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Expression Levels of miR-126, Mitochondrial Components, and Adhesion Molecules in Peripheral Blood Endothelial Microparticles from Patients with Acute Myocardial Infarction and Their Clinical Significance (Postprint)

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Abstract

Background Acute myocardial infarction (AMI) is a major cause of morbidity and mortality from cardiovascular disease worldwide. Despite the widespread application of biomarkers for myocardial necrosis, the incidence and mortality of AMI remain high.

Objective To investigate the expression levels and clinical significance of miR-126, mitochondrial components, and adhesion molecules contained within endothelial microparticles (EMPs).

Methods Fifty AMI patients, 50 stable coronary artery disease (SCAD) patients, and 50 healthy individuals who visited the People' s Hospital of Xinjiang Uygur Autonomous Region from September 2021 to September 2022 were enrolled. Both AMI and SCAD patients were hospitalized and underwent percutaneous coronary intervention (PCI) at our hospital, while healthy individuals were evaluated through our hospital' s physical examination center. Peripheral blood samples and general data were collected from the three groups. Transmission electron microscopy was used to observe the morphology of microparticles, flow cytometry was used to identify EMPs levels, fluorescence quantitative PCR was used to detect miR-126 expression in EMPs, and ELISA was used to detect mitochondrial reactive oxygen species (ROS) and adhesion molecules [vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), E-selectin, P-selectin] levels in EMPs.

Results Transmission electron microscopy observation revealed that the isolated MPs had intact membrane structures with diameters of 100-400 nm. Compared

with the control group, the expression level of miR-126 in plasma EMPs of the AMI group decreased (Z=4.979, P<0.001), while the expression levels of ROS (Z=9.651, P<0.001), VCAM-1 (Z=2.336, P=0.019), ICAM-1 (Z=5.894, P<0.001), E-selectin (Z=2.730, P=0.019), and P-selectin (Z=6.470, P<0.001) increased. Multivariate logistic regression analysis showed that decreased miR-126 expression level (OR=0.026, 95%CI=0.003-0.210, P=0.001) was a protective factor for AMI, while elevated ROS (OR=1.009, 95%CI=1.005-1.013, P<0.001) and P-selectin expression levels (OR=1.063, 95%CI=1.022-1.105, P=0.002) were risk factors for AMI. Receiver operating characteristic (ROC) curve analysis showed that the area under the curve (AUC) for miR-126 in diagnosing AMI was 0.816, for ROS was 0.892, for P-selectin was 0.728, and for the combined diagnosis of miR-126, ROS, and P-selectin was 0.950.

Conclusion miR-126, ROS, and P-selectin in EMPs, as well as their combined indicator, all have diagnostic value for AMI, with the combined indicator showing the highest diagnostic value, suggesting that they may serve as potential diagnostic markers for AMI patients.

Full Text

Title and Authorship

Levels of Endothelial Cell Microparticles miR-126, Mitochondrial Components and Adhesion Molecules in Peripheral Blood of Patients with Acute Myocardial Infarction and Their Clinical Significance

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Abstract

Background: Acute myocardial infarction (AMI) is the leading cause of cardiovascular disease morbidity and mortality worldwide. Despite the widespread use of biomarkers for myocardial necrosis, the morbidity and mortality of AMI remain high.

Objective: To investigate the expression levels and clinical significance of miR-126, mitochondrial components, and adhesion molecules in endothelial microparticles (EMPs).

Methods: A total of 50 AMI patients, 50 patients with stable coronary artery disease (SCAD), and 50 healthy subjects were enrolled at the People' s Hospital of Xinjiang Uygur Autonomous Region from September 2021 to September 2022. AMI and SCAD patients were hospitalized and received percutaneous coronary intervention (PCI), while healthy subjects were evaluated by the hospital' s physical examination center. Peripheral blood samples and general data were collected from all three groups. Microparticle morphology was observed by transmission electron microscopy (TEM), EMPs levels were identified by flow cytometry, miR-126 expression in EMPs was detected by fluorescence quantitative PCR, and ELISA was used to detect levels of mitochondrial reactive oxygen species (ROS) and intracellular adhesion molecules [vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, and P-selectin] in EMPs.

Results: TEM observation revealed that isolated MPs had intact membrane structures with diameters ranging from 100 to 400 nm. Compared with the control group, the AMI group showed significantly decreased miR-126 expression ($Z=4.979$, $P<0.001$) and significantly increased ROS expression ($Z=9.651$, $P<0.001$) in plasma EMPs. VCAM-1 expression was increased ($Z=2.336$, $P=0.019$), as were ICAM-1 ($Z=5.894$, $P<0.001$), E-selectin ($Z=2.730$, $P=0.019$), and P-selectin ($Z=6.470$, $P<0.001$). Multivariate logistic regression analysis showed that decreased miR-126 expression (OR=0.026, 95%CI=0.003-0.210, $P=0.001$) was a protective factor for AMI, while increased ROS (OR=1.009, 95%CI=1.005-1.013, $P<0.001$) and P-selectin (OR=1.063, 95%CI=1.022-1.105, $P=0.002$) were risk factors. Receiver operating characteristic (ROC) curve analysis showed that the area under the curve (AUC) for miR-126 in diagnosing AMI was 0.816, for ROS was 0.892, for P-selectin was 0.728, and for the combined diagnosis of miR-126, ROS, and P-selectin was 0.950.

Conclusion: miR-126, ROS, P-selectin, and their combined indicator in EMPs all have diagnostic value for AMI, with the combined indicator showing the highest diagnostic value, suggesting they may serve as potential diagnostic biomarkers for AMI patients.

Keywords: Acute myocardial infarction; Endothelial cell; Microparticles; miR-126; Mitochondria; Adhesion molecules; Clinical significance

Introduction

Acute myocardial infarction (AMI) represents a major global health crisis, causing over 3 million deaths worldwide annually. As the most common emergency form of ischemic heart disease, AMI is triggered by the rupture of vulnerable atherosclerotic plaques with superimposed thrombosis, leading to coronary artery occlusion and cell death in hypoperfused regions. The disease onset is abrupt and severe, making individual patient events difficult to predict. Therefore, early diagnosis of AMI is critically important but remains largely unmet in clinical practice.

Microparticles (MPs) are vesicles measuring 100-1,000 nm in diameter, including endothelial cell-derived microparticles (EMPs), monocyte-derived microparticles, and platelet-derived microparticles. These vesicles carry various bioactive molecules such as miRNAs and play crucial roles in cell communication. Endothelial dysfunction is particularly critical in activating immune-inflammatory responses and tissue damage. Endothelial cell-associated miR-126 is one of the most abundant miRNAs in endothelial cells and has anti-inflammatory and anti-cardiomyocyte apoptosis effects. Our previous research found that reduced levels of MP-associated miR-126 in coronary blood of AMI patients may be associated with acute coronary thrombotic events. Additionally, miRNAs regulate oxidative stress by targeting reactive oxygen species (ROS) pathways and antioxidant effectors. Based on domestic and international research, MPs and their miRNA components promote adhesion molecule expression through mitochondrial ROS-dependent pathways, with monocyte adhesion, recruitment, and migration to damaged sites exacerbating tissue injury. However, the role of EMPs and miRNAs as intermediate communication mediators affecting myocardial injury after AMI remains unclear. Therefore, further elucidating the role of EMPs and their miR-126 in adhesion molecule expression provides new insights for exploring the microenvironment conducive to myocardial repair following AMI.

This study collected peripheral blood from AMI patients, stable coronary artery disease (SCAD) patients, and healthy individuals before coronary angiography. MPs were detected by transmission electron microscopy, EMPs were labeled and quantitatively analyzed by nanoparticle tracking, and the expression levels of miR-126, ROS, and surface adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), P-selectin, and E-selectin on EMPs were measured. The study analyzed the levels of miR-126, mitochondrial components, and adhesion molecules in EMPs and their clinical significance, exploring the role of EMPs and miR-126 in regulating adhesion molecule expression through ROS-dependent pathways after AMI to provide a scientific basis for effectively extending the therapeutic time window and reducing complications.

Methods

1.1 Study Subjects and Grouping

A total of 50 AMI patients (AMI group), 50 SCAD patients (SCAD group), and 50 healthy subjects (Control group) treated at the People' s Hospital of Xinjiang Uygur Autonomous Region from September 2021 to September 2022 were enrolled. AMI and SCAD patients were hospitalized and received percutaneous coronary intervention (PCI), while healthy subjects were evaluated by the hospital' s physical examination center. All participants had complete baseline data, and general information and biochemical indicators were collected. This study was approved by the Ethics Committee of the People' s Hospital of Xinjiang Uygur Autonomous Region (Approval No.: KY2020041046), and all patients provided informed consent.

1.1.1 Inclusion Criteria

(1) AMI was defined as elevated or decreased cardiac biomarkers (preferably troponin) with at least one value exceeding the 99th percentile upper reference limit plus at least one of the following: signs of myocardial ischemia; new or presumably significant ST-segment changes or new left bundle branch block; pathological Q waves on electrocardiogram; imaging evidence of new loss of viable myocardium or new regional wall motion abnormality; angiographic or anatomical evidence of intracoronary thrombus. All patients had coronary artery disease confirmed by coronary angiography (refer to "Guidelines for the Diagnosis and Treatment of Acute ST-Segment Elevation Myocardial Infarction" and the 2020 ESC Guidelines for Non-ST-Segment Elevation Acute Coronary Syndromes).

(2) SCAD was defined as a clinical syndrome of transient myocardial ischemia and hypoxia caused by increased myocardial demand on the basis of fixed severe coronary stenosis. Patients diagnosed with coronary heart disease during coronary angiography who required stent implantation were selected (refer to "Guidelines for the Diagnosis and Treatment of Stable Coronary Artery Disease").

(3) Healthy subjects were those with normal chest X-ray, electrocardiogram, liver and kidney function, and biochemical indicators during physical examination, and no history of other diseases.

1.1.2 Exclusion Criteria

(1) Severe hepatic or renal insufficiency; (2) Malignant tumors; (3) Hematopoietic system diseases; (4) Rheumatoid arthritis, systemic lupus erythematosus, or Sjögren' s syndrome; (5) Cerebral infarction or pulmonary embolism.

1.1.3 Sample Collection Peripheral blood was collected from the Control group at rest. For AMI and SCAD groups, peripheral blood was collected at rest before coronary angiography (5-10 mL). For AMI patients, blood was collected within 24 hours of chest pain onset using sodium citrate anticoagulation tubes (BD, USA). Specimens collected in sodium citrate tubes were centrifuged at 3,000×g for 10 minutes, and plasma was aliquoted into three small tubes and stored at -80°C for subsequent EMPs identification, miR-126 quantitative analysis, and measurement of mitochondrial components and adhesion molecule levels.

1.2 Main Instruments and Reagents

Main instruments: Microscope (NIKON, H550S), microplate reader (Thermo Fisher, Multiskan 51119000), tissue stretcher (Zhejiang Kedi Equipment, KD-P), and electrothermal constant temperature incubator (Changzhou Zhiborui, SHZ-82), among others.

Reagents: Anhydrous ethanol (Sinopharm Chemical Reagent, 100092683), xylene (Sinopharm Chemical Reagent, 1330-20-7), hematoxylin (Zhuhai Besso, BA4097), eosin stain (Zhuhai Besso, BA4099), among others.

1.3 Experimental Methods

1.3.1 Transmission Electron Microscopy (TEM) Detection of MPs Plasma samples were centrifuged at 1,550×g for 15 minutes at room temperature. The plasma supernatant was transferred to a new centrifuge tube and centrifuged at 18,800×g for 30 minutes. The supernatant was removed, the pellet was resuspended in phosphate-buffered saline (PBS), and centrifuged again at 18,800×g for 30 minutes. The resulting pellet was MPs, which were fixed with glutaraldehyde for later use. For TEM analysis, 5-10 L of MPs solution was applied to Formvar-carbon-coated copper grids. The grids were washed by floating on 100 L PBS drops (Formvar membrane side down) on parafilm. Grids were then placed on 50 L of 1% glutaraldehyde for 5 minutes, followed by eight washes on 100 L ddH_2O drops for 2 minutes each. Subsequently, grids were placed on 50 L of uranyl oxalate solution (pH 7.0) for 5 minutes, then on 50 L of methylcellulose solution for 10 minutes on ice. Excess liquid was blotted with filter paper, and grids were air-dried for 5-10 minutes before being placed in sample boxes. Electron micrographs were captured using a TECNAI 10 transmission electron microscope.

1.3.2 Nanoparticle Tracking Analysis (NTA) of MPs MPs were resuspended in 1 mL PBS. A 50 L sample of MPs was diluted with 100 L PBS and added to the nanosight particle analysis detection platform for MPs size distribution analysis.

1.3.3 Flow Cytometry Detection of EMPs Plasma samples were removed from -80°C storage and thawed at room temperature for 10 minutes. After com-

plete thawing, 400 L of plasma sample was transferred to a new EP tube and centrifuged at 1,550×g for 15 minutes at room temperature. The supernatant was transferred to a new EP tube and centrifuged at 18,800×g for 30 minutes. The supernatant was removed, and the pellet was resuspended in 500 L PBS buffer, followed by centrifugation at 18,800×g for 30 minutes at room temperature. The resulting pellet was isolated MPs. MPs were resuspended in 400 L PBS-1% BSA solution. A 100 L aliquot was transferred to a new EP tube, and FITC-CD31 and PE-CD42b antibodies were added at a 1:100 ratio. After gentle mixing, the antibodies were incubated at room temperature for 1 hour in the dark. Following incubation, samples were centrifuged at 18,800×g for 30 minutes at room temperature. The pellet was resuspended in 400 L PBS buffer and immediately analyzed by flow cytometry. During flow cytometry analysis, forward scatter (FSC) and side scatter (SSC) were used to observe the size and complexity distribution of MPs. The MPs scatter cluster was gated, and FL1 (CD31) and FL2 (CD42b) channels were used to analyze the proportion of $CD31^+$ cells among all MPs, including $CD31^+/CD42b^-$ MPs, $CD31^-/CD42b^+$ MPs, and $CD31^+/CD42b^+$ MPs.

1.3.4 PCR Detection of miR-126 in EMPs A 200 L plasma sample was centrifuged at 1,550×g for 15 minutes at room temperature. The plasma supernatant was transferred to a new centrifuge tube and centrifuged at 18,800×g for 30 minutes. The supernatant was removed, the pellet was resuspended in PBS, and centrifuged again at 18,800×g for 30 minutes. The resulting pellet was MPs. RNAiso plus (0.5 mL) was added for complete lysis before transfer to an EP tube. Chloroform (100 L) was added, vigorously shaken for 15 seconds, left at room temperature for 15 minutes, then centrifuged (4°C, 12,000×g, 15 minutes). The supernatant was mixed with isopropanol and precipitated. The pellet was dissolved in 50 L DEPC water, concentration was measured by UV spectrophotometry, and samples were stored at -80°C.

Reverse transcription was performed using the miRcute Enhanced miRNA cDNA First Strand Synthesis Kit. The reverse transcription system consisted of: Total RNA (1 g), 2×miRNA RT Reaction Buffer (5 L), miRNA RT Enzyme Mix (1 L), and DEPC water to a final volume of 10 L. After mixing, the reaction was performed in a PCR instrument at 42°C for 60 minutes and 95°C for 3 minutes. Fluorescence quantitative PCR was performed using: cDNA (2 L), PCR forward primer (0.4 L), PCR reverse primer (0.4 L), SYBR Green solution (10 L), sterile double-distilled water (7.2 L), for a total volume of 20 L. Reaction conditions were: 95.0°C for 2 minutes; 95.0°C for 3 seconds, 60.0°C for 30 seconds, for 40 cycles; 95.0°C for 15 seconds, 60.0°C for 1 minute, 95.0°C for 15 seconds.

1.3.5 ELISA Detection of Mitochondrial ROS in EMPs Plasma samples were removed from -80°C storage and thawed at room temperature for 10 minutes. After complete thawing and gentle mixing, 200 L of plasma sample was transferred to a new EP tube and centrifuged at 1,550×g for 15 minutes

at room temperature. The supernatant was transferred to a new EP tube and centrifuged at 18,800×g for 30 minutes. The supernatant was removed, and the pellet was resuspended in 500 L PBS, followed by centrifugation at 18,800×g for 30 minutes at room temperature. The resulting pellet was isolated MPs. A 96-well plate was placed in a fluorescence microplate reader, and the fluorescence OD value of each sample was measured at an excitation wavelength of 396 nm and emission wavelength of 610 nm.

1.3.6 ELISA Detection of Adhesion Molecules in EMPs Plasma samples were removed from -80°C storage and thawed at room temperature for 10 minutes. After complete thawing and gentle mixing, 400 L of plasma sample was transferred to a new EP tube and centrifuged at 1,550×g for 15 minutes at room temperature. The coated 96-well plate was removed, and standards and samples were added with sealing film. The plate was incubated in a 37°C incubator for 2 hours, then the solution was removed. Each well was washed, and 100 L of biotin-labeled antibody was added with sealing film before incubation at 37°C. Subsequently, 90 L of TMB substrate was added to each well with sealing film and incubated at 37°C in the dark. Finally, 50 L of stop buffer was added to each well, gently mixed, and absorbance was measured at 450 nm using a microplate reader.

1.4 Statistical Methods

Statistical analysis was performed using SPSS 26.0 software. Normality tests were conducted for measurement data. Normally distributed data were expressed as mean ± standard deviation ($\bar{x}\pm s$) and compared between two groups using t-tests or among multiple groups using one-way ANOVA. Non-normally distributed data were expressed as median (P25, P75) and compared using rank-sum tests. Count data were expressed as percentages and compared using ^{2} tests. $P<0.05$ was considered statistically significant.

Results

2.1 Comparison of General Data and Laboratory Indicators Among Three Groups

Significant differences were observed among the three groups in smoking history, hypertension history, diabetes history, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), glucose (GLU), creatinine (Crea), and medication history including aspirin, clopidogrel, β-blockers, ACEI/ARB, and statins ($P<0.05$). No significant differences were found in age, gender, BMI, drinking history, platelet count (PLT), C-reactive protein (CRP), fibrinogen (FIB), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), or blood urea nitrogen (BUN) ($P>0.05$). See Table 1

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2.2 Morphological Characteristics of MPs Under Transmission Electron Microscopy

Transmission electron microscopy revealed that isolated MPs had intact membrane structures with clear contours and uniform disc-shaped, spherical morphology. The diameter varied from 100 to 400 nm. See Figure 1 [Figure 1: see original paper].

2.3 NTA Analysis of MPs

Nanoparticle tracking analysis showed that MPs in the Control group were predominantly below 200 nm, while both AMI and SCAD groups had additional MPs in the 600-800 nm range. See Figure 2 [Figure 2: see original paper].

2.4 Flow Cytometry Analysis of EMPs

Flow cytometry analysis demonstrated that isolated MPs were positive for TSG101 and HSP70 proteins, confirming their identity as EMPs. See Figure 3 [Figure 3: see original paper]. The results showed significant differences among the three groups in $CD31^+/CD42b^-$ MPs, $CD31^-/CD42b^+$ MPs, and $CD31^+/CD42b^+$ MPs levels (P<0.05). Specifically, $CD31^+/CD42b^-$ EMPs levels in the AMI group were significantly higher than those in both the Control and SCAD groups (P<0.005). See Figure 4 [Figure 4: see original paper] and Table 2 .

2.5 Comparison of miR-126 Levels in EMPs Among Three Groups

The expression level of miR-126 in plasma EMPs was 0.97 (0.67, 1.53) in the Control group, 1.22 (0.63, 2.12) in the SCAD group, and 0.45 (0.32, 0.73) in the AMI group. Significant differences were observed among the three groups (Z=40.459, P<0.001), with AMI patients showing significantly lower miR-126 expression compared to both Control and SCAD groups (P<0.001).

2.6 Comparison of Mitochondrial ROS Levels in EMPs Among Three Groups

ROS expression levels in plasma EMPs were (180,627.41$±98, 230.28)*intheControlgroup*, (454, 913.43±159, 697
in the AMI group. Significant differences were observed among the three groups (Z=94.296, P<0.001). ROS levels were higher in the SCAD group compared to the Control group, and highest in the AMI group compared to both other groups (P<0.001).

2.7 Comparison of Adhesion Molecule Levels in EMPs Among Three Groups

Significant differences were observed among the three groups in VCAM-1, ICAM-1, E-selectin, and P-selectin expression levels ($P<0.05$). Both SCAD and AMI groups showed higher expression of VCAM-1, ICAM-1, E-selectin, and P-selectin compared to the Control group. Additionally, ICAM-1 expression was significantly higher in the AMI group than in the SCAD group ($P<0.05$). See Table 3 .

2.8 Logistic Regression Analysis

Using AMI occurrence as the dependent variable (assignment: yes=1, no=0), univariate logistic regression analysis of miR-126, ROS, VCAM-1, ICAM-1, E-selectin, and P-selectin revealed that miR-126, ROS, ICAM-1, and P-selectin were statistically significant ($P<0.001$). Multivariate logistic regression analysis was then performed including miR-126, ROS, ICAM-1, P-selectin, and clinically relevant variables such as smoking, age, gender (male=1, female=0), BMI, TC, TG, diabetes (yes=1, no=0), and hypertension (yes=1, no=0). The results showed that miR-126 was a protective factor, while ROS and P-selectin were independent risk factors for AMI ($P<0.05$). See Table 4 .

2.9 Diagnostic Value of miR-126, ROS, and P-selectin for AMI

ROC curve analysis revealed that the AUC for miR-126 in diagnosing AMI was 0.816, for ROS was 0.892, for P-selectin was 0.728, and for the combined indicator of miR-126, ROS, and P-selectin was 0.950. See Figure 5 [Figure 5: see original paper].

Discussion

AMI is a severe cardiovascular disease (CVD) and a leading cause of morbidity and mortality globally. Accumulation of fatty acids and cholesterol participates in the atherosclerotic process, while subsequent inflammatory and immune system involvement drives the development of advanced fibrous plaques. Ulceration or rupture of accumulated lesions with acute thrombosis leads to AMI. Despite its high incidence, early diagnosis of AMI remains a major clinical challenge.

This study analyzed components of EMPs in peripheral blood of AMI patients, suggesting potential pathogenic mechanisms underlying acute AMI events and providing new directions for understanding the disease. Considering clinical differences between AMI and SCAD patients, which primarily relate to the pathophysiology of acute versus stable states, SCAD patients and healthy individuals were selected as respective controls. Our previous research demonstrated that MPs play an extremely important role in the occurrence and development of

CVD. Therefore, this study analyzed levels of miR-126, mitochondrial components, and adhesion molecules in EMPs from AMI patients and explored the role of EMPs and miR-126 in regulating adhesion molecule expression through ROS-dependent pathways after AMI, providing a scientific basis for effectively extending the therapeutic time window and reducing complications.

We first identified MPs in peripheral blood of AMI patients, SCAD patients, and healthy individuals. Transmission electron microscopy revealed that isolated MPs had intact membrane structures with diameters ranging from 100-400 nm, confirming the presence of MPs in peripheral blood of AMI patients. The intact membrane structure of MPs is essential for delivering various substances (including nucleic acids) to local and distant cells to influence cellular phenotypes. MPs are released from various activated and apoptotic cells, including EMPs. Since surface markers on MPs reflect their parental cell origin, these proteins can be used for selective isolation and identification of cell type-specific MPs. In this study, isolated MPs were positive for TSG101 and HSP70 proteins, confirming their identity as MPs. Endothelial cell-derived EMPs are small membrane vesicles shed from endothelial cells due to activation (CD62e) or apoptosis (CD31/CD42b). Using $CD31^+/CD42b^-$ to label EMPs of endothelial origin, we found that $CD31^+/CD42b^-$ EMPs levels were significantly elevated in plasma of AMI patients compared to SCAD patients and healthy individuals.

Studies have shown that miRNAs play important roles in the occurrence and development of various diseases, and changes in CVD-related miRNA levels have attracted attention due to their significant diagnostic value. This study found that miR-126 levels in EMPs obtained from peripheral blood of AMI patients were significantly lower than those in SCAD patients and healthy individuals. However, the specific molecular mechanisms underlying this phenomenon remain unknown and require further investigation. Mitochondrial dysfunction during acute myocardial ischemia is a critical determinant of cell death after AMI, as mitochondria play essential roles in producing ATP required for normal cardiac contractile function. This study found that ROS levels in EMPs from AMI patients were significantly higher than those in SCAD patients and healthy individuals. We speculate that after AMI, EMPs deliver miR-126, activating mitochondria and inducing increased ROS levels, thereby mediating endothelial cell premature senescence.

Interestingly, coronary endothelial cells in AMI patients release more inflammation-related MPs with abundant VCAM-1 surface expression. Studies have found that VCAM-1 is a direct target gene of miR-126, which inhibits VCAM-1 protein and mRNA expression by binding to the 3' untranslated region of VCAM-1, reducing monocyte adhesion and recruitment to endothelial cells and thereby exerting anti-inflammatory repair effects. This study found that VCAM-1, ICAM-1, E-selectin, and P-selectin expression were upregulated in plasma EMPs of AMI patients compared to healthy individuals. JASIEWICZ et al. demonstrated that circulatory failure in acute coronary syndrome directly affects hepatic blood flow, leading to hepatocellular dysfunction and elevated

AST. Smoking, age, gender, BMI, TC, TG, diabetes, and hypertension are known to play important roles in AMI pathogenesis. In this study, miR-126, ROS, P-selectin, and ICAM-1 were included in univariate logistic regression analysis. Multivariate logistic regression including miR-126, ROS, P-selectin, ICAM-1, smoking, age, gender, BMI, TC, TG, diabetes, and hypertension showed that miR-126, ROS, and P-selectin were independently associated with AMI occurrence, with miR-126 being a protective factor and ROS and P-selectin being risk factors. ROC curve analysis demonstrated that miR-126, ROS, P-selectin, and their combined indicator in EMPs all have diagnostic value for AMI, with the combined indicator showing the highest diagnostic value, suggesting they may serve as potential diagnostic biomarkers.

However, this study has limitations. Blood samples were collected from peripheral blood without repeated monitoring of EMPs-related miR-126, ROS, and P-selectin levels. Additionally, the sample size was small, and we plan to expand it in future studies to ensure robust results. Our research team also aims to collect follow-up data to investigate the relationship between EMPs-related miR-126, ROS, and P-selectin levels and prognosis in AMI patients. Furthermore, molecular mechanisms linking miR-126, ROS, and P-selectin levels in coronary blood EMPs to acute thrombotic events in AMI require further investigation.

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