

Original Article

Plasma miR-302b is a promising biomarker for diagnosis of acute myocardial infarction

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Abstract: Alteration in expression of circulating microRNAs (miRNAs) has been shown to be involved in the initiation and development of cardiovascular diseases. The purpose of current study aimed to determine whether plasma miR-302b can be used as novel indicators for detection of acute myocardial infarction (AMI). Plasma samples from 76 patients with AMI and 30 healthy adults were collected. Quantitative real-time PCR and ELISA assays were used to evaluate the expression level of plasma miR-302b and conventional biomarkers, respectively. Receiver-operator characteristic curve (ROC) analysis was conducted to evaluate the diagnostic ability of plasma miR-302b for AMI. The expression level of plasma miR-302b was significantly upregulated in AMI patients ($P<0.01$). In addition, plasma miR-302b expression level was positively correlated with cardiac troponin I (cTnI, $r=0.9189$, $P<0.0001$), creatine kinase (CK, $r=0.7902$, $P<0.0001$) as well as CK-MB ($r=0.7858$, $P<0.0001$). ROC analysis indicated that plasma miR-302b had a high accuracy in diagnosing AMI (AUC=0.952). Taken together, plasma miR-302b was significantly upregulated in AMI, indicating that it may represent a novel and promising biomarker for early diagnosis of AMI.

Keywords: Acute myocardial infarction, miR-302b, plasma

Introduction

Acute myocardial infarction (AMI) remains one of the leading causes of morbidity and mortality worldwide [1]. Diagnosis of AMI at an early stage with high accuracy is crucial to minimizing myocardial injury and preserving heart function [2]. In addition to clinical symptoms and electrocardiographic findings, biomarkers associated with myocardial necrosis such as cardiac troponins (cTnI/cTnT) and creatine kinase are commonly used for detection of AMI [3, 4]. However, these biomarkers are not specific enough for AMI from other diseases such as chronic kidney failure and septic shock [5, 6]. Therefore, it is necessary and important to explore more sensitive and specific novel biomarkers for the diagnosis of AMI.

MicroRNAs (miRNAs) are short (approximately 19-25 nucleotides long), single-stranded RNA molecules that regulate gene expression at the posttranscriptional level by binding to 3'UTR of mRNA [7]. MiRNAs have been shown to play an

important role in a wide range of biological processes such as cell cycle control, proliferation, differentiation, survival and migration [8]. Deregulation of miRNAs involve in the initiation and progression of many diseases including cardiovascular diseases [9, 10]. Recent studies showed that miRNAs are highly stable in the body fluids such as serum and plasma, indicating that circulating miRNAs might be used as biomarkers for early detection as well as monitoring the progression of the diseases [11]. Wang et al recently the expression level of plasma miR-19b-3p, miR-134-5p and miR-186-5p were significantly increased in early stage of AMI. In addition, all three miRNAs have a positive correlation with cTnI and high accuracy in distinguishing between AMI and controls, suggesting miR-19b-3p, miR-134-5p and miR-186-5p might play an important role in the pathogenesis of AMI and serve as promising biomarkers [12]. Similarly, miR-1 level was significantly increased in plasma from AMI patients compared with the controls and its level returned to normal range following therapy. In addition, increased circulating miR-1 was correlated with QRS and had high efficiency in separation of

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Table 1. Clinical characteristics and parameters of study population

Parameter	Control group (n=30)	AMI group (n=76)	P
Age	62.3 ± 10.5	63.6 ± 11.7	0.56
Gender (Male/Female)	19/11	48/28	0.99
Smoking history (Yes/No)	14/16	45/31	0.24
Alcohol history (Yes/No)	15/15	34/42	0.62
HDL C (mmol/L)	1.28 ± 0.25	1.05 ± 0.32	0.28
LDL C (mmol/L)	2.45 ± 0.39	2.71 ± 0.41	0.09
TG (mmol/L)	1.42 ± 0.34	1.48 ± 0.29	0.78
TC (mmol/L)	4.55 ± 0.54	4.28 ± 0.47	0.62
SBP (mmHg)	115 ± 18	132 ± 26	0.16
DBP (mmHg)	81 ± 11	83 ± 13	0.69
Cr (umol/L)	55 ± 29	82 ± 42	0.15
DM (Yes/No)	1/29	8/68	0.23
Hypertension (Yes/No)	9/21	32/44	0.25

DM, diabetes mellitus; TC, total cholesterol; HDL C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL C, low-density lipoprotein cholesterol; Cr, creatinine; TG, total triglyceride.

AMI and non-AMI patients as well as AMI patients under hospitalization and discharge [13].

Although the expression level of miR-302b was significantly increased in the tissue samples with myocardial infarction [14], its plasma expression level and potential clinical significance remains largely unknown. Therefore, the aim of the present study was to compare the plasma levels of miR-302b between patients following AMI and healthy subjects, and evaluate its usefulness as a biomarker for AMI detection.

Materials and methods

Study population and sample collection

Seventy-six patients with AMI and thirty healthy adults were enrolled in this study. All subjects or their guardians gave written informed consent and the study was approved by the ethics committee of the Hunan Provincial People's Hospital. AMI was diagnosed based on combination of several parameters: clinical symptoms plus electrocardiographic changes, elevated plasma levels of markers of cardiac necrosis biomarkers (cTnI, CK and CKMB). Up to 5 ml whole blood was drawn from the participants. Then the cells are removed from plasma by centrifugation at 2,000×g for 10 minutes at

4°C. The plasma samples were stored at -80°C until use.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

About 2 mL of plasma was used for the miRNA analysis. Total RNA was extracted from plasma samples using the mirVana PARIS kit (Ambion, Applied Biosystem, Foster City, USA). The synthesis of first strand cDNA was performed with the miRcute miRNA First-Strand cDNA Synthesis Kit (Tiangen, Beijing, China). The miRcute miRNA qPCR Detection kit (SYBR Green) was used in real-time PCR and the amplification process was performed using the LightCycler 480® II real-time PCR System (Roche Diagnostics, Penzberg, Germany). U6 RNA was used as a miRNA internal control and the $2^{-\Delta\Delta Ct}$ method was used to analyze the relative expression of miRNA.

Plasma cTnI/CK/CK-MB determination

The concentrations of plasma cTnI/CK/CK-MB were measured by ELISA assay according to the manufacturer's protocol (Beckman Coulter, USA).

Statistical analysis

All data were represented as the mean ± standard deviation (SD). As the data were subjected to normal distribution, the expression levels of plasma miR-302b in patients with AMI and the control subjects were compared using unpaired two sample Student t test. Pearson's correlation coefficient was performed to evaluate the association between plasma miR-302b and conventional biomarkers. Receiver operating characteristic (ROC) curves and the area under the ROC curves (AUC) were used to determine the efficiency of plasma miR-302b to discriminate AMI patients from control subjects. SPSS 21.0 (Chicago, IL, USA) was used to perform the statistical analyses and a P value less than 0.05 was considered to be statistically significant.

Results

Statistical analysis of patients' characteristics

No statistical differences were found between the control group and the AMI patients for any of the considered variables including LDL cholesterol, total cholesterol, total triglycerides,

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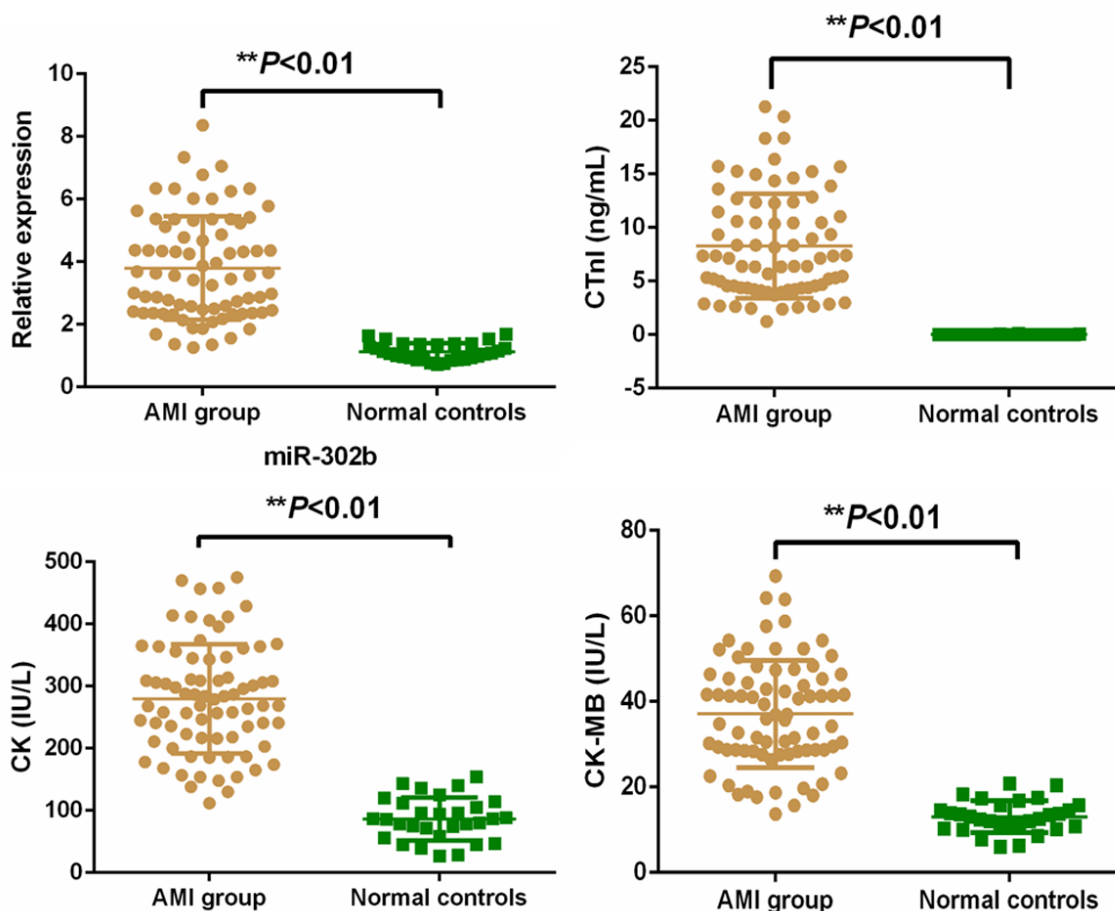


Figure 1. Expression level of miR-302b, cTnI, CK and CK-KB between patients and normal controls.

HDL cholesterol, systolic blood pressure, diastolic blood pressure, creatinine, diabetes, alcohol history and smoking status (**Table 1**).

Expression levels of plasma miR-302b and traditional biomarkers in AMI patients and the controls

Our results showed that the expression level of plasma miR-302b was significantly increased in patients with AMI in comparison with control subjects. The expression of circulating miR-302b in AMI patients relative to the controls was upregulated by 3.80 ± 1.64 fold ($P < 0.01$). Similarly, the concentrations of cTnI, CK and CK-MB were significantly increased in AMI patients compared to the controls ($P < 0.01$) (**Figure 1**).

Association between plasma miR-302b and traditional biomarkers

We then aimed to investigate whether there was any relationship between plasma miR-

302b and traditional biomarkers. A significantly positive correlation was found between plasma miR-302b and cTnI ($r = 0.9189$, $P < 0.0001$) (**Figure 2**). Similarly, plasma miR-302b expression level also highly correlated with CK ($r = 0.7902$, $P < 0.0001$) as well as CK-MB ($r = 0.7858$, $P < 0.0001$) concentrations.

Diagnostic value of plasma miR-302b for AMI

Receiver-operator characteristic (ROC) curve analysis was performed to evaluate the predictive power of circulating miR-302b levels for AMI. The results revealed the plasma miR-302b could distinguish AMI patients from healthy volunteers with high specificity (93.3%) and sensitivity (88.2%) with an area under curve (AUC) of 0.952 (95% CI, 0.892-0.984) (**Figure 3**).

Discussion

Early detection of AMI with high accuracy is extremely important because it not only imp-

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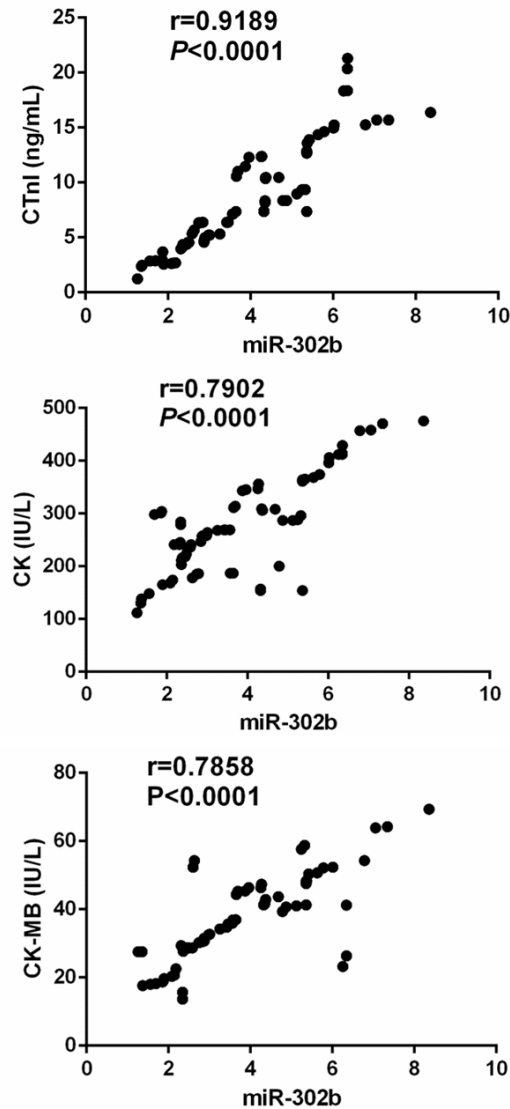


Figure 2. Correlation between plasma miR-302b and conventional biomarkers including CTnl, CK and CK-MB.

roves the clinical outcome but also reduces the total medical costs when treating patients. The commonly used method for diagnosis of AMI is the combination of physical examination together with electrocardiogram and the measurement of cardiac biomarkers [15]. However, this approach has low sensitivity and specificity for the diagnosis of AMI for some patients. Recently miRNAs have been demonstrated to be promising biomarkers for many diseases including cardiovascular disease [16].

In the present study, our results revealed that plasma miR-302b expression was significantly

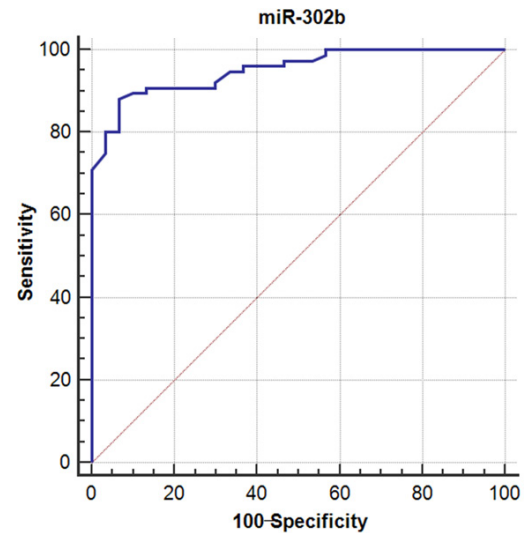


Figure 3. Diagnostic value of plasma miR-302b in AMI.

increased in patients with AMI patients. In addition, plasma miR-302b expression level was positively associated with the concentrations of conventional biomarkers. Moreover, ROC analysis indicated that plasma miR-302b had a high accuracy in diagnosing AMI. Thus, plasma miR-302b might play an active role in the initiation and progression of AMI, and is a promising biomarker for detecting AMI. Consistent with our findings, Wang et al compared the differentially expressed tissue RNAs and miRNAs between AMI and non-AMI patients by profiling genome-wide transcripts and miRNAs. miR-302b was found to be significantly increased in the infarcted heart tissues, suggesting it might involve in the pathogenesis of AMI [14]. Chen et al showed that overexpression of miR-302b could enhance the all-trans retinoic acid mediated cytotoxicity, leading to glioblastoma cell apoptosis and death. Contrary results were also found when miR-302b expression level was downregulated. In addition, E2F3 was identified as a potential target of miR-302b, indicating the miR-302b might play an important role in regulating the apoptosis process [17]. Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant hereditary neuromuscular disorder associated with chromatin relaxation of the D4Z4 macrosatellite array on chromosome 4. Apoptosis of muscle cells is increased in patients with FSHD. Dmitriev et al compared the differentially expressed miRNAs between

FSHD and normal myoblast. miR-302b expression level was significantly upregulated in FSHD myoblast compared with the controls, indicating miR-302b might involve in the pathogenesis of FSHD by increasing the apoptosis of muscle cells [18]. We propose that the increased miR-302b expression in patients with AMI can lead to the apoptosis of cardiac myocytes.

The limitations of this study are the following. First, the sample size was relative small, and a large sample size is needed for further corroborating the clinical significance of plasma miR-302b for detection of AMI in the future. Second, currently no direct evidence is available to demonstrate that miR-302b involves in the initiation and progression of AMI. Therefore, elucidating the molecular mechanisms accounting for the increase of miR-302b during the pathogenesis of AMI is important and necessary.

In conclusion, this study has shown that the plasma miR-302b level was significantly increased in AMI patients. In addition, the level of plasma miR-302b was positively correlated with concentrations of conventional AMI biomarkers. Moreover, plasma miR-302b could discriminate AMI patients from normal subjects with high accuracy. Our findings suggest plasma miR-302b might serve as a novel biomarker for detection of AMI.

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Disclosure of conflict of interest

None.

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