Review Article

Circulating microRNAs, novel biomarkers of acute myocardial infarction: a systemic review

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BACKGROUND: MicroRNAs (MiRNA) are a novel class of non-coding RNAs involved in the regulation of gene expression post-transcriptionally by cleavage or translational repression of their specific target miRNAs. Numerous studies have demonstrated that circulating miRNAs are stable and abundant in blood and aberrantly expressed under pathological conditions, including cardiovascular diseases. The implications of circulating miRNAs in acute myocardial infarction have recently been recognized. This review will highlight the potential role of miRNA as a novel class of biomarkers in acute myocardial infarction.

METHODS: This systemic review is based on our own work and other related reports.

RESULTS: During diseases circulating miRNAs are derived from not only circulating blood cells but also other tissues affected by ongoing diseases. These disease-related miRNAs in the blood can serve as potential biomarkers.

CONCLUSION: The circulating miRNAs can be used as novel biomarkers potentially offering more sensitive and specific tests than those currently available for diagnosis of acute myocardial infarction.

KEY WORDS: MicroRNAs; Acute myocardial infarction; Biomarkers

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INTRODUCTION

A rapid diagnosis of acute myocardial infarction (AMI) is critical for appropriate management of patients with chest pain. Clinical presentation of echocardiography findings and cardiac markers are often nonspecific in patients with chest pain.^[1] Therefore the ideal biomarker for rapid and reliable diagnosis of AMI is still lacking. Human serum and other body fluids are rich resources for the identification of novel biomarkers, which can be measured in routine clinical diagnosis. Over many decades, it has been shown that cell-free DNA and RNA are present in serum and other body fluids and that these circulating nucleic acids may represent potential biomarkers.^[2,3] MicroRNAs (MiRNAs) are a novel class of

non-coding RNAs that are widely expressed in plants and animals to regulate gene expression post-transcriptionally by cleavage or translational repression of their specific target miRNAs.^[4] The expression of miRNAs is often tissue-specific or developmental specific and thus miRNAs play an important role in repression of gene expression at specific stages in various biological processes.

In the past years, we have witnessed the rapid development of many creative-innovative-inventive techniques and methodologies pertinent to miRNA research and applications in cardiovascular disease.^[5,6] These technologies have convincingly demonstrated their efficacy and reliability in being novel biomarkers of cardiovascular disease. Here we summarize the rapidly

expanding knowledge of the circulating miRNAs in acute myocardial infarction.

miRNA biogenesis and function

The human genome has been estimated to encode up to 1000 miRNAs that are predicted to regulate a third of human genes. According to the current understanding, miRNAs originate from a primary transcript (hundreds to thousands of nucleotides long) called a pri-miRNA which is transcribed by RNA polymerase II and regulated by transcription factors as same as conventional mRNAs; after nuclear cleavage by Drosha (a ribonuclease III) the pri-miRNA binds protein DGCR8/Pasha to generate a hairpin-shaped pre-miRNA;^[7,8] the nuclear export factor exportin-5 transports the pre-miRNA to the cytoplasm;^[9,10] and within the cytoplasm the ribonuclease III Dicer and its cofactors (PACT and TRBP) process the precursors into 19- to 25-nucleotide miRNA duplexes. The double-stranded RNA molecule dissociates and one strand is incorporated into the RNA-induced silencing complex (RISC). The miRNA-loaded RISC is capable of bringing the target mRNAs to inhibit their translation by cleavage and degradation of mRNA or by blocking translation through several different mechanisms.^[11]

Stabilization and possible release mechanisms

While miRNA presence is relevant for the regulation of several genes in tissues the possibility to extract and reliably determine cell-free miRNA content in body fluids like serum as was shown in many studies.^[12,13] The findings of the studies confirmed that miRNAs are enriched in the small RNA fraction isolated from serum samples. Because of the high levels of RNase activity in plasma synthetic miRNAs were quickly degraded,^[14] but cell-free miRNAs was stable under harsh conditions including boiling low/high pH extended storage and multiple freeze-thaw cycles.^[11-14] Filtering and differential centrifugation experiments suggest that miRNAs are not derived from cells circulating in the blood.^[15] At present there are at least two possible explanations for the origin of circulating miRNAs:

One hypothesis is that miRNAs are released during tissue injury. For example, miRNA-208, which exclusively expressed in the heart, was measured in the serum after heart tissue injury.^[16] Alternatively circulating miRNAs might have biological functions, e.g. acting as long-distance signals as is known from plants.^[17]

MiRNAs are detected in serum or plasma in a remarkably stable form and are resistant against RNase-mediated degradation.^[14,18] Increasing evidence suggests

that there are several different mechanisms that protect miRNAs from degradation. One possible mechanism is that the miRNAs are incorporated in lipid vesicles to avoid degradation. Several types of small lipid vesicles released by cells are described: microvesicles/microparticles (relatively large 100 nm to 1 μ m) are shed from the cell plasma membrane into the extracellular space and released into the blood stream under physiological and pathological conditions. The exosomes (smaller membrane fragments 30–100 nm) are derived from the endosomal compartment. In addition, apoptotic bodies (larger up to 4 μ m) are released when cells are undergoing apoptotic death.^[19]

Functional roles of circulating miRNAs

Increasing studies suggest that circulating miRNAs can regulate target gene expression. Cells can take up extracellular RNAs as shown for RNA which had been incorporated into microvesicles. In physiological and pathological conditions secreted microvesicles containing miRNAs can transfer the miRNAs to recipient cells and regulate target gene expression.^[20]

Darnell et al^[21] confirmed that the expression of miR-1 coincided with the onset of cardiomyocyte differentiation in the developing heart tube and somitic myotome development in chick embryos. In a study^[22] exosomes were identified as an active component of conditioned medium and cardiomyocyte progenitor cells released exosomes to stimulate the migration of endothelial cells. The authors of the study also indicated that endothelial cell-specific miR-126 plays an essential role in neoangiogenesis after MI. Moreover, it was shown that glioblastoma-derived microvesicles enriched with mRNAs and miRNAs can be taken up by brain microvascular endothelial cells with subsequent alteration of the genetic endothelial program.^[23]

Circulating miRNAs are stable and abundant in blood. It is believed that circulating extracellular miRNAs play important roles in cell-cell communication. However, the functional roles of circulating miRNAs and proposed mechanisms remain to be determined.

Circulating miRNAs reflecting physiological and pathological changes

With direct cloning and microarray-based profiling, a large set of miRNAs expressed in the heart have been identified *in vivo* and *ex vitro*. MiR-1 and miR-133 were found to be highly enriched in cardiac and skeletal muscle. Increasingly, miR-1 and miR-133 involved in cardiac hypertrophy were identified.^[24] da

Costa Martins et al^[25] demonstrated that miR-199b is a direct calcineurin/NFAT target gene which increases in expression in mouse and human heart failure.

Multiple miRNAs aberrantly expressed in infarcted hearts are responsible for cardiac remodeling after MI or I/R injury. Ren et al^[26] determined the miRNA signature in ischemic hearts by a mouse model of cardiac I/R *in vivo* and *ex vivo*, and they found that miR-320 expression was consistently dysregulated after ischemia. Further studies from the same group showed that the miR-144/451 cluster also protected against simulated I/R-induced cardiomyocyte death.^[27] It was found that some miRNAs such as miR-29, miR-199 and miR-24 were downregulated after myocardial infarction.^[28–30]

MiRNAs also regulate the expression of molecules involved in regulating action potentials and cardiac conduction. In a study,^[31] miR-1 was found to be upregulated in the patients with coronary artery disease (CAD) and in rat ischemic hearts and confirmed its involvement in arrhythmogenesis. Recently, Lu et al^[32] reported that miR-328 contributes to adverse electrical remodeling in atrial fibrillation (AF). These studies strongly highlight the potential of miRNAs as a novel biomarker.

Circulating miRNAs as biomarkers for acute myocardial infarction

Muscle-specific and cardiac-specific miRNAs in plasma of AMI patients were significantly higher than those of healthy subjects coronary heart disease (CHD) patients without AMI or patients with other cardiovascular diseases. miRNA-208b and miR-499 are expressed by myosin heavy chains in cardiac or skeletal muscle and both are used for detecting cardiac damage.^[33,34] In a study,^[35] miRNA-208b and miR-499 were highly increased in MI patients (>105-fold, $P < 0.001$) and they were detected in healthy controls. One hour early after the onset of chest pain, the two miRNAs were detected and their diagnostic accuracy is robust. In addition, in patients who presented less than 3 hours after the onset of chest pain, miR-499 was positive in 93% of the patients and hs-cTnT in 88% ($P = 0.78$). Patients with ST-elevation MI ($n = 397$) had a higher miRNA concentration than those with non-ST-elevation MI ($n = 113$) ($P < 0.001$).

Recent studies revealed that other miRNAs which were not muscle specific or cardiac specific have been overlooked. MiR-328 is ubiquitously distributed in many tissues and has been found to involve in many pathological conditions. Wang et al^[36] found that the miR-133 and miR-328 levels in plasma from AMI patients exhibited respectively a 4.4-fold and 10.9-fold

increase compared with those from healthy controls. The elevated circulating miR-133 and miR-328 levels were recovered to the control levels at 7 days after AMI. ROC analysis revealed that the AUCs of miR-328 in plasma and whole blood were 0.810 and 0.872, suggesting that the increased miR-328 level might be associated with AMI. In other studies, miR-1, miR-129, miR-663, miR-145 and miR-30c levels were increased in both experimental AMI models and/or in patients with AMI.^[37–38]

In conclusion, the blood contains large amounts of stable miRNAs derived from various tissues/organs and circulating miRNAs are resistant to RNaseA digestion and other harsh conditions. However, the mechanism of resistance of miRNAs to RNase requires further study. Studies unequivocally showed that circulating miRNAs can be used as a novel biomarker potentially offering more sensitive and specific tests than those currently available for diagnosis of acute myocardial infarction.

Since the results of studies have clearly indicated the expression of the miRNAs in blood from patients with AMI, their physiological functions and relationship with genesis need further investigation. The results also strongly suggest that during diseases circulating miRNAs are derived from not only circulating blood cells but also other tissues affected by ongoing diseases and that these disease-related miRNAs in the blood can serve as potential biomarkers. These novel biomarkers have the potential to revolutionize the present clinical management. Given the fact that miRNAs are identified as the first class of RNAs stably present in the blood, it would be of great interest to understand the biological functions of circulating miRNAs and their other application.

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Contributors: Chen Y proposed the study and wrote the first draft. All authors read and approved the final version.

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