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REVIEW

Diagnosis, Prognosis and Therapeutic Role of Circulating miRNAs in Cardiovascular Diseases

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Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in the world. Although much progress has been made for cardiovascular diseases in diagnosis, treatment and prognosis during the past two decades, the clinical need for a novel diagnostic biomarker and new therapeutic interventions to decrease the cardiovascular disease incidence is ongoing.

MicroRNAs (miRNAs) are endogenous, small (~22 nucleotides), single-stranded, non-coding RNAs that regulate gene expression and are detectable in whole blood, serum, plasma, urine and other body fluids in a highly stable form. Accumulating evidence suggests that miRNAs are potential novel biomarkers with high sensitivity for early diagnosis and modern treatment for cardiovascular diseases.

Altered circulating miRNAs expressions have been reported in acute myocardial infarction (AMI), acute coronary syndrome (ACS), stable coronary artery disease, heart failure, atherosclerosis, essential hypertension and stroke. In the present review, we examine more recent data regarding circulating miRNAs and their potential roles in diagnosis, prognosis and therapeutic strategies for cardiovascular diseases. In addition, we briefly present our own recent experience in detecting circulating miRNAs, and the significance of these miRNAs in AMI prognosis.

Keywords Cardiovascular disease (CVD) • Circulating miRNAs • Biomarkers • Prognosis • Treatment

Introduction

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in the world. Acute myocardial infarction (AMI) is the single largest cause of death in the United States and responsible for one of every six deaths [1]. Over the past two decades there have been dramatic changes in the diagnosis, treatment and prognosis of cardiovascular disease, which help to reduce the mortality rate in the future. However, there is still a clinical need for a novel diagnostic biomarker and new therapeutic interventions to decrease the cardiovascular disease incidence. miRNAs are potential novel biomarkers with high sensitivity for early diagnosis and modern treatment for cardiovascular diseases [2].

miRNAs are involved in multiple functions such as proliferation, migration, differentiation, secretion, excitation, conduction, cell cycle, ageing and apoptosis. The cardiovascular system is extremely sensitive to changes in miRNAs

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level [3,4]. In the last few years, the dysregulation of miRNAs expression in tissues has been reported to directly link to cardiovascular diseases. In addition to existence in tissues, more recent studies have demonstrated that miRNAs also exist in serum, plasma, urine and other body fluids in highly stable forms that are protected from endogenous RNase activity [5]. Altered levels of circulating miRNAs have been found in patients with AMI [6], acute coronary syndrome (ACS) [7], stable coronary artery disease [8], heart failure [9], essential hypertension [10], and stroke [11]. In this review, we focus on more recent data regarding circulating miRNAs and their potential roles in diagnosis, prognosis and therapeutic strategies for cardiovascular diseases.

Expression and Stability of Circulating miRNAs

miRNAs are endogenous, small (~22nucleotides), single-stranded, non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to the 3′untranslated regions (UTRs) of their target mRNAs [12]. miRNAs that are detected in serum or plasma are collectively called circulating miRNAs, which may fulfill biological functions outside the cell and act as potential biomarkers for cardiovascular diseases [13]. Despite intense research, the origin of circulating miRNAs remains poorly understood. The source of circulating miRNAs might be vesicles (exosomes, microparticles), protein complexes or lipoprotein complexes. Although various tissues such as heart, lung, liver and kidney contribute to the circulating miRNA pool, most of the miRNAs are derived from blood cells [14,15]. The human genome is estimated to encode approximately 1000 miRNAs. Among them, more than 100 miRNAs have been identified in serum from healthy subjects [16]. Different from miRNAs in tissues, circulating miRNAs are extremely stable in boiling water. Prolonged room temperature incubation or freeze-thawed multiple times also show little effect on circulating miRNAs isolation. It has been reported that endogenous plasma miRNAs exist in a form that is resistant to plasma RNase activity [17]. When synthetic miRNAs such as cel-miR-39, cel-miR-54, and cel-miR-238 were added into human plasma, they were very rapidly degraded by endogenous RNase[18]. Although circulating microRNAs are very stable, a high concentration of proteins present in human plasma or serum might potentially interfere with sample preparation and miRNAs detection. Fortunately, many commercial kits are available now in the market for detection of circulating microRNAs.

Measurement of Circulating miRNAs

A phenol-based (TRIzol®/TRI Reagent®) extraction method is commonly used to isolate intact RNA from serum, plasma, and cerebrospinal fluid. To determine the level of a specific circulating miRNA, it is the first key step to isolate high quality of total miRNAs. However, reproducible isolation of cell-free miRNAs with high purity is a technical challenge for numerous reasons: 1) mature miRNAs are short, lack a common sequence [e.g. poly(A) tail]; 2) plasma or serum contains very low amounts of miRNA, which falls below the limit of accurate quality control by regular methods for RNA isolation; 3) high plasma level of proteins might interfere with sample preparation and the following measurement. For these reasons, it is easy to understand that the different quality of total miRNAs isolated by different research groups may account for, at least partially, the variability in the data from various studies. Fortunately, some companies have developed kits that are specifically designed for the isolation of high quality circulating miRNAs [19], which present a good opportunity for reduction of the discrepancy in circulating miRNAs reports among different groups caused by variations in miRNAs preparation.

Absence of a commonly accepted internal control, reference or comparator miRNA in serum samples is another major reason for the variability in the data from different labs. Quantitative real time PCR (q-PCR) is the most commonly used method to quantify circulating miRNAs level and usually performed with a SYBR Green fluorescent dye. In this method, some large small RNA species (such as U6 RNA, cel-miR-39) are often used as a control to normalise the q-PCR data. Unfortunately, the recovery efficiency of these large exogenous small RNA from plasma is actually pretty low [20]. Therefore, finding commonly acceptable controls is a guarantee for reproducible results in circulating miRNAs measurement. Depending on our own experience, the usage of synthetic mimic miR-156 instead of commonly used cel-miR-39 during RNA extraction from plasma of AMI patients can obtain much higher quality of total RNA (OD ratio: 1.8-2.2). Additionally, the usage of endogenous miR-156 instead of commonly used U6 for normalisation control during real time-PCR can achieve more reproducible results (ct value: 18-25) (unpublished data). More information on methodological issues for miRNA isolation and detection is available in a recent review article [21].

Circulating miRNAs and Cardiovascular Diseases

Recently, numerous studies have shown that plasma levels of many miRNAs were significantly changed in cardiovascular diseases. Some of them might become novel biomarkers for early diagnosis and new drug targets for cardiovascular diseases such as AMI, heart failure, hypertension or stoke, whereas some of them may have therapeutic potential against cardiovascular diseases. Table 1 summarises the most important circulating miRNAs and their role in cardiovascular diseases. Among them, ~ 50 circulating miRNAs displayed here are believed to be associated with cardiovascular diseases and some of them (such as miR-208, miR-499 and miR-1) will be discussed in detail in the