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Circulating microRNAs as novel and sensitive biomarkers of acute myocardial Infarction

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Abstract

Coronary artery disease and acute myocardial infarction (AMI) are the leading causes of death for both men and women. Serum cardiac-specific troponin level is now used for the “early” diagnosis of AMI. However, due to the “delayed” release of troponin, an earlier, more sensitive and specific biomarker is urgently demanded to further reduce AMI mortality. Recent studies have found that circulating microRNAs (miRNAs) are closely linked to myocardial injury. Due to the cell-specific physiological functions and the stability of miRNAs in plasma, serum, and urine, they are emerging as sensitive biomarkers of AMI. This review summarizes the latest insights into the identification and potential application of plasma and serum miRNAs as novel biomarkers for diagnosis and prognosis of AMI.

Keywords

MicroRNA; Biomarker; Circulating; Acute myocardial infarction

Introduction

Coronary artery disease (CAD) and acute myocardial infarction (AMI) are the leading causes of death in developed and developing countries [1]. According to the American Heart Association, mortality caused by CAD in United States of America was 406,351 in 2007, accounting for about 1 in every 6 deaths. It is estimated that approximately every minute, someone in the USA dies from a heart attack [2]. AMI accounts for most of the mortality due to CAD. However, the mortality from AMI in the USA has been declining, partly due to earlier recognition and effective revascularization therapy, including percutaneous coronary intervention (PCI) and coronary artery bypass surgery (CABG) [3]. Circulating biomarkers of myocardial damage, especially cardiac-specific troponin, have led to an early diagnosis of AMI, maximizing the benefits of revascularization therapy. In AMI patients, troponin levels rise as early as 3.5 hours (hrs) after the onset of chest pain. However, due to the relative

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"delayed" release time of troponin, earlier biomarkers with both high sensitivity and specificity are urgently demanded to further reduce the AMI mortality [4].

In addition to high sensitivity and specificity, the ideal biomarker for AMI should meet three criteria [5]. (1) Good accessibility: samples must be easily obtained by the least invasive methods, such as those from the body fluids, including plasma, serum and urine. (2) Predictability: the biomarkers should have a relative long half-life in the blood, which ensures the predictability of detection. The expression signatures and levels of biomarkers should closely correlate with the extent and healing of myocardial injury. (3) Robust reliability: the detecting methods should be rapid, accurate, sensitive, inexpensive, without special requirement for instrument or equipment, which is essential for point-of-care, and wide-spread popularization. Most of the currently available biomarkers with clinical applications are proteins and polypeptides [6]. Novel biomarkers, such as molecular and genetic biomarkers, are under investigation [7]. In recent years, miRNAs have emerged as novel biomarkers due to their diverse but tissue- or cell-specific biological and pathological functions [8–10].

In this review, we summarize recent information on biomarkers for AMI, focusing on the latest insights in the identification and potential use of miRNAs in the plasma and serum as novel specific and sensitive biomarkers for AMI.

The biogenesis of circulating miRNAs

MicroRNAs are short (17–25 nucleotides) non-coding RNAs that regulate gene expression at the post-transcriptional level, either by inhibiting mRNA translation or inducing its degradation [11]. It is estimated that the human genome encodes more than 1000 miRNAs [12,13]. Indeed, up to 1424 miRNAs are in the miRBase Database (release 17.0, April, 2011) [14,15]. Most of the microRNA genes are located in the introns of protein-coding genes or as independent entities in between the genes [16–19]. As shown in Fig. 1, the primary miRNA (primiRNA) is transcribed from the microRNA genes in the nucleus by RNA polymerase II [20]. The pri-miRNAs are processed by the Drosha–Dgcr8 enzyme complex, generating a ~70-nucleotide long hair-pin-folded precursor miRNA (pre-miRNA), which is subsequently exported into the cytoplasm by the nuclear export factor, exportin-5, in a Ran-GTP-dependent manner [21–23]. In the cytoplasm, the pre-miRNAs are cleaved by an RNAse III enzyme complex, Dicer, into 20 to 24 nucleotides mature miRNAs, and subsequently incorporated into the RNA-induced silencing complex (RISC) [24]. If the miRNAs bind perfectly or almost perfectly to the target mRNAs, the 3′- or 5′-untranslated regions of the mRNAs can be cleaved and degraded [25]. If the miRNAs bind imperfectly to the mRNAs, the translation of mRNA is suppressed without degrading the target mRNAs (Fig. 1). More than 30% of human genes are predicted to be miRNA target genes [26]. The miRNA gene network consists of thousands of miRNAs and their target genes. A single miRNA can regulate one or more distinct target genes, while a single protein-coding gene can be regulated by several different miRNAs [27]. Therefore, miRNAs have diverse functions in the regulation of cellular function, including proliferation, apoptosis, and necrosis [28–30].

The existence of miRNAs in the plasma and serum has been recently demonstrated and become the focus of translational research. More than 100 circulating miRNAs have been sequenced in healthy individuals [31]. The exact origins and biological functions of circulating miRNAs under physiological and pathological conditions remain to be elucidated. Normally, most of the circulating miRNAs are derived from the liver, lung, kidney, and blood cells. Dead and living cells may release miRNAs into circulation. Extracellular non-membrane binding circulating miRNAs may be the by-products of dead

cells or cellular response after biological stimulation that is released into the extracellular space [32–34].

The miRNAs in the plasma or serum are resistant to RNAase digestion and remain stable in the RNAase-rich environment of blood. MiRNAs are stable even stabilize to extreme conditions, including boiling, very high or low pH, extended storage, and freeze-thaw cycles [32]. The circulating miRNAs per se are not intrinsically resistant to RNase digestion, their resistance with the formation of a protein–miRNA complex or by chemical modification. Circulating miRNAs tend to bond to lipid-based carriers and exist mainly in two forms: nonvesicle and vesicle-associated forms. The non-vesicle form accounts for 80% of total circulating miRNA. Argonaute 2 (Ago2) protein, a part of RNA-induced silencing complex, forms a highly stable complex with miRNA, remain stable as the by-products of dead cells, and can be detected in the circulation [33]. Nucleophosmin 1 (NPM1), primarily located in the nucleolus and involved in ribosomal processing, is also involved in the packing and exporting of circulating miRNAs [34]. High density lipoproteins (HDL), which are important reverse transporters of endogenous cholesterols [35,36], also transport miRNAs from the peripheral cells to the hepatocytes and act as mediators of cell-to-cell communication [37].

Microvesicles, also called exosomes and microparticles, contribute to contain a small portion of circulating miRNAs [38–40]. Neutral sphingomyelinase 2, which regulates the biogenesis of ceramide, is responsible for the secretion of exosomes to the extracellular matrix [41,42]. Microvesicles contain more than 1200 mRNAs and approximately 121 miRNAs and can be delivered from one cell to another. Circulating miRNA may be physiologically active and play a pivotal role in cell-to-cell communication [43].

Circulating miRNAs in acute myocardial infarction

More than 200 miRNAs exist in the heart [44–47]. The most abundant miRNAs in cardiac muscles are miR-1, let-7, miR-133, miR-126-3p, miR-30c and miR-26a; arterial smooth muscle cells have miR-145, let-7, miR-125a, miR-125b, miR-23 and miR-143 [48,49]. Some studies have reported that circulating miRNAs may be novel biomarkers for CAD and AMI [50,51]. For example, Fichtlscherer et al. [52] reported that in patients with stable CAD, plasma levels of miR-126, miR-17, miR-92a, and miR-155 are decreased, while miR-133a and miR-208a are increased. Hoekstra et al. [53] reported that stable and unstable angina pectoris could be distinguished from each other based on different miRNA profiles. Wang et al. [54] found that plasma miR-133 and miR-328 are increased 11- to 16-fold in Chinese with AMI relative to controls. Similar results were reported in Italian and Japanese; miRNA-133 is increased 12-fold (relative to values in controls) 156 minutes after the onset of AMI, a time course earlier than traditional AMI biomarkers, such as troponin I [55,56]; MiR-1 and miR-499-5p are also found to be increased while miR-122 and miR-375 are decreased relative to controls [55].

Animal studies have corroborated the elevated circulating miRNA levels found in humans with AMI. For example, the levels of miR-1, miR-133a, miR-208a, and miR-499 in the infarcted mouse myocardium are decreased; while their circulating levels are increased, indicating that the elevated circulating miRNAs are released from the infarcted heart. The elevated cardio-specific miRNAs are localized in the exosomes [56]. In adult pigs, circulating miR-1, miR-133a, miR-208b, miR-499-5p levels are rapidly increased by 70–4000-fold after induction of AMI by inflation of a balloon in the coronary artery [57]. The increased levels of circulating miRNAs after AMI are a reflection of myocardial injury, and parallel the extent of myocardial damage as measured by cardiac troponin [58] (Table 1).

miR-1

MiR-1 is primarily expressed in cardiac and skeletal muscles and consists of two subfamilies, miR1-1 and miR1-2. The two subfamilies have identical sequences, but are encoded by two distinct genes in chromosomes 18 and 20, respectively. MiR-1, involved in cardiogenesis and muscle differentiation, may regulate cardiac arrhythmogenicity by repressing the expression of the gap junction protein, connexin (GJA1) and the inward-rectifier potassium ion channel (KCNJ2) [59,60].

In the rat, the circulating miR-1 level is rapidly increased 1 hr after coronary artery ligation, and peaked at 200-fold higher than baseline 6 hrs after AMI. The elevated miR-1 level returned to basal levels 3 days after AMI, a time course earlier than traditional AMI biomarkers, such as troponin [54,61]. In the mouse, miR-1 began to rise at 6 hrs after coronary artery ligation, suggesting possible species-specific releasing time-course [55].

In a small cohort of 31 AMI patients and healthy controls, a nearly 100-fold elevation in serum miR-1 was detected 6 hrs after AMI [61]. In a larger cohort with 159 patients with or without AMI, plasma miR-1 level was found to be significantly higher in AMI patients, and the level returned to normal on discharge. The increased miR-1 level was not associated with age, gender, diabetes or the other well established AMI biomarkers. The area under the receiver operating characteristic (ROC) curve was 0.774 for separating AMI from non-AMI patients [62]. Cheng et al. [61] reported a positive relationship between the elevated miR-1 and serum creatine kinase-MB (CKMB). In Gidlöf et al. research, they found that miR-1 and miR-133a levels are strongly correlated with the renal glomerular filtration rate, indicating that the renal elimination might affect the plasma miRNA levels [57].

miR-133

MiR-133 is expressed in smooth, skeletal, and cardiac muscles, and consists of miR-133-a and miR-133-b [63]. MiR-133, a key regulator of vascular smooth muscle cell phenotypic switch, contributes to the progression of atherosclerosis [64].

In a rat AMI model, circulating miR-133a levels began to rise within 1 hr after AMI and peaked at 3 hrs to more than 1000-fold higher than baseline [54]. In contrast, in the mouse AMI model, the circulating miR-133a levels peaked at 6 hrs to approximately 13-fold above baseline [55]. Although miR-133 is also highly expressed in the skeletal muscles, acute limb ischemia does not result in increased circulating levels suggesting that the release of miRNA into the circulation after an insult is tissue-specific [65].

The above-mentioned results were also found in human studies. D'Alessandra et al. [55] investigated 33 patients with AMI and found that plasma miR-133a and miR-133b levels are at their peak about 156 minutes after the onset of symptoms. In a larger cohort comprised of 444 patients with acute coronary syndrome (ACS), the study design of which differs from others by using ACS subtypes as comparisons. The plasma miR-133a and miR-133b levels were independently associated with increased high sensitive cardiac troponin T (hsTnT) levels [65]. MiR-133a levels were significantly associated with the risk of death but lost the independent association with the all-cause mortality after adjusting for hsTnT levels, suggesting that the net reclassification improvement after incorporating the miRNA data to the traditional AMI marker, hsTnT, seemed to be minor [65]. Wang et al. [54] also reported a 4.4 fold increase of miR-133 in plasma from AMI patients and the area under ROC curve was 0.89. A positive correlation was also reported in the elevated miR-133 and cardiac troponin I. This report also indicated that miR-133 may be superior to cardiac troponin I due to the some confounding factors that may affect troponin I levels [66]. For example, in the end-stage renal disease, the lower glomerular filtration would increase the troponin I level in the plasma [67].

MiR-208

MiR-208, a miRNA mainly expressed in the heart, is encoded by an intron of the α-myosin heavy chain gene [68]. The miR-208 family includes two subfamilies: miR-208a and miR-208b. MiR-208 is involved in cardiomyocyte hypertrophy, fibrosis, and regulation of other cardiac muscle gene expression and function [69].

In a rat AMI model, plasma miR-208a began to increase 1 hr after coronary artery occlusion, peaked at 3 hrs to approximately 1000-fold above baseline, and returned to basal levels within 24 hrs [54].

Circulating miR-208 is immeasurable in healthy humans. Wang et al. [54] reported that miR-1, miR-133a, miR-499, and miR-208a are increased after AMI. Among these four miRNAs, miR-208a displayed the highest sensitivity and specificity for AMI. Widera et al. [65] confirmed the association between elevated miR-208b and AMI in a large cohort with 444 patients with ACS, and further indicated that miR-208b levels were predictive of 6 month mortality. The associations remained significant after adjustment for age and gender, but not after the adjustment for admission hsTnT levels, indicating that incorporating the circulating miRNA information to the traditional prognostic factors failed to increase the net reclassification improvements. Other similar studies failed to confirm the utility of circulating miR-208b levels in AMI patients, partly due to the rapid clearance and low concentrations of miR-208b in the circulation [70].

MiR-499 and other miRNAs

MiR-499 is also a cardiac-specific miRNA [71]. The increased levels of circulating miR-499 in patients with AMI were corroborated in the rat and mouse models of AMI [54,70]. Similar to miR-208a, plasma miR-499 concentrations are below the lower limit of detection in healthy subjects but are elevated in patients with AMI [70]. Other circulating miRNAs have also been reported to be increased in patients with AMI; miR-1291 and miR-663b are claimed to have the highest sensitivity and specificity in discriminating cases from controls [72]. These authors also reported that circulating miR-30c and miR-145 levels correlated with infarct size, estimated by troponin T release [72].

Current limitations and future directions

In recent years, miRNAs have been suggested as biomarkers for AMI diagnosis and prognosis. However, some critical issues have to be resolved before they can be applied into clinical practice. First, all the above-mentioned studies were done in a small number of cases. Therefore, global and large-scale clinical studies are required to confirm the correlation with and establish the time-related circulating miRNA levels after AMI. Second, the values of circulating miRNAs should be reevaluated when incorporating the traditional biomarkers including troponin into the current diagnostic and prognostic model. The multiple biomarker strategy might be an effective way for assessing the improvements of risk stratification beyond the risk assessment based on established risk factors [73,74]. Third, the cost involved in measuring circulating miRNAs is also very important consideration before for incorporating new biomarkers to clinical management. Fourth, some technical issues should be taken into consideration. Comparing circulating miRNA levels across studies can be confounded by multiple technical factors in the pre-analytical and analytical procedures [75,76]. A unified standard analytical method should be formulated to minimize inter-procedure bias. The current golden standard for quantifying circulating miRNA is real-time PCR. However, the procedure is specific to a specific miRNA and the analysis can take 2–3 hrs. This is a critical limitation because the time needed for quantifying circulating miRNA far exceeds the reperfusion time recommended by the latest global AMI management guidelines [77]. Therefore, the development of fast,

highly sensitive, and specific methods for the detection and quantification of ultra-low levels (femtomolar) of miRNA in blood samples is critical for the clinical application of the circulating miRNAs as biomarkers for AMI diagnosis and prognosis.

Conclusions

Circulating miRNAs are emerging as novel biomarkers for AMI. However, more large-scale multi-centered clinical studies are needed before they can be used in clinical practice. The development of fast, highly sensitive, specific, and effective methods for the detection of the circulating miRNA at ultra-low levels may accelerate the pace of the application of circulating mRNAs biomarkers in guiding the diagnostic, therapeutic, and prognostic strategies of AMI.

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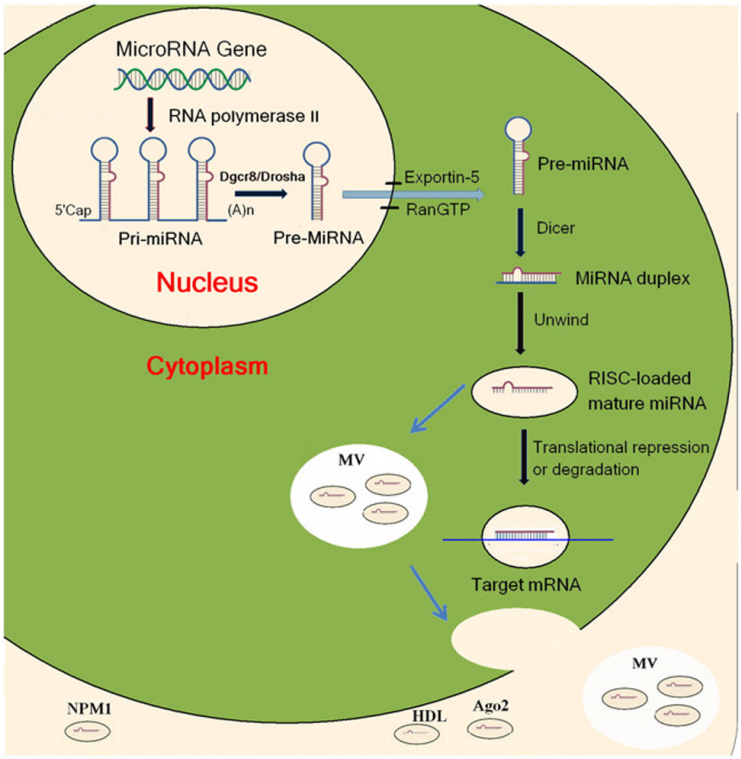

Fig. 1.

Biogenesis of miRNA and transfer to the circulation. Pri-miRNAs are transcribed from miRNA genes in the nucleus by RNA polymerase II and then processed by the Drosha-Dgcr8 enzyme complex to generate pre-miRNAs. Exportin-5 recognizes the pre-miRNAs and exports them to the cytoplasm in a Ran-GTP-dependent manner. In the cytoplasm, the pre-miRNAs are subsequently cleaved by Dicer to produce miRNA duplex. The duplex is separated and usually one strand is translated into the mature miRNA while the other strand is degraded. The mature miRNAs couple with a multiple protein nuclease complex to form RISC. The miRNAs bind to the target mRNAs to repress translation or induce degradation. MiRNAs in the cells can be incorporated into microvesicles (MV) or miRNA-binding proteins and secreted into the circulation. Argonaute (Ago2) complexes may be vesicle-independent carriers of circulating miRNA. Nucleophosmin 1 (NPM1) participates in the packing and exporting of circulating miRNA. High density lipoproteins (HDL) may act as endogenous transporters of miRNAs to the recipient cells.

Table 1

Summary of circulating miRNA levels in AMI.

miRNA	Source	Species	Patient number (n)	Control number (n)	Basal level (fold)	Time of measurable increase	Peak level (fold)	Peak Time (hr)	Duration (hr)	Reference
miR-1	Serum	Human	31	20	1	-	100	6–72	168	[61]
	Plasma	Human	33	17	1	-	15	<3	<5	[55]
	Plasma	Human	93	66	-	-	-	-	-	[62]
	Plasma	Human	33	30	1	-	-	-	-	[54]
	Plasma	Human	444	-	-	-	-	-	-	[65]
	Plasma	Human	25	-	-	<12h	~100	<12	>72	[57]
	Plasma	Rat	-	-	1	11h	~800	3	>24	[54]
	Serum	Rat	-	-	1	11h	260	6	72	[61]
	Plasma	Mice	-	-	1	6h	6	18	72–120	[55]
	Plasma	Pig			1	90 min	1.6	2	-	[57]
miR-499	Plasma	Human	14	10	undetectable	<48h	-	6–12	Before discharge	[70]
	Plasma	Rat	-	-	1	11h	~40–60	3	>24	[54]
miR-499-5p	Plasma	Human	33	17	1	9–11 h	100	9–11	>72	[55]
	Plasma	Mice	-	-	1	<15min	70	24	72	[55]
	Plasma	Pig	25	-	undetectable	60 min	1.6	>2	-	[57]
miR-133a	Plasma	Human	33	30	1	-	-	-	-	[54]
	Plasma	Human	444	-	-	-	-	-	-	[65]
	Plasma	Human	51	28	-	-	-	-	<168	[10]
	Plasma	Rat	-	-	1	11h	1000	3	>24	[54]
	Plasma	Mice	33	17	1	6h	13	6	>72	[55]
miR-133b	Plasma	Human	33	17	1	-	60	<3	>72	[55]
	Plasma	Mice	-	-	1	6h	13	6	28–72	[55]
miR-208a	Plasma	Human			undetectable	-	-	-	-	[54]
	Plasma	Rat	-	-	1	11h	~800–900	3	12	[54]
miR-208b	Plasma	Human	33	30	-	-	-	-	-	[66]
	Plasma	Human	25	-	-	<12h	~1,000	<12	>72	[57]
	Plasma	Pig	-	-	1	11h	1.75	2	-	[57]

miRNA	Source	Species	Patient number (n)	Control number (n)	Basal level (fold)	Time of measurable increase	Peak level (fold)	Peak Time (hr)	Duration (hr)	Reference
miR-30c	Whole blood	Human	22	20	-	-	-	-	-	[72]
miR-145	Whole blood	Human	22	20	-	-	-	-	-	[72]