potential tumor suppressor for PCa and was involved in the cancer progression. Since the expression of *miR-372* was abnormal in PCa serum and its potential functional role has ever been demonstrated in cancer cells, we further explored its clinicopathological significance in diagnosis of PCa through ROC analysis. The results suggested that *miR-37*2 expression was a high sensitive and specific diagnostic biomarker to distinguish prostate cancer cases from healthy individuals.

Although we have investigated the expression pattern and diagnostic value of *miR-372* in PCa patients, more further studies are still needed to confirm our findings. In the study of Yu et al., they showed that serum or tissue *miR-372* levels were significantly up-regulated in CRC patients and it could be a noninvasive biomarker for the early detection and prognosis of CRC [29]. Therefore, the role of *miR-372* might be varying in different cancers.

Conclusion

Taken together, all data in our study demonstrated the downregulated expression of *miR-372* was involved in the progression of PCa, and had high diagnostic value for patients with PCa. The molecular mechanisms of *miR-372* acting in PCa remain further studies.

Abbreviations

Prostate cancer (PCa)

microRNAs (miRNAs)

MicroRNA-372 (miR-372)

quantitative real-time PCR (qRT-PCR)

receiver operating characteristics (ROC)

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.

Consent for publication

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

Data availability

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

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Funding information is not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

S.L. design of the work; X.R. the acquisition, analysis, T.L. interpretation of data; S.L. the creation of new software used in the work; S.L., X.R., T.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

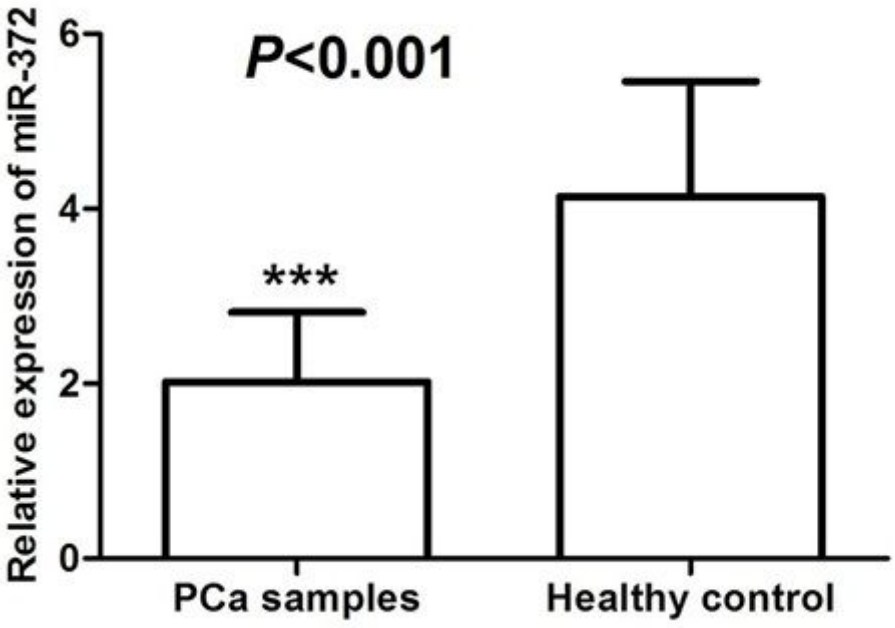

Figure 1

Serum expression of miR-372 in PCa patients and healthy controls. Decreased miR-372 expression was found in PCa serum specimens compared with the healthy controls (P<0.001).

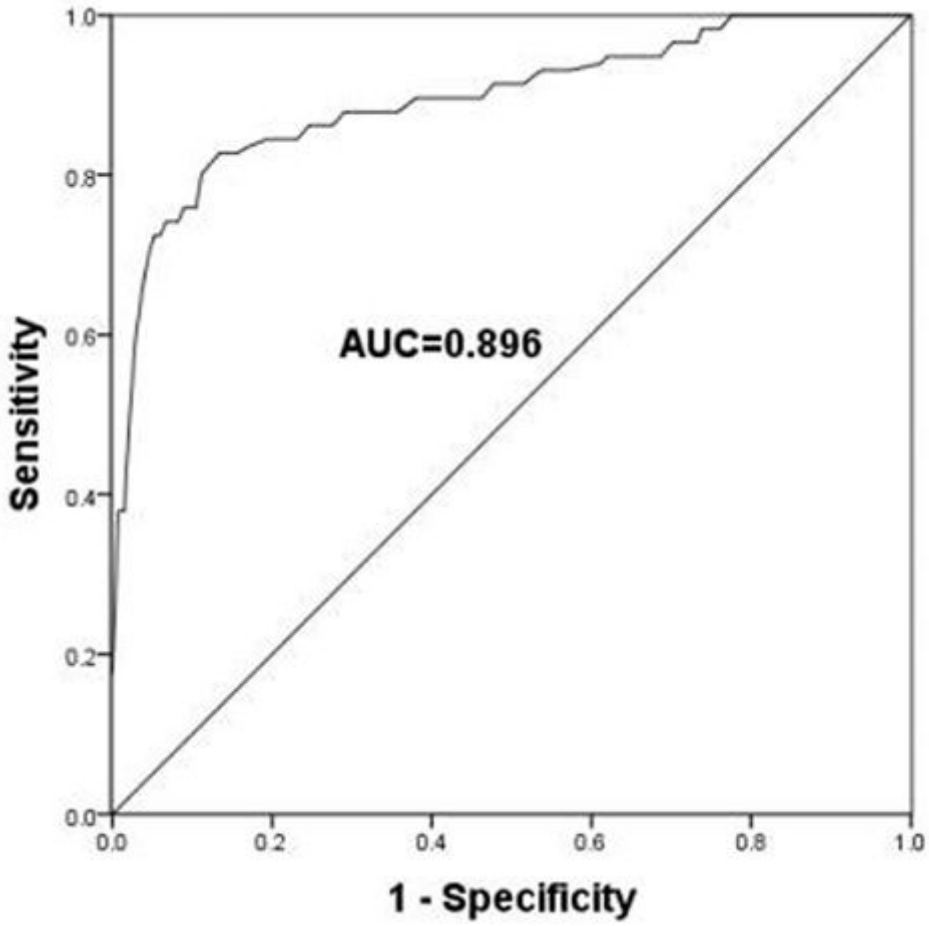

Figure 2

ROC curve based on the expression of miR-372. The AUC value of 0.896 suggested the high diagnostic accuracy of miR-372 in patients with PCa.