Plasma MicroRNA 499 as a Biomarker of Acute Myocardial Infarction

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BACKGROUND: MicroRNAs (miRNAs) are endogenous small RNAs 21–25 nucleotides in length. Recently, we reported that miRNA 208 (miR-208) is produced exclusively in the rat myocardium and that plasma miR-208 is a biomarker of myocardial injury in rats. In the present study, we assessed the hypothesis that plasma concentrations of myocardial-specific miRNAs can be used to diagnose myocardial injury in humans.

METHODS: We used array analysis of microRNA production in various human tissues to identify heart-specific miRNAs. We assessed the plasma concentrations of miR-499 in 14 individuals with acute coronary syndromes, 15 individuals with congestive heart failure, and 10 individuals without cardiovascular diseases. Plasma miR-499 concentrations were measured with a real-time reverse-transcription PCR method that used an artificial small RNA as an internal calibrator.

RESULTS: The miRNA array analysis of various human tissues indicated that miR-499 was produced almost exclusively in the heart. Plasma miR-499 concentrations were measurably increased in all individuals with acute myocardial infarction but were below the limit of detection for all individuals in the other patient groups.

CONCLUSIONS: The plasma concentration of miR-499 may be a useful biomarker of myocardial infarction in humans.

MicroRNAs (miRNAs),3 endogenous small RNAs 21–25 nucleotides in length, can pair with the 3’ untranslated region sites in mRNAs of protein-coding genes to downregulate their expression (1), and they play important roles in various physiological and pathologic processes (2, 3). More than 500 human miRNAs have been identified (4), and most human protein-coding genes appear to be targeted by these miRNAs (5, 6). miRNAs appear to function as rheostats to fine-tune adjustments in the protein output (7, 8).

The presence of miRNAs in various body fluids has recently been reported (9–11), and we recently reported that the plasma concentration of miRNA 208 (miR-208), a myocardial-specific miRNA in rats, is a useful biomarker of myocardial injury (12). Other groups have also reported that plasma miRNAs are sensitive and specific biomarkers of various tissue injuries (13, 14). In the present study, we examined which human tissues produced miR-499 and assessed whether the plasma concentration of miR-499 is a useful biomarker of myocardial injury in humans.

We collected blood samples from 29 inpatients and 10 healthy asymptomatic outpatients at the National Cardiovascular Center Hospital after obtaining their written informed consent. This study was approved by the Ethics Committee of the National Cardiovascular Center.

The acute coronary syndromes group consisted of 9 patients with acute myocardial infarction (AMI) and 5 patients with unstable angina pectoris. All acute coronary syndrome patients underwent coronary angiography and percutaneous coronary intervention. The blood samples from the acute coronary syndrome patients were obtained within 48 h of the last onset of chest pain. We also obtained blood samples from AMI patients before their final discharge when their clinical status was stable. The congestive heart failure (CHF) group consisted of 8 patients with old myocardial infarction [New York Heart Association (NYHA) class III], 4 patients with dilated cardiomyopathy (NYHA class II), and 3 patients with valvular diseases (1 patient in NYHA class III and 2 in NYHA class II). The blood samples of patients in the CHF group were obtained while they were in NYHA functional class II or III. The control individuals consisted of asymptomatic healthy and/or borderline hypertensive outpatients who were visiting the hospital for regular health checkups. Creatine kinase MB was increased in the patients with AMI and not in the patients with unstable angina pectoris (Table 1).

We isolated total plasma RNA with the mirVana™ PARIS Kit (Ambion) according to the manufacturer’s protocol. Before purification, we added a fixed amount of a small synthetic RNA to the plasma samples for a dual assay to verify the RNA-purification procedures. Details of the procedure are described in the Supplemental Data file available in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol56/issue7.
To identify myocardial-specific miRNAs, we used the ABI TaqMan MicroRNA Array kit (Applied Biosystems) according to the manufacturer’s protocol for profiling the production of miRNAs in various human tissues and cultured cells.

To measure miR-499 concentrations, we used a TaqMan microRNA real-time RT-PCR kit (Applied Biosystems) according to the manufacturer’s protocol. We simultaneously assessed the concentration of the internal reference small RNA in a single tube. The limit of detection for miR-499 was 240 copies/100 µL. All assays were performed in duplicate. Calibration assays with various amounts of synthetic miR-499 were performed on each assay plate. Details of the statistical analyses are described in the Supplemental Data file in the online Data Supplement.

The miRNA array analyses of 671 species of miRNAs in various tissues and cells indicated that miR-499 is produced almost exclusively in the human heart (see Supplemental Table in the online Data Supplement). miR-208a and miR-208b concentrations appear to be very low in the human heart (see Supplemental Table in the online Data Supplement), and these 2 miRNAs appear not to be useful as plasma biomarkers.

Fig. 1 summarizes the data for plasma miR-499 concentrations in the study population. Plasma miR-499 concentrations were assessed by real-time reverse-transcription PCR with a synthetic miRNA included as an internal calibrator. Values are expressed as log miR-499 copies/100 µL. Concentrations were measured in patients with AMI [repeatedly measured in samples obtained within 48 h (AMI_1) and at just before hospital discharge (AMI_2)], in patients with unstable angina pectoris (UAP), in CHF patients in NYHA class III (CHF_3), in CHF patients in NYHA class II (CHF_2), and in healthy control individuals (Normal). An ANOVA indicated that the mean miR-499 values were significantly different among the groups (P < 0.0001). The subsequent Dunnett test indicated that values in the AMI_1 group were significantly higher than those of the other groups (P < 0.0001 for all comparisons).

To identify myocardial-specific miRNAs, we used the ABI TaqMan MicroRNA Array kit (Applied Biosystems) according to the manufacturer’s protocol for profiling the production of miRNAs in various human tissues and cultured cells.

Table 1. Patient characteristics.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>AMI(^b) (n = 9)</th>
<th>UAP (n = 5)</th>
<th>CHF_III (n = 9)</th>
<th>CHF_II (n = 6)</th>
<th>Normal (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/M sex, n</td>
<td>3/6</td>
<td>2/3</td>
<td>2/7</td>
<td>2/4</td>
<td>5/5</td>
</tr>
<tr>
<td>Age, years</td>
<td>66.8 (9.28)</td>
<td>70.2 (16.2)</td>
<td>71.6 (6.6)</td>
<td>61.5 (16.4)</td>
<td>41.5 (8.0)</td>
</tr>
<tr>
<td>CKMB, U/L(^c)</td>
<td>122.2 (124.9)</td>
<td>18.9 (6.6)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>BNP, ng/L(^c)</td>
<td>ND</td>
<td>ND</td>
<td>674 (341)</td>
<td>175 (142)</td>
<td>ND</td>
</tr>
<tr>
<td>Log miR-499 copies/100 µL</td>
<td>4.19 (0.24)</td>
<td>&lt;2.38</td>
<td>&lt;2.38</td>
<td>&lt;2.38</td>
<td>&lt;2.38</td>
</tr>
</tbody>
</table>

\(^a\) Data are expressed as the mean (SD) where indicated.
\(^b\) AMI, acute myocardial infarction; UAP, unstable angina pectoris; CHF_III, congestive heart failure in NYHA class III; CHF_II, congestive heart failure in NYHA class II; Normal, healthy control individuals; CKMB, creatine kinase MB; BNP, brain natriuretic peptide; ND, not determined.
\(^c\) CKMB (reference interval, 0–23 U/L) and BNP (reference interval, <18.4 ng/L) were measured in the AMI groups (AMI and UAP) and the CHF groups, respectively.

A positive correlation between creatine kinase MB activity and plasma miR-499 concentration was clearly of the onset of myocardial infarction (data not shown). Plasma miR-499 concentrations were assessed by real-time reverse transcription PCR with a synthetic miRNA included as an internal calibrator. Values are expressed as log miR-499 copies/100 µL. Concentrations were measured in patients with AMI [repeatedly measured in samples obtained within 48 h (AMI_1) and at just before hospital discharge (AMI_2)], in patients with unstable angina pectoris (UAP), in CHF patients in NYHA class III (CHF_3), in CHF patients in NYHA class II (CHF_2), and in healthy control individuals (Normal). An ANOVA indicated that the mean miR-499 values were significantly different among the groups (P < 0.0001). The subsequent Dunnett test indicated that values in the AMI_1 group were significantly higher than those of the other groups (P < 0.0001 for all comparisons).
observed in individuals with AMI (see Supplemental Data in the online Data Supplement).

The present study is the first to confirm that a cardiac-specific miRNA, miR-499, can be a biomarker of myocardial infarction in humans. The next question is whether this assessment of the plasma miR-499 concentration has any clinical significance. We expected the PCR-based assay of plasma miR-499 to detect plasma miR-499 concentrations reliably in CHF patients. A more sensitive assay to detect plasma miR-499 can be developed, however, and it might establish miR-499 as a new biomarker of cardiovascular diseases in the same way that the recently developed high-sensitivity assays for troponins have become very useful for evaluating patients with cardiovascular diseases (16).

Accumulating evidence suggests the usefulness of circulating miRNAs as stable blood-based biomarkers for various diseases (9–11). The present study has confirmed, for the first time, that the plasma miR-499 concentration may be a biomarker of myocardial infarction in humans. Our array data indicate other intriguing candidates for clinical applications, including miR-124a for the central nervous system, miR-122 for the liver, and miR-133a for skeletal muscle. These observations await further clinical investigations.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References


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