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Serum microRNA-302b: the novel biomarker for diagnosis of acute myocardial infarction

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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]. However, these are not specific enough for diagnosing AMI from other diseases, such as chronic kidney failure and septic shock [4]. Therefore, it is necessary and important to explore more sensitive and specific novel biomarkers for the diagnosis of AMI.

MicroRNAs (miRNAs) are short (19–25 nucleotides), single-stranded RNA molecules that regulate gene expression at the post-transcriptional level by binding to 3'UTR of mRNA [5]. Several studies have identified miRNAs as potential biomarkers for cancer diagnosis, prognosis and therapy [6–8]. Wang et al. recently noted that plasma miR-19b-3p, miR-134-5p and miR-186-5p levels are significantly increased in early stages of AMI, correlated positively with cTnI and had high accuracy in distinguishing between AMI and controls, suggesting they might play an important role in the pathogenesis and serve as promising biomarkers [9]. Similarly, plasma miR-1 was significantly increased in AMI patients compared to controls and its level returned to normal following therapy. In addition, increased circulating miR-1 correlated with QRS changes and had high efficiency in separation of AMI and non-AMI patients as well as AMI patients under hospitalization and discharge [10].

Although the expression level of miR-302b is significantly increased in the tissue samples following myocardial infarction [11], its plasma 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 so evaluate its usefulness as a biomarker for AMI detection.

Seventy-six patients with AMI and 30 healthy adults were enrolled. All healthy adults, who had the same exclusion criteria and no history of any cardiovascular disease, came from the medical examination centre of Central Hospital of Linyi. Patients >18 years of age presenting to the emergency department with symptoms suggestive of AMI with an onset or peak within the last 24 h were recruited after providing written informed consent. AMI was diagnosed based on combination of clinical symptoms, electrocardiographic changes and elevated plasma levels of markers of cardiac necrosis (cTnI, CK and CKMB). All subjects gave written informed consent and the study was approved by the ethics committee of the Central Hospital of Linyi.

Blood samples were collected in EDTA-K2 tubes and processed within 1 h of collection by centrifugation at 1200 g for 10 min at 4 °C. Supernatants were transferred to microcentrifuge tubes, followed by a second centrifugation at 12,000 g for 10 min at 4 °C. The supernatants were transferred to RNase-free tubes and stored at −80 °C. Total RNA was extracted from supernatants samples using the mirVana PARIS kit (Ambion, Applied Biosystem, Foster City, USA). About 2 µg of total RNA was reverse transcribed into cDNA using random primers and miRcute miRNA First-Strand cDNA Synthesis Kit (Tiangen, Beijing, China) at 37 °C for 120 min. Afterwards, real-time RT-PCR was performed, using 1µL of cDNA product, mir-302b LNA™ primers (Exiqon, Denmark) and SYBR Green master mix (Exiqon, Denmark). The U6 sn-RNA gene was used as an internal control. PCR reactions were conducted at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min using the LightCycler 480® II real-time PCR System (Roche Diagnostics, Penzberg, Germany). All real-time PCR reactions were performed in duplicate. To ensure that the RNA samples were not

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Table 1. Clinical and demographic characteristics of the subjects.

Parameter	Controls ($n = 30$)	AMI ($n = 76$)	P
Age	62.3 ± 10.5	63.6 ± 11.7	0.56
Male/Female	19/11	48/28	0.99
Smoking (Yes/No)	14/16	45/31	0.24
HDL C (mmol/L)	1.31 ± 0.25	1.15 ± 0.32	0.26
LDL C (mmol/L)	2.45 ± 0.39	2.71 ± 0.41	0.09
TG (mmol/L)	1.42 ± 0.34	1.76 ± 0.48	0.29
TC (mmol/L)	4.67 ± 0.56	4.48 ± 0.53	0.42
SBP (mmHg)	115 ± 18	132 ± 26	0.16
DBP (mmHg)	81 ± 11	83 ± 13	0.69
DM (Yes/No)	1/29	8/68	0.23
miR-203b*	1.06 ± 0.08	3.80 ± 1.64	0.0027
cTnI(ng/mL)	0.06 ± 0.01	8.63 ± 2.18	<0.001
CK(IU/L)	40.5± 17.5	286.7 ±60.4	<0.001
CK-MB(IU/L)	38.4 ± 9.52	161.2 ± 3.18	<0.001

Notes: 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. *relative expression.

contaminated with genomic DNA, a no reverse transcriptase control (no RT) was included during each run of real-time RT-PCR. Furthermore, to check the accuracy of amplifications, we included a negative control in each run by eliminating the cDNA sample in the tube. miR-302b specific primer (F: 5′- ATCCAGTGCGTGTCGTG-3′, 5′- TGCTTAAGTGCTTCCATGTT-3′) and U6 specific primer(5′- TGCGGGTGCTCGCTTCGGCAGC-3′, 5′- CCAGTGCAGGGTCCGAGGT-3′) were purchased from Qiagen (Shanghai, China). The thresholds were manually selected at the lowest point of each amplification curve to obtain threshold cycle (Ct) values of the reaction tube. Data were analyzed using $2^{-\Delta\Delta Ct}$ method, $2^{-\Delta\Delta Ct}$represents ratios of gene expression between the experimental group and the control group, expressed as the following formula: $\Delta\Delta Ct = [Ct_{(\text{target gene})} - Ct_{(\text{reference gene})}]$ experimental group $- [Ct_{(\text{target gene})} - Ct_{(\text{reference gene})}]$ the control group. The concentrations of plasma cTnI/CK/CK-MB were measured by ELISA assay according to the manufacturer's protocol (Beckman Coulter, USA).

All data were represented as the mean ± standard deviation (SD) and compared using 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 ability of plasma miR-302b to discriminate AMI patients from control subjects**.** A multivariate logistic regression analysis (MVLRA) was performed to evaluate the diagnostic accuracy of circulating serum markers. SPSS 21.0 (Chicago, IL, USA) was used to perform the analyses. $P < 0.05$ was considered significant.

No statistical differences were found between the control group and the AMI patients for any risk factors. The level of plasma miR-302b, cTnI, CK and CK-MB were significantly increased in AMI patients compared to the controls (Table 1). A significantly positive correlation was found between plasma miR-302b and cTnI ($r = 0.92$ $P < 0.0001$) (Figure 1). Similarly, plasma miR-302b also highly correlated with CK and CK-MB (both $r = 0.79$, $P < 0.0001$).

ROC curve analysis was performed to evaluate the predictive power of circulating miR-302b, cTnI, CK and CK-MB levels for AMI. Plasma miR-302b, cTnI, CK and CK-MB distinguished AMI patients from healthy volunteers with specificity 93.3,89.4, 76.6 and 81.4% and a sensitivity of 88.2,95.3, 73.1, and 80.9% with an area under curve (AUC) of 0.95 (95% CI, 0.89–0.98, $P = 0.0012$), 0.91 (95% CI, 0.80–0.94, $P = 0.002$), 0.72 (95% CI, 0.63–0.90, $P = 0.02$) and 0.82 (95% CI, 0.73–0.92, $P = 0.006$), respectively. These data suggested miR-302b and cTnI have the highest sensitivity and specificity. Univariate analysis revealed that CK-MB, cTnI and miR-203b were significantly associated with AMI ($P < 0.001$). In a MVLRA, only miR-302b levels were significantly independently diagnostically associated (HR, 2.46; 95% CI, 1.66–3.65; $P = 0.002$).

Early detection of AMI with high accuracy is extremely important because it improves the clinical outcome. AMI is commonly diagnosed by the combination of physical examination, electrocardiogram changes and cardiac biomarkers [12]. However, this approach has less than perfect sensitivity and specificity for the diagnosis of AMI. Recently miRNAs have been demonstrated to be promising biomarkers for many diseases including cardiovascular disease [6–10,13].

Our results show that plasma miR-302b levels are significantly increased in patients with AMI, and positively associate with conventional biomarkers. Moreover, ROC analysis indicated that plasma miR-302b had a high accuracy in diagnosing AMI. Thus, we speculate that 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 be involved in the pathogenesis of AMI [11]. 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. In addition, E2F3 was identified as a potential target of miR-302b, indicating the latter might play an important role in regulating apoptosis [14]. 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 be involved in the pathogenesis of FSHD by increasing the apoptosis of muscle cells [15]. We propose that the

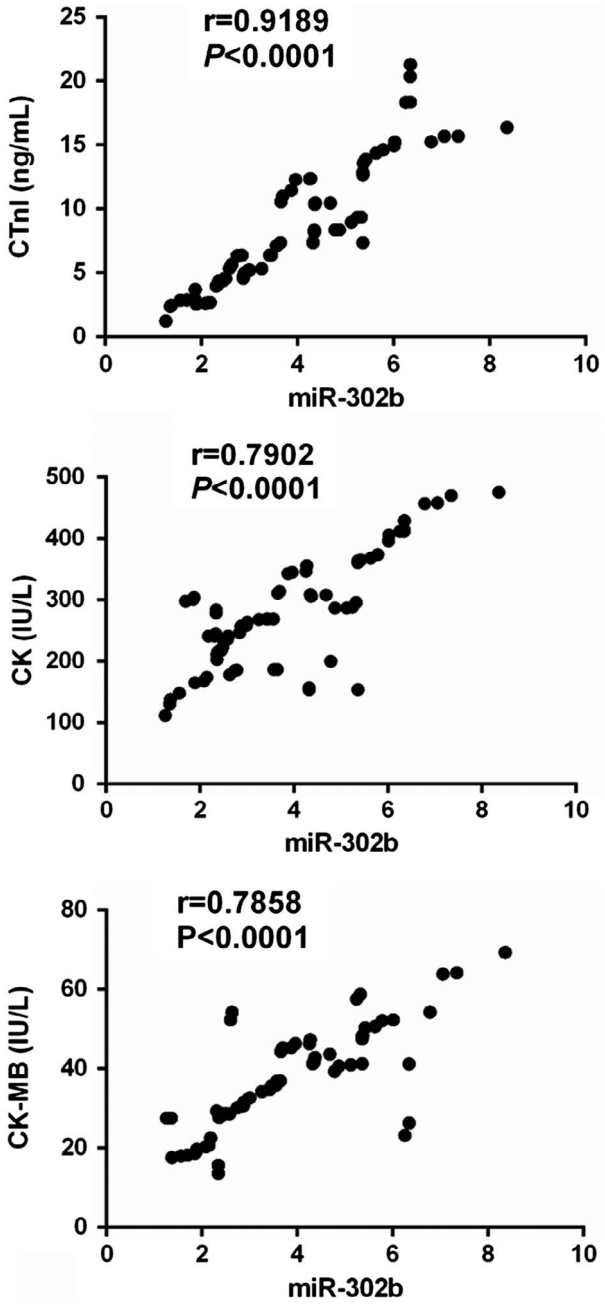

Figure 1. Correlation between relative expression of plasma miR-302b and CTnI.

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. Secondly, currently no direct evidence is available to demonstrate that miR-302b is involved 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.

This work represents an advance in biomedical science because it shows that plasma miR-302b discriminates AMI patients from normal subjects with high accuracy, and so may represent a novel and promising biomarker for early AMI diagnosis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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