Circulating miR-499 as a potential biomarker for acute myocardial infarction

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Abstract: Acute myocardial infarction (AMI), a common heart disease that may lead to chronic heart failure, is the leading cause of morbidity and mortality worldwide. MicroRNAs (miRNAs) are small non-coding RNAs that mediate the expression of target genes. Recently, a number of miRNAs are emerging as potential biomarkers of AMI. MiRNA-499 is a newly discovered member of miRNAs, and is mainly expressed in myocardium, the circulating levels of miRNA-499 was increased in AMI patients. This review summarizes the latest advances in the miRNA-499 study and discusses the potential of miRNA-499 to be a biomarker of AMI.

Keywords: Acute myocardial infarction (AMI); miRNA-499; biomarker

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Acute myocardial infarction (AMI) is one of the leading causes of morbidity and mortality worldwide, thus an effective and accurate diagnostic biomarker would be needed to help decrease the mortality of AMI patients. One of the traditional biomarkers for AMI is troponin. Circulating troponin levels rise around 3 hours after the onset of chest pain, due to the relative delayed release time of troponin. It is widely believed that a rapid and correct diagnosis of AMI has an important impact on patients' treatment and prognosis. Therefore, earlier biomarkers than troponin with both high sensitivity and specificity remain to be needed.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs of less than 22 nucleotides that are implicated in nearly all cellular events including cell differentiation and proliferation as well as the pathogenesis of certain human diseases (1,2). Recently, a number of findings support the notion of the implication of miRNAs in the development and progression of cardiovascular diseases (3). The plasma levels of miRNA-1, -133a, -133b, and -499-5p are up-regulated in AMI patient, and represent novel biomarkers of cardiac damage. Several reviews have provided an over-view of biology of miRNA-1, miRNA-133b, -499-5p and their involvement in cardiovascular physiology and pathology, and discussed the potential of these miRNAs to become a novel biomarker of AMI and even an effective therapeutics agent (4-8). miRNA-499 is a newly discovered member of miRNAs encoded by myosin gene family, and its expression level in plasma was elevated in the patients with AMI, a common heart disease that is the leading cause of morbidity and mortality worldwide (9). A previous study compared the diagnostic performance of miRNA-499 and some traditional biomarkers like SMB, cTnI, cTnT, CK-MB, CK, LDH, and concluded that miRNA-499 was present in plasma earlier than other conventional biomarkers of AMI, pointing to the likelihood that miR-499 can be a vital molecule in the early diagnosis of AMI. This study also found that the circulating level of miR-499 correlated well with circulating troponin I

and CK-MB, two traditional biomarkers of AMI (10,11).

In this review, we will summarize the latest advances in the study of miR-499, particularly those related to AMI patients, and highlight the potential of miR-499 to be a novel biomarker of AMI.

Biology of miR-499 in cardiomyocyte development

Cardiomyocytes, which stem from embryonic mesoderm during development, express distinct genes that encode structural proteins and transcription factors governing cardiomyocyte development (12). For example, the expression of muscle-related genes is regulated coordinately by well-known transcriptional factors including serum response factor (SRF), myocyte enhancer factor 2 (MEF2) and other transcription factors (13).

miRNA-499 was a newly discovered member of miRNAs encoded by myosin gene family, and is located in an intron of the Myh7b gene. miRNA-499a-5p, miR-499a-3p, miR-499b are three family members exhibiting high sequence homology. Recent studies have shown that miRNA-499, which is highly conserved across species, inhibits cardiomyocyte progenitor cells proliferation and promotes cell differentiation (14). There is a positive feedback between the expression of miRNA-499 and some cardiac enriched transcription factors. For example, the overexpression of miRNA-499 increased the expression of MEF2, SRF and other cardiac transcription factors that activate the expression of cardiac genes encoding many contractile proteins (15), while MEF2 was found to bind to the intronic enhancer of miRNA-499 to activate its expression in ventricular myocytes (16,17). In addition, miRNA-499 was reported to induce structural and functional differentiation of cardiac stem cells (CSCs) into cardiomyocytes, therefor promoting the recovery of heart after injury (18).

Plasma level of miRNA-499 and AMI diagnosis

Several recent studies have demonstrated that miRNAs play an important role in the development and progression of AMI. MiRNA-1, miRNA-133a, miRNA-208 and miR-499 were found to be expressed mainly in myocardium and elevated in plasma of AMI patients, thus pointing to the possibility of these miRNAs to become novel biomarkers for the diagnosis of AMI (19).

Recently, Zhang *et al*. reported that circulating miRNA-499 was substantially elevated in AMI patients as compared with non-AMI group and healthy control group, miRNA-499 was already detectable in the plasma 1 h after onset of chest pain in AMI patients, and increased continually and gradually within 9 h after onset of AMI. This study also demonstrated that miRNA-499 was highly positively correlated with the serum level of CK-MB and cTnI, as evidenced by the area under the curve (AUC) of miRNA-499 for the diagnosis of AMI 0.86 (20). Another study showed that the plasma level of miRNA-499 was significantly higher in the AMI patients than that unstable angina patients and healthy controls immediately after admission, these findings reinforce the assumption that miRNA-499 may be a valuable biomarker of AMI. Moreover, miRNA-499 levels in AMI patients with two- and three-vessel coronary artery disease (CAD) were higher than those in patients with single-vessel CAD and the plasma level of miRNA-499 at admission was significantly higher than that 24 h after PCI in AMI patients, suggesting that the level of miRNA-499 is linked to the severity of AMI, indeed, it was found that miR-499 was positively correlated with Gensini scores, which are used to evaluate the severity of coronary stenosis (21).

Circulating miRNA-499-5p, a family member of miRNA-499, was shown to have the highest increase in NSTEMI patients by ~80-fold, and exhibited the greatest discrimination between NSTEMI and CHF patients groups, in the total population, the expression levels of miRNA-499-5p was significantly correlated with cTnT, the diagnostic accuracy of miRNA-499-5p was evaluated with ROC analyses, and was comparable to that of cTnT (22). In the elderly patients with acute NSTEMI, a study showed that circulating miRNA-499-5p had better accuracy in the diagnosis compared with cTnT (23). In line with the above findings, another recent study demonstrated that plasma level of miRNA-499-5p was increased distinctly in AMI patients, suggesting that circulating miR-499-5p could be used for the evaluation of mortality risk (24).

Possibility of miR-499 as a biomarker for diagnosis of AMI

The circulating miR-499 was found to be increased in AMI patients and could serve as a potential biomarker for diagnosis of AMI, some limitations of the studies in the field need to be pointed out. Intriguingly, miR-499 was found to be expressed in skeletal muscle, Wang *et al*. reported that miRNA-208a, another muscle-enriched miRNA, had a high specificity (100%) and sensitivity (90.9%) in the diagnosis of AMI, however, the sensitivity of miRNA-499 was only 36.4% (25,26). Thus, whether miRNA-499 can be a highly

reliable and specific biomarker for diagnosis of AMI awaits further investigation.

Although several studies suggested that miRNA-499 existed in micro vesicles, and were likely to be released into the blood in physiological and pathological situations like in tumor (27,28), the findings from miRNA array analysis have shown that mir-499 is nearly specifically expressed in cardiac cell (29), and can be released into circulation when cardiac cells are injured. Moreover, miRNA-499 is a sensitive and stable marker for cardiac injury, thus offering a comparable diagnostic value for AMI (30).

However, there are two major limitations for miR-499 in clinical practice. First, miR-499 can only be detected by PCR, which is time and labor consuming, and is not facilitated in early diagnosis of AMI (31). Second, the internal reference for circulating microRNAs tests is still an issue. For instance, U6, which is usually used as an internal reference for circulating microRNA test, is not a suitable one as suggested by some study (32).

Conclusions

Since its discovery, miRNA-499 has already been shown to be potentially associated with some human diseases including atrial fibrillation, cardiomyopathy and AMI (21,33,34), and its genetic polymorphisms are also potentially linked to cancer development and autoimmune diseases (35,36). In the present review, we focused on the potential of miRNA-499 to become a highly reliable, sensitive and specific diagnostic marker for AMI. From a research perspective, all recent researches on the clinical application of miRNA-499 are still in the primary stage, and some other biological functions and regulation mechanism of miRNA-499 remain to be elucidated. From a clinical perspective, larger trials are required to compare the performance of miRNA-499 and other traditional biomarkers such as cTnT and establish a reliable reference value for circulating miRNA-499 in the early diagnosis of AMI. Although a number of challenges remain to be overcome, with more studies to be performed focusing on miRNAs to be biomarkers of human diseases and rapid advances in the field, we believe whether miRNA-499 can be a valuable biomarker for the early diagnosis of AMI in clinic as expected will be uncovered in the foreseeable future.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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