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Circulating miR-23b as a Novel Biomarker for Early Risk Stratification After ST-Elevation Myocardial Infarction

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Study Design A
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Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: miR-23b overexpression can promote cardiomyocyte apoptosis and reduce cell growth under hypoxic conditions, suggesting that miR-23b acts as a biomarker for ST-elevation myocardial infarction (STEMI). The aim of this study was to investigate the effect of miR-23b on STEMI patients.





Material/Methods: We enrolled 80 eligible patients with STEMI and 60 control subjects. Blood samples were obtained at 6 h, 12 h, 24 h, 48 h, 3 days, and 7 days after the onset of symptoms. Another blood sample was collected before and after percutaneous coronary intervention (PCI). The samples were used for real-time quantitative PCR analysis. A Siemens Immulite2000 detector (Germany) was used for cTnI detection, and the serum CK-MB content was detected by electrochemical luminescence method.

Results: The expression level of miR-23b was increased in patients with STEMI ($P < 0.05$). No significance difference was observed among risk factors, although the clinical data was comparable ($P > 0.05$). The level of miR-23b in STEMI patients after PCI was lower ($P < 0.05$). The ROC curve of plasma miR-23b showed a separation, with an AUC of 0.809 (95%CI, 0.737–0.936, $P < 0.05$), compared to CK-MB with an AUC of 0.753 (95%CI, 0.707–0.896) and cTnI with an AUC of 0.783 (95%CI, 0.723–0.917).

Conclusions: The present study reveals that miR-23b is a useful biomarker of STEMI, providing a novel insight for the diagnosis for STEMI.

MeSH Keywords: Anterior Wall Myocardial Infarction • Creatine Kinase, MB Form • MicroRNAs • Troponin I

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Background

Acute myocardial infarction (AMI) is a severe cardiovascular disease caused by acute artery occlusion, characterized by high mortality and poor prognosis [1]. The diagnosis of patients with ST-elevation myocardial infarction (STEMI) mainly depends on clinical manifestation and myocardial enzyme changes, among which the level of troponins (cTns) is widely regarded as the criterion standard [2]. However, previous studies show that cTns is significantly increased in the treatment of various diseases [3,4]. Given that the specificity of cTns for STEMI is not sufficient, it is of great importance to seek more targeted STEMI diagnostic markers with higher sensitivity and specificity.

MicroRNAs are a class of highly conservative non-coding small RNAs (about 21–24 nucleotides) that regulate gene expression in signal pathways, which play an important role in maintaining the homeostasis [5]. It regulates post-transcriptional gene silencing, promotes mRNA degradation or inhibits the transcription by recognizing the 3'UTR of target mRNA, thus controlling protein expression level, which participates in a series of reactions such as cell differentiation, metabolism, proliferation, and apoptosis [6].

He et al. found that miR-23b overexpression promotes cardiomyocyte apoptosis and reduces cell growth under hypoxic conditions [7], suggesting that miR-23b is a biomarker for STEMI. Thus, we aimed to investigate the level of miR-23b in STEMI patients and to characterize miR-23b as a novel biomarker for STEMI.

Material and Methods

Patients

We enrolled 80 eligible patients with STEMI and 60 control subjects from January 2015 to January 2017 at The First Hospital of Hebei Medical University. All STEMI patients were first diagnosed by percutaneous coronary intervention (PCI). All participants provided informed consent, and this study was approved by the Ethics Committee of The First Hospital of Hebei Medical University.

Samples collection

Blood samples were obtained at 6 h, 12 h, 24 h, 48 h, 3 days, and 7 days after the onset of symptoms, and another blood sample was collected at 24 h before and after PCI. The samples were centrifuged at 10 000 g for 15 min within 2 h of collection, followed by plasma separation and freezing at -80°C .

Real-time quantitative PCR analysis

The total RNA was isolated from the plasma by use of TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocols, then we assessed the quality and quantity of RNA using NanoDrop (NanoDrop Products, Wilmington, Del., USA). cDNA was synthesized from 50 ng of total RNA using an miRCURY LNA Universal cDNA synthesis kit (Exiqon, Vedbaek, Denmark). The expression level of miR-23b in plasma was determined by qRT-PCR, which was carried out in triplicate reactions with the SYBR Green PCR SuperMix Kit (TransGen Biotech, Beijing, China). The primer of miR-23b was ATCACATTGCCAGGGATTA.

CK-MB, cTnI detection

The Siemens Immulite2000 detector (Germany) was used for cTnI detection and the serum CK-MB content was detected by electrochemical luminescence method. Results were subjected to statistical analyses.

Statistical analysis

The data were analyzed using SPSS17.0 software and GraphPad. Normal distribution measurement data was calculated as the mean \pm SD, and the *t* test was used for comparison between variable groups. Categorical variables were compared using the chi-square test. The correlation analysis of continuous variables was based on the Spearman test correlation method. The diagnostic efficacy of the plasma miR-23b content was analyzed with the ROC curve, and the area under the ROC curve (AUC) was calculated. $P < 0.05$ was regarded as statistically significant.

Results

The expression level of miR-23b was increased in patients with STEMI

In this study, we selected 80 STEMI patients and 60 healthy volunteers. The baseline clinical characteristics of the patients and control groups are shown in Table 1. There were no significant differences between STEMI patients and controls in the baseline clinical data, including age, sex, blood pressure, body mass index, and other biochemical parameters. Plasma miR-23b expression levels detected immediately after admission were higher in the STEMI group than in the control group (Figure 1A, $P < 0.05$). Blood samples obtained from STEMI patients at 0 h, 6 h, 12 h, 24 h, 48 h, 3 days, and 7 days were analyzed to investigate changes in miR-23b in STEMI patients. As presented in Figure 1B, the concentrations of miR-23b in STEMI patients were significantly elevated after the onset of symptoms, and achieved a peak at 48 h and nearly returned

Table 1. The clinical information of two groups.

Variable	STEMI (n=80)	Control (n=60)	P value
Male	46 (57.5%)	35 (58.3%)	0.921
Age	62.83±7.52	63.15±7.32	0.723
BMI (kg/m ²)	24.09±2.45	24.06±2.35	0.855
Risk factors			
Hypertension	50 (62.5%)	32 (53.3%)	0.276
Diabetes mellitus	31 (38.8%)	19 (31.7%)	0.387
Dyslipidemia	48 (60.0%)	31 (51.7%)	0.325
Smoking	39 (48.8%)	24 (40.0%)	0.303

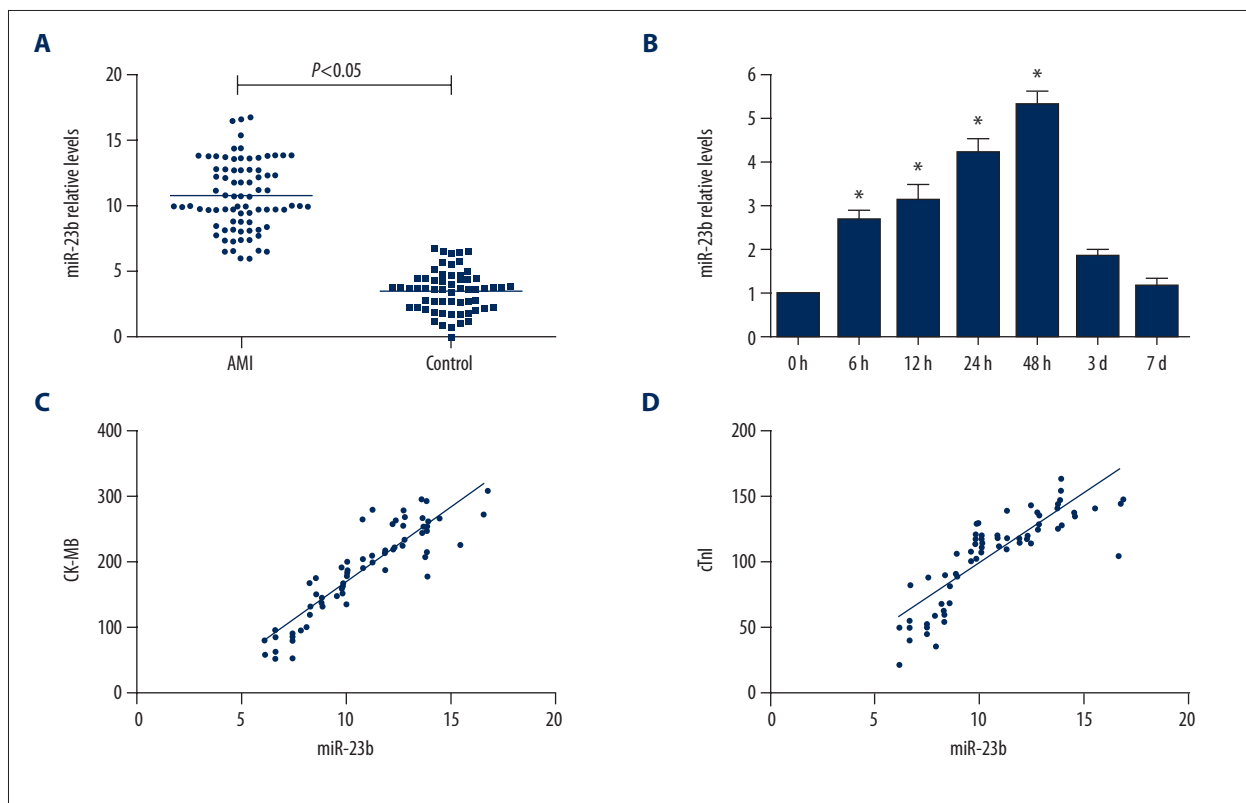


Figure 1. miR-23b expression level in AMI and healthy controls. (A) Plasma level of miR-23b in AMI is significantly elevated compared with control group. (B) Plasma level of miR-23b in AMI patients at the indicated time point. Data are presented as the mean ±SD; $P < 0.05$ versus health control. (C) Correlation between miR-23b and CK-MB in plasma from AMI patients. (D) The correlation between miR-23b and cTnI in plasma from AMI patients.

to the control levels after 7 days. CK-MB and cTnI concentrations were also detected, and were found to be positively correlated with miR-23b. Moreover, the results show that the relative miR-23b levels in STEMI patients were positively correlated with the serum concentrations of CK-MB ($r=0.905$, $P < 0.05$) and cTnI ($r=0.849$, $P < 0.05$) (Figure 1C, 1D).

The level of miR-23b in sub-groups of patients with STEMI

The main risk factors of acute cardiac infarction are age, smoking, blood pressure, blood glucose, and blood lipid. Thus, we investigated the expression of miR-23b in STEMI patients under different conditions. No significant difference was observed in different risk factors, although the clinical data were comparable ($P > 0.05$; Figure 2).

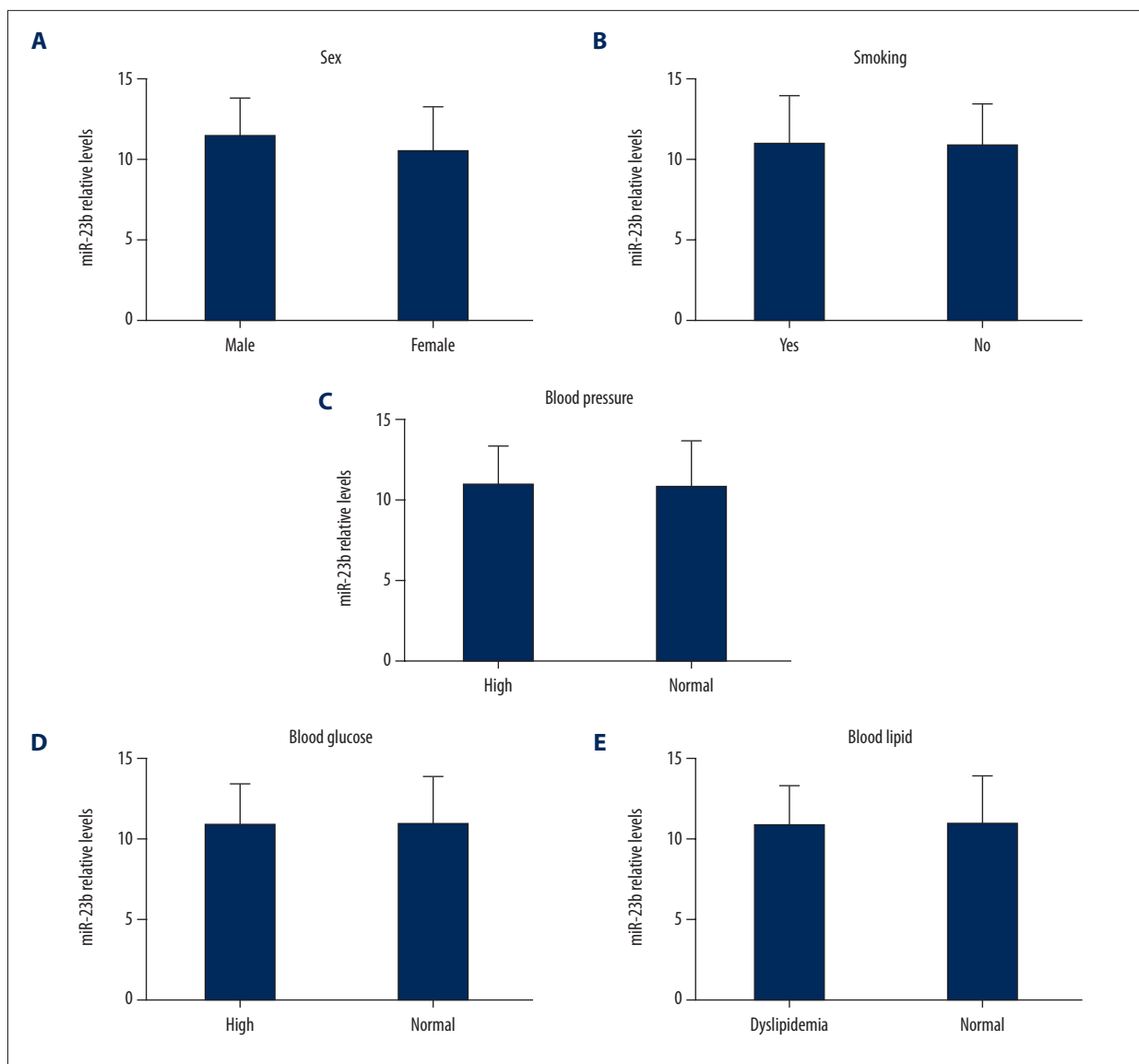


Figure 2. No significant differences were observed in sex, smoking, blood pressure, blood glucose, and blood lipid in patients with STEMI.

The level of miR-23b in STEMI patients after PCI

Plasma miR-23b level in the before PCI group was significantly higher than in the control group. As shown in Figure 3A, miR-23b levels were significantly higher in STEMI patients with 2- and 3-branch lesions ($P < 0.05$). The expression of miR-23b in STEMI patients was significantly downregulated after PCI ($P < 0.05$; Figure 3B). Furthermore, correlation analysis revealed that miR-23b levels were negatively correlated with left ventricular ejection fraction ($r = -0.861$, $P < 0.05$; Figure 3C).

The plasma miR-23b is a sensitive biomarker for STEMI

To evaluate the potential role of miR-23b as a biomarker for STEMI, ROC analysis was performed to determine the suitability of miR-23b. The ROC curve of plasma miR-23b showed a separation, with an AUC of 0.809 (95%CI, 0.737–0.936, $P < 0.05$), compared to CK-MB with an AUC of 0.753 (95%CI, 0.707–0.896) and cTnI with an AUC of 0.783 (95%CI, 0.723–0.917) (Figure 4). Taken together, these data suggest that miR-23b is a sensitive and specific biomarker for STEMI.

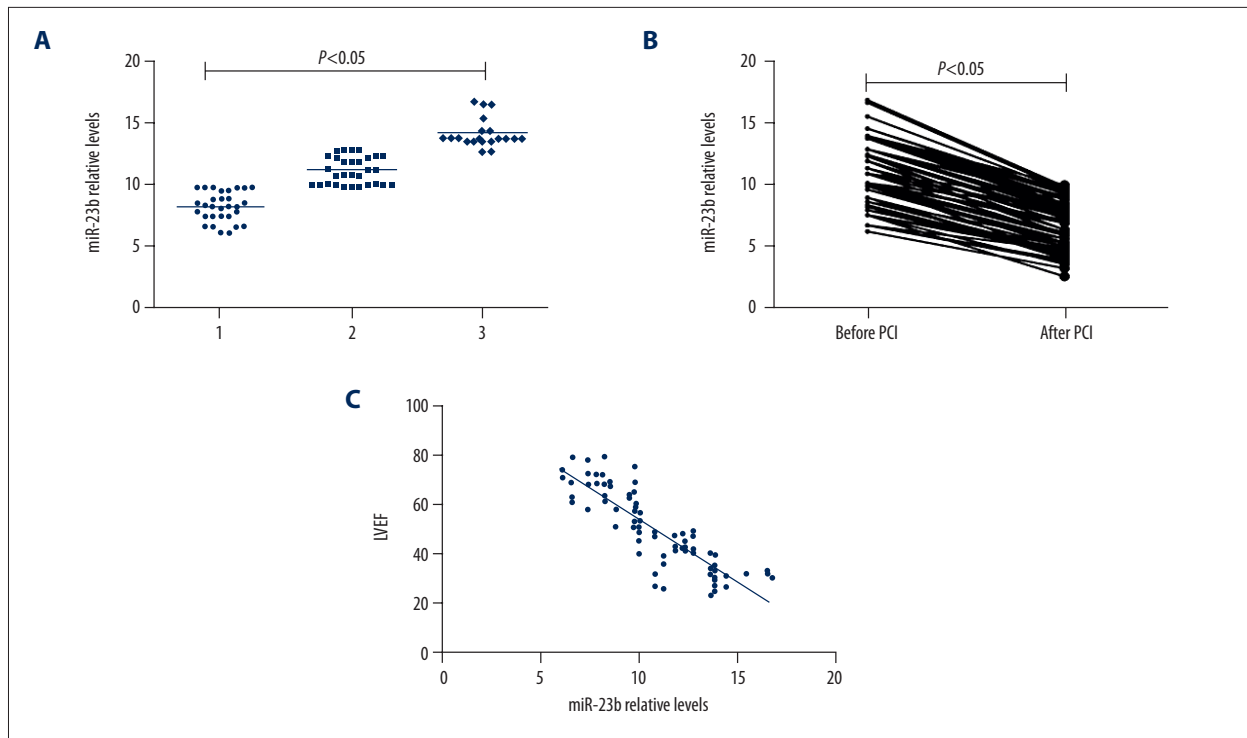


Figure 3. (A) Plasma miR-23b level at indicated time points after PCI. (B) Plasma miR-23b levels in AMI patients after PCI were significantly lower than in the before PCI group ($P < 0.05$). (C) Correlations between plasma miR-23b levels and LVEF in STEMI patients ($r = -0.861$, $P < 0.05$).

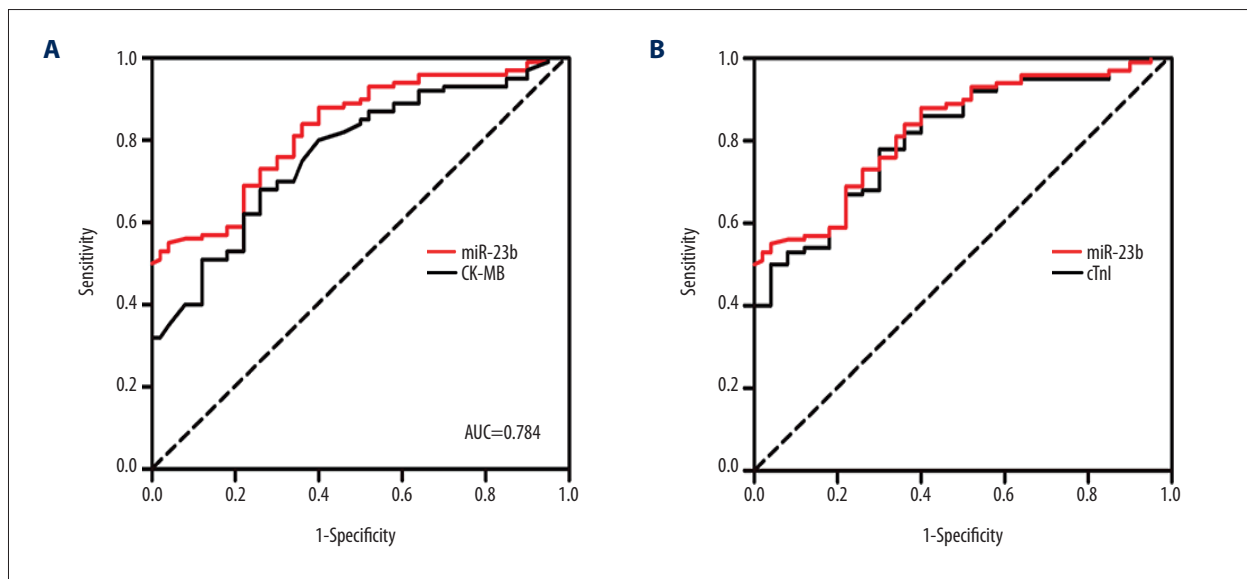


Figure 4. ROC analysis of miR-23b level as STEMI prediction. (A) The ROC curve of plasma miR-23b showed a separation, with an AUC of 0.809 (95%CI, 0.737–0.936, $P < 0.05$), compared to CK-MB with an AUC of 0.753 (95%CI, 0.707–0.896). (B) The ROC curve of plasma miR-23b showed a separation, with an AUC of 0.809 (95%CI, 0.737–0.936, $P < 0.05$), compared to cTnI with an AUC of 0.783 (95%CI, 0.723–0.917).

Discussion

Some microRNAs are highly expressed in the cardiovascular system and play an important role in the regulation of cardiovascular development, as well as being involved in the pathogenesis of related diseases [8–12]. It has been reported that some microRNAs may be potential biomarkers for the diagnosis of AMI, such as miR-1, miR-133, miR-208, and miR-499 [13,14]. High-throughput miRNA profiling shows overexpression of miR-23b in failing human myocardium [15]. miR-23b was found to be highly expressed in the heart and is considered to be a modulator in many cancers and other biological processes [16].

Major specific serum cardiac markers include troponins and CK-MB. An elevation in cardiac markers is required for the diagnosis of AMI [17]. Although troponins are more specific and sensitive in the diagnosis of myocardial injury compared to CK-MB [18], elevated troponins and CK-MB have been detected in many other diseases [4,19].

We investigated the dynamics of miR-23b in patients with STEMI, finding that the level of miR-23b was significantly elevated in plasma of STEMI patients compared to healthy controls. Moreover, the level of miR-23b achieved a peak at 48 h and the correlation analysis revealed the positive association between miR-23b and CK-MB or cTnI.

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Male sex [20], smoking [21], hypertension [22], hyperglycemia [23], and dyslipidemia [24] were the main risk factors associated with progression of AMI, but the differences were not statistically significant, perhaps because of the small sample size.

The ROC curve of plasma miR-23b showed a separation compared to CK-MB and cTns, which are commonly considered the criterion standard biomarkers for AMI, suggesting it is possible that miR-23b is another diagnostic biomarker. However, based on our data, we could not determine if miR-23b is released at the time of symptom onset, nor could we determine the details of the microenvironment within the release; many microRNAs show a restricted expression pattern temporally and spatially [25]. Further studies of molecular mechanisms are needed to thoroughly characterize the role of miR-23b in STEMI.

Conclusions

The present study reveals the biomarker of miR-23b in STEMI, providing a novel insight for the diagnosis for STEMI.

Conflict of interest

None.

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