Abstract. – OBJECTIVE: Previous studies have demonstrated that microRNA-379 (miR-379) was involved in regulating cell proliferation. The present study aimed to investigate its potential role in the diagnosis of acute myocardial infarction (AMI).

PATIENTS AND METHODS: Plasma samples from 30 patients with AMI and 30 healthy adults (non-AMI controls) were collected. The abundance of circulating miR-379 was determined using quantitative Real-time PCR (qRT-PCR). Receiver-operator characteristic (ROC) analyses were performed by GraphPad Prism 5.0 and the areas under the curve (AUC) were calculated. The proliferative ability and cell cycle progression of vascular smooth muscle cell (VSMC) were measured by CCK-8 and flow cytometry, respectively.

RESULTS: We found that the plasma miR-379 level was significantly decreased in patients with AMI compared with healthy people. Further studies demonstrated the miR-379 was negatively correlated with creatine kinase-MB (CK-MB) and cTns in study subjects. Finally, ROC analysis revealed an AUC value of 0.751 in discriminating AMI patients from healthy controls. Function assay in vitro further indicated miR-379 inhibited cell proliferation and induced cell cycle G0/G1 arrest in VSMCs.

CONCLUSIONS: Our results suggest that miR-379 may be a novel biomarker for the diagnosis of AMI by affecting VSMC cell function, which could be used in the early diagnosis of AMI.

Key Words: miR-379, AMI, Diagnosis, VSMC, Cell proliferation, Cell cycle.

Introduction

Acute myocardial infarction (AMI) is one of the most frequently occurring cardiovascular diseases with high morbidity and mortality, which is considered to be a severe health threat worldwide. It is estimated that 16 million people will suffer from AMI in 2020 and 23 million in 2030. Thus, a rapid and accurate diagnosis of AMI is required to control the progression of AMI and improve its treatment and survival rate in patients. At present, a spectrum of biochemical markers, including lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase MB isoform (CDK-MB) and cardiac troponin I (cTnI), have been widely applied to the clinical diagnosis of AMI, but they are insufficient for early detection of AMI for limitations in specificity. Therefore, it is necessary to seek more sensitive and specific novel biomarkers to detect early AMI.

MicroRNAs (miRs) are non-coding small RNA with 17-27 nucleotides in length. Various studies have demonstrated that miRs play an important role in numerous biological processes, including cell growth, differentiation, proliferation and apoptosis. In addition, they have been implicated in various cardiovascular diseases. For example, the plasma levels of miR-1, miR-133a, miR-133b and miR-499-5p are involved in cardiovascular physiology and pathology. Circulating miR-19a was found to be significantly increased in AMI plasma and could be a candidate diagnostic biomarker for AMI. MiR-21, miR-499 and miR-486 were also shown to be involved in AMI and other cardiovascular diseases, indicating that circulating miRs are ideal biomarkers of AMI. Li et al. reported that miR-379 inhibited cell proliferation, migration and invasion by targeting insulin-like factor-1 in vascular smooth muscle cells. However, the specific expression patterns of circulating miR-379 in patients with AMI and their clinical significance remain unknown. Thus, the present work intended to detect miR-379 levels in AMI patients and investigated whether it has diagnostic value in AMI. Furthermore, we also investigated the function of miR-379 in cardiovascular diseases in vitro. Our results highlight the potential of miR-379 to be a novel biomarker of AMI.
Patients and Methods

Patient Characteristics
A total of 30 AMI patients and 30 healthy control (Ctrl) subjects were enrolled in this study from the Community Health Service Center of Laoshan District (Qingdao, China) between June 2015 and October 2016. The present study was approved by Ethics Committee of Community Health Service Center of Laoshan District. All participants provided informed consent. AMI was diagnosed based on combination of several parameters, including at least 50% stenosis in at least one of the coronary arteries, increased cTnI, CK-MB, myoglobin, and brain natriuretic peptide (BNP) levels. Baseline ECG was recorded in all patients. In addition, Ctrl subjects needed to attend routine medical examinations and were confirmed to have no clinical manifestation or medical history of heart disorders, family history of coronary heart disease, or abnormal ECG. Individuals with any acute or chronic infections, hematological disease, tumor, severe liver or renal function defects, inflammatory diseases, as well as valvular heart disease such as heart failure, arrhythmia and cardiomyopathies, were excluded from this study.

Blood Collection
A total of 2 mL of venous blood was collected from each individual and stored in EDTA-anticoagulant tubes containing sodium citrate at 4°C overnight. Then, the blood samples were centrifuged at 3000 rpm for 100 min at 4°C. The obtained supernatant (plasma) was transferred into an RNAase-free Eppendorf tube (Hamburg, Germany) and stored at -80 °C for the following analysis.

Cell Culture and Transfection
VSMC cells were provided by the cell bank of Chinese Academy of Science (Shanghai, China), cultured in Dulbecco’s modified Eagle’s medium (DMEM, HyClone, Logan, UT, USA) containing 10% fetal bovine serums (FBS) and maintained in an incubator with 5% CO₂ at 37°C. Next, cells were transfected with miR-379 mimics or scrambled miRNA purchased from GenePharma (Shanghai, China) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Thus, cells were classified as three groups, including non-transfected cells (Control), transfected with scrambled miRNA (negative control, NC) and transfected with miR-379 mimics (miR-379 mimics).

Cell Proliferation Analysis
Cell proliferation analysis was performed in VSMC cells using CCK-8 assay. Briefly, cells were seeded in 96-well plates at density of 3000 cells per well and incubated from 24 h. Then, 10 μl CCK-8 solution was added to each well at the indicated time-point and cells were incubated for another 2 h at 37°C. Finally, the optical density (OD) value was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow Cytometric Analysis of Cell Cycle
Cell cycle was analyzed using flow cytometric methods. Transfected cells were digested with trypsin and centrifuged at 1200 rpm for 5 min. Cells were washed with phosphate-buffered saline (PBS) three times and fixed in 75% ethanol. After further washing with PBS, cells were stained with 500 μl PI staining solution for 15 min at room temperature. Stained cells were analyzed by flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer’s instructions.

Detection of miR-379 Expression
Total RNA was isolated from the 2 mL of plasma samples using the mirVana PARIS kit (Ambion, Applied Biosystem) according to the manufacturer’s protocol. Reverse transcription of miRNA was performed using the TapMan Reverse Transcription Kit (Tiangen, Beijing, China). For miR-379 mRNA, quantitative Real-time PCR was performed using miRscript SYBR Green PCR Kit according to the protocol of the manufacturer (TaKaRa, Dalian, China). The primers for miR-379 and U6 used were as follow: miR-379 forward, 5'-GCTACATGATACAGTGCAAA-3', miR-379 reverse, 5'-AGTTTGCTTGATCCCTCTTCAG-3'; U6 forward, 5'-GCTTCGGCAGCACATATACTAA-3', U6 reverse, 5'-AACGCTTCACGAATTTGCGT-3'. U6RNA was used as a miRNA internal control. The relative expression values of miR-379 to U6 were calculated using the 2⁻ΔΔCt method.

Statistical Analysis
The baseline characteristics were expressed as means or medians. Quantitative data were presented as mean of three independent experiments ± SD. Student’s t-test was used to evaluate the difference between two groups, including the healthy control and AMI, as well as the NC group and miR-379 mimics group. Correlation between miR-379 and biochemical parameters was per-
Table I. Clinical characteristics of the study population.

<table>
<thead>
<tr>
<th>Variables</th>
<th>AMI (n = 30)</th>
<th>Control (n = 30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.35 ± 8.65</td>
<td>57.64 ± 5.91</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>75/45</td>
<td>61/59</td>
<td></td>
</tr>
<tr>
<td>CK-MB</td>
<td>38.95 ± 1.35</td>
<td>18.23 ± 3.64</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>cTns</td>
<td>1.29 ± 3.67</td>
<td>0.17 ± 7.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP</td>
<td>123.5 ± 9.34</td>
<td>120.31 ± 8.64</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>DBP</td>
<td>83.64 ± 3.57</td>
<td>75.68 ± 6.94</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>115.7 ± 5.67</td>
<td>58.64 ± 10.20</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BNP</td>
<td>923.4 ± 1.34</td>
<td>896.1 ± 1.06</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DM</td>
<td>12</td>
<td>20</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

SBP: systolic blood pressure; DBP: diastolic blood pressure; BNP: brain natriuretic peptide; DM: diabetes mellitus.

Results

Clinical Characteristics of the Study Population

In the present study, we enrolled 30 patients with AMI and 30 controls. The baseline clinical characteristics of the study subjects are listed in Table I. Significant differences were found in some risk indicators of AMI, including CDK-MB, cTns, myoglobin and BNP, which were confirmed significantly upregulated in AMI plasma compared with control groups. There were no significant differences in age, sex, systolic blood pressure (SBP), diastolic blood pressure (DBP) and diabetes mellitus (DM) between AMI patients and control subjects.

The Pattern of Plasma miR-379 Levels in AMI

Using quantitative Real-time PCR, we determined the expression levels of miR-379 in AMI patients and controls. As shown in Figure 1, the relative expression levels of circulating miR-379 in AMI group were significantly lower than that in control groups (p < 0.001), suggesting its potential value in predicting coronary heart disease.

Correlation Between Plasma miR-379 and Conventional Biomarkers

To assess the significance of decreased levels of circulating miR-379 following AMI, we analyzed whether the levels of miR-379 correlated with CDK-MB and cTns, respectively. Our data revealed that the level of miR-379 was significantly negatively correlated with CDK-MB (Figure 2A, p < 0.05) and cTns (Figure 2B, p < 0.05).

Plasma miR-379 Might Be a Potential Diagnostic Marker of AMI

To further evaluate the predictive power of circulating miR-379 for AMI, ROC curve and areas under ROC curve (AUC) analyses were performed. As shown in Figure 3, the AUC was 0.751 (95% CI = 0.675-0.820, p < 0.05), which suggested that miR-379 had marked sensitivity and specificity for AMI. However, whether it was superior to cTns or CDK-MB for the diagnosis of AMI still needed to be further investigated.

Effects of miR-379 on Cell Proliferation and Cell Cycle Progression

We then investigated the functional role of miR-379 in VSMC cells by gain-of-function assays. As...
shown in Figure 4A, miR-379 mimics transfection significantly elevated the expression level of miR-379 in VSMC cells (\(p < 0.001\)). Next, cell proliferation assay was performed by using CCK-8 kit. Compared to their respective control groups, the proliferative ability of miR-379 mimics-transfected VSMCs were significantly inhibited compared with control or NC groups (Figure 4B, \(p < 0.01\)). Cell cycle assay was performed to further examine if miR-379 could affect cell cycle distribution. As shown in Figure 4C, the percentage of cells in G0/G1 phase was significantly increased, while cells in S phase was remarkably decreased in miR-379 mimics groups in comparison with control or NC groups, which indicated that upregulated miR-379 could inhibit cell proliferation through inducing cell cycle G0/G1 phase arrest. These in vitro assays further demonstrated that miR-379 plays an important role in the progression of AMI by regulating VSMC cell growth.

**Discussion**

In this study, we found the expression of miR-379 was significantly decreased in patients’ plasma after AMI compared with that in healthy volunteers. This data supported our previous hypothesis that miR-379 was highly associated with human AMI indeed. Numerous studies demonstrated that circulating miRs are important indicators specifically in the heart for its highly stable and resistant to plasma RNase activity\(^1\),\(^2\). Further correlation analysis showed a negative correlation between circulating levels of miR-379 and CDK-MB, as well as cTns, which were identified as established biomarkers for the early diagnosis of AMI\(^3\). Then, ROC curve of miR-379 was plotted to investigate the accuracy for AMI diagnosis. Our results showed that AUC of circulating miR-379 for the diagnosis of AMI was 0.751, suggesting that miR-379 could act as useful indicator for AMI.

AMI is mainly caused by atherosclerosis and considered a chronic inflammatory\(^4\). Studies have demonstrated that abnormal proliferation of vascular smooth muscle cells (VSMCs) plays a critical role in the pathogenesis of cardiovascular diseases\(^5\),\(^6\). To further elucidate the mechanisms of miR-379 in AMI, we determined the function of miR-379 in VSMCs. Consistent with Li et al\(^7\) findings, we found upregulation of miR-379 could inhibit cell proliferation through inducing cell cycle arrest.

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**Figure 2.** Correlation of the miR-379 with CK-MB and cTns in AMI patients.

**Figure 3.** Evaluation of the sensitivity and specificity of the diagnosis by plasma miR-379 in the AMI patients. ROC curves were constructed to evaluate the diagnostic values of miR-379 for the AMI patients. AUC; area under the ROC curve.
cycle G0/G1 phase arrest. In addition, miR-379 was found to be closely associated with cancer cell proliferation in vitro. For example, miR-379 could suppress breast cancer cell proliferation by regulating cyclin B1 expression. In malignant pleural mesothelioma, miR-379 inhibited cell growth by targeting interleukin 18. This evidence suggested that miR-379 is crucial for the development of AMI and plays a suppressive role in AMI progression.

**Conclusions**

Circulating miR-379 might be a promising biomarker for AMI identification and highly...
Circulating miR-379 as a potential novel biomarker for diagnosis of acute myocardial infarction

Conflict of Interest
1. Authors’ declaration of personal interests: (i) Jie Yi performed the experiment and was responsible for data acquisition. (ii) Jie Yi and Yi An conceived and designed the study. (iii) Jie Yi was responsible for data analysis and Yi An was responsible for statistical analysis. (iv) Jie Yi drafted the manuscript and Yi An revised it accordingly. They have read and approved the final manuscript.

2. Declaration of funding interests: This study was supported by no foundation.

References


