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A subset of circulating microRNAs are predictive for cardiac death after discharge for acute myocardial infarction ☆

Sen Matsumoto [a,1], Yasuhiko Sakata [a,b,c,1,*], Daisaku Nakatani [a,1], Shinichiro Suna [a,1], Hiroya Mizuno [a,b,1], Masahiko Shimizu [a,1], Masaya Usami [a,1], Tatsuya Sasaki [d,1], Hiroshi Sato [e,1], Yukio Kawahara [f,1], Toshimitsu Hamasaki [g,1], Shinsuke Nanto [a,b,1], Masatsugu Hori [h,1], Issei Komuro [a,1]

[a] Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan
[b] Department of Advanced Cardiovascular Therapeutics, Osaka University Graduate School of Medicine, Suita, Japan
[c] Department of Evidenced-Based Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan
[d] Cardiovascular Division, Osaka Minami Medical Center, National Hospital Organization, Kawachinagano, Japan
[e] School of Human Wilfare Studies Health Care Center and Clinic, Kwansei Gakuin University, Nishinomiya, Japan
[f] Laboratory of RNA Function, Osaka University Graduate School of Medicine, Suita, Japan
[g] Department of Biomedical Statistics, Osaka University Graduate School of Medicine, Suita, Japan
[h] Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

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ABSTRACT

To investigate the prognostic impact of circulating microRNAs (miRs) in patients who survived acute myocardial infarction (AMI), we compared the circulating miR signature at the time of survival discharge among samples in the serum bank of the Osaka Acute Coronary Insufficiency Study. Using a high-throughput array consisting of 667 miRs, 11 miRs were found to be differentially expressed in the serum among patients at high-risk for cardiac death. Real-time RT-PCR confirmed that the serum levels of miR-155 and miR-380* were approximately 4- and 3-fold higher, respectively, in patients who experienced cardiac death within 1 year after discharge. Accordingly, a subset of circulating miRs might be predictive for cardiac death in post-AMI patients.

1. Introduction

MicroRNAs (miRs) are small endogenous noncoding RNAs that regulate gene expression by targeting the degradation or translational repression of mRNA. Recently, it has been demonstrated that circulating miRs in the blood are useful biomarkers for cardiovascular disease [1] as well as certain forms of cancer [2]. For example, Wang et al. [3] reported that miR-208a is an excellent diagnostic marker for AMI, as demonstrated by its sensitive detection in AMI patients within 4 h of the onset of symptoms. The authors also revealed that miR-208a had high sensitivity and specificity for diagnosing AMI by receiver operating characteristic curve analysis [4]. Kuwabara et al. [5] recently reported that circulating miR-133a serves as a useful marker for cardiomyocyte death and thus, can be used for the detection of several cardiovascular diseases, including acute myocardial infarction (AMI), and unstable angina, and takotsubo cardiomyopathy.

In patients with malignancy, the usefulness of circulating serum miRs as markers for prognosis and diagnosis has been established for several types of cancers. Few reports, however, have examined the predictive value of serum miRs in the field of cardiovascular medicine, particularly in the setting of secondary prevention after AMI. Here, we therefore investigated whether circulating miRs collected during the convalescent stage of AMI could predict cardiac death in post-AMI patients registered in the Osaka Acute Coronary Insufficiency Study (OACIS) database.

2. Materials and methods

2.1. OACIS registry

The OACIS is a prospective, multicenter observational study enrolling consecutive AMI patients in 25 collaborating hospitals from the Osaka region of Japan, and is registered with the

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AMI, acute myocardial infarction; ARB, angiotensin receptor blocker; CI, confidence interval; HR, hazard ratio; miR, microRNA.

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* Corresponding author. Address: Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita 565-0871, Japan. Fax: +81 6 6879 6613.
E-mail address: sakatayk@cardiology.med.osaka-u.ac.jp (Y. Sakata).

[1] On behalf of The Osaka Acute Coronary Insufficiency Study (OACIS) Group (listed in the Appendix A).

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University Hospital Medical Information Network Clinical Trials Registry, Japan (ID: UMIN000004575). A detailed description of the OACIS has been published elsewhere [6]. The present study protocol was approved by the ethics committee of each participating hospital.

2.2. Patients

Among 8603 patients with AMI who were registered in the OACIS between 1998 and 2009, we firstly selected 4160 consecutive patients fulfilling the following criteria: (1) discharged alive and (2) provided written informed consent for serum analysis at the time of registration. Among the selected patients, 60 cardiac deaths occurred after discharge. In the discovery phase, we randomly selected 7 patients who died of cardiac cause within a year after discharge and another 7 patients who did not experience any cardiovascular events during a 3-year follow-up period using propensity score-based matching of age, gender, classical coronary risk factors, infarction size, reperfusion therapy, and medical treatment at discharge. In the validation phase, we increased the number of patients in the cardiac death and survival groups to 19 and 21, respectively.

2.3. Serum collection

At each hospital, fasting blood samples were collected into serum separator tubes, which were then centrifuged at 1430g for 15 min at 4 °C to separate the clots. Serum was removed from the tubes and stored at −80 °C until the time of the assay.

2.4. RNA isolation and miR analysis

Total RNA was isolated from 1 ml of serum using a mirVana Paris kit (Life Technologies Co., Carlsbad, CA). Reverse transcription and preamplification steps were performed with a TaqMan MicroRNA RT kit (Life Technologies Co.) and Megaplex Primers (Life Technologies Co.). To identify miRs that could serve as predictive markers of cardiac death at 1 year, the expression levels of 667 miRs were compared between groups using TaqMan Human MicroRNA A and B Arrays, version 2.0 (Life Technologies Co.) (discovery phase). To confirm the results from the discovery phase, the expression levels of candidate miRs were examined by real-time PCR using a 7900HT Fast Real-Time PCR system (validation phase).

2.5. Data collection

Research cardiologists and trained research nurses or coordinators recorded data concerning sociodemographic variables, medical history, therapeutic procedures, and clinical events during patients' hospital stays. Clinical data after discharge were obtained at 3 and 12 months after the onset of AMI, and annually thereafter. The incidence of cardiac death was the clinical endpoint of the study.

2.6. Statistical analysis

To adjust for potential confounding factors, we selected two groups for the discovery and validation phases using a propensity score-based method. Briefly, a propensity score for cardiac death within 1 year after discharge was calculated using logistic regression analysis that included age, gender, diabetes mellitus (DM), hypertension (HT), dyslipidemia, smoking, previous MI, Killip class $\geqslant$ II at admission, infarction size, reperfusion therapy, and medication at discharge (ACEI or ARB, and statin) as variables. For the analysis, we first selected seven patients who died of cardiac cause within 1 year after discharge and another seven patients who did not experience any cardiovascular events during a 3-year follow-up period. We then selected 19 patients who died of cardiac cause after discharge and 21 patients who did not experience any cardiovascular events during a 2-year follow-up period. For the two sets of groups, patient backgrounds were compared using the χ^2 test. Expression levels of miRs between the two groups were analyzed by the Mann–Whitney U test. Associations were considered significant if the p value was <0.05. All statistical analyses were performed using SPSS software (SPSS Japan, Inc., Tokyo, Japan).

3. Results

3.1. Discovery phase

To investigate whether serum miRs could predict prognosis in the convalescent stage of AMI, we compared circulating miR signatures at the time of survival discharge using the OACIS serum bank. As shown in Table 1, patient backgrounds were well matched between patients who died of cardiac cause within 1 year after discharge ($N = 7$) and those who did not experience any cardiovascular events during the 3-year follow-up period ($N = 7$) in the discovery phase. High-throughput array analysis revealed

Table 1
Baseline characteristics in the discovery phase.

Variable	Cardiac death ($N = 7$)	Event free ($N = 7$)	p Value
Age (years)	68 ± 8	67 ± 7	0.810
Men (%)	86	71	1.000
Diabetes mellitus (%)	57	29	0.592
Hypertension (%)	83	57	0.559
Dyslipidemia (%)	71	57	1.000
Smoking (%)	57	86	0.559
Previous MI (%)	14	0	1.000
Peak CK $\geqslant$ 3000 IU/L (%)	14	43	0.559
Killip class $\geqslant$ II on admission (%)	43	43	1.000
Reperfusion therapy (%)	100	100	–
ACEI or ARB (%)	71	67	1.000
Statin (%)	57	67	1.000

ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker, CPK: creatinine phosphokinase, MI: myocardial infarction.

Upregulated miRs	**hsa-miR-134, hsa-miR-155, hsa-miR-18a, hsa-miR-192, hsa-miR-380***
Downregulated miRs	**hsa-miR-125a-5p, hsa-miR-212, hsa-miR-331-3p, hsa-miR-223*, hsa-miR-190b, hsa-miR-93***

Fig. 1. High-throughput array analysis revealed that the levels of 5 miRs were increased and those of 6 miRs were decreased in the cardiac death group.