Micrornas as Mediators and Novel Biomarkers of Cardiovascular Disease

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Abstract

Among the various strategies demonstrated for the post-transcriptional silencing of genes, micro RNA (miRNA or miR) has a central pivotal role in the epigenetic regulation of gene expression. Recent studies demonstrated that miRs have role as mediators and novel biomarkers in the detection of Cardiovascular Disease (CVD). In general, the myocardium-derived miR such as miR-1, miR-133a and 133b, miR-208 and miR-499 were found to be increased in CVD. Among the various miR involved in the CVD, level of miR-1, miR -122, miR -126, miR -133a, miR -133b, and miR-199a were positively modulated in both unstable and stable angina patients. A positive modulation of miR-337-5p instable angina and miR-145 in unstable angina was also demonstrated. While endothelium derived miR such as miR-126 was reduced. Furthermore, the plasma level of miR-1, miR-30a and miR-133a, were described as novel markers of arterial remodeling. Stability of miR in the circulation as well as the sensitivity of the detection contributed their significant role as biomarkers in CVD. The most common sensitive and accurate method for the detection is real-time RT-PCR. However, lack of consistency between the reported miR markers certainly gives rise to concern about the pre-examination, examination and post-examination phases of the analysis. Furthermore, currently no known extracellular housekeeping RNAs that can be used for normalization is reported. This review article discusses the role of miR as mediators and novel biomarkers in CVD.

Keywords: Micro RNA; Cardiovascular disease; Stable angina; Unstable angina; Myocardial infarction

Introduction

Cardiovascular Disease (CVD) is one of the leading causes of approximately 30% mortality worldwide [1]. Biomarkers for the arterial remodeling in CVD can significantly contribute to its intervention. microRNAs (miRNA or miR), small non-coding RNAs of 18-25 nts length belonging to the category of naturally occurring small interfering RNA, are synthesized from the human genome. They are involved in a wide variety of physiological processes including cell proliferation and differentiation, immunity, metabolism and programmed cell death [2]. They mediate intercellular communications and were also involved in the transmission of biological signals between the cells. Most of the biological effects of miR are attributed to their role as the major post-transcriptional inhibitory regulators of gene expression by binding to complementary messenger RNA (mRNA). Role of miR were found in various metabolic diseases like type 2 diabetes, atherosclerosis, obesity and metabolic syndrome. Previous studies emphasize the role of circulating miR as bio markers in cancer, neurodegenerative disorders and diabetes [3,4]. The role of miR as novel biomarkers in cancer diagnosis and prognosis was recently reviewed [5]. Despite a few studies, the association of miR with circulating lipoproteins and formation of coronary artery disease are fragmentary [6]. Considering the important functions in the regulation of endothelial cells (ECs) and myocardiocytes, they might be involved in the pathophysiology of atherosclerosis. Araldi et al. [7] recently demonstrated the association of miR with the complication of arterial fibrillation or remodeling [7]. Several miR were involved in the biological processes and have direct relevance to cardiovascular complications especially associated with the diabetes. However, the role of extracellular miR in the regulation has not yet been elucidated completely [8]. This review article discusses the recent update on the role of miR as mediators and novel biomarkers of CVD.

Formation of Micro RNA and Post Transcriptional Silencing of mRNA

Lee et al. [9] discovered the miR in 1993 since then studies demonstrated their expression as diagnostic as well as prognostic marker in several human ailments [9]. The formation of miR is
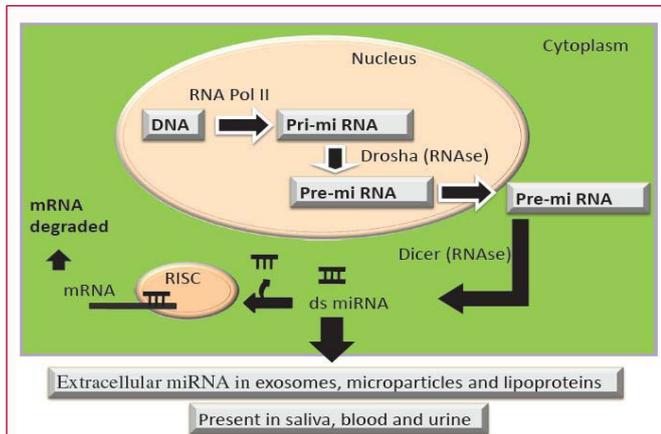
Figure 1: Formation of micro RNA (miRNA). It is formed in the nucleus and translocated to cytosol and later forms double stranded miRNA. One of the single strands of miRNA incorporate in *RNA-induced silencing complex (RISC). RISC processes the mRNA to degrade.*

Figure 2: Role of micro RNA (miR) as mediators of cardiovascular disease. Pathways mediated by molecules such as *Transforming growth factor beta* (TGF-beta)/Smad3, phosphoinositide 3-kinase (*PI3K*)-AKT and mitogen-activated protein kinase (raf-MEK-ERK) are involved in the inflammatory, proliferative, fibrosis and apoptosis mechanisms associated with Atherogenesis. MI (Myocardial infarction) can also initiate the inflammation and further fibrotic changes which are inhibited by various miR.

depicted in Figure 1 [10]. About 5% of the total genes in human genome are transcribed in the nucleus mainly by RNA pol II as a long primary (pri)-miR. This is subjected to Drosha, an RNase III action to form pre-miR of ~70 nts. They are further cleaved in the cytosol by another RNase III, Dicer to form 18-25 nts long mature dsRNA. One of the strands of this mature miR will be incorporated in the ribonucleoprotein complex called RNA-Induced Silencing Complex (RISC), while the other strand degraded in the cytoplasm. The RISC complex will direct the binding of miR to the 3' or 5' untranslated regions of the targeted mRNA which results in the degradation of mRNA and, thereby, the post transcriptional silencing of the mRNA. Cellular miR which are exported in vesicles and in circulation they are transported by membrane-derived vesicles such as exosomes, microparticles, lipoproteins and other ribonucleoprotein complexes. Thus, they kept them away from the degradation by extracellular RNase. The lipoprotein particles bound miR can be transferred to recipient cells where they change the gene expression by intercellular communication [11]. In serum/plasma or urine they remain stable can withstand the repetitive freezing and thawing cycles. The presence of miR in ECF such as saliva, blood and urine can be detected by quantitative real-time polymerase chain reaction.

Role of miRNA as Mediators of Cardiovascular Disease

The miR expression profile is unique to a tissue. Clauss et al. [12] demonstrated that miR was differentially expressed in the plasma of marathon runners. The plasma level correlates with the left atrial diameter suggesting that circulating miR could potentially serve as biomarkers of atrial remodeling in athletes [12]. A few of the miRs and their role in the cell proliferation, fibrosis, differentiation and apoptosis during the inflammatory process associated with atherosclerosis is depicted in Figure 2. The role of miR in the vascular remodeling associated with the atheroma formation, differentiation of cells such as macrophages of the immune system and smooth muscle cells of the arterial intimal region were demonstrated as well. miR-1 which is found in the skeletal and smooth muscle cells is closely related to the physical and pathological processes of vascular modeling, heart development, arrhythmias, ischemia, myocardial infarction and cardiac hypertrophy. miR-1 inhibits the cardiac hypertrophy thus protecting the heart from hypertrophy-related injury [13]. Therefore, over expression of miR-1 may inhibit the hypertrophy which was
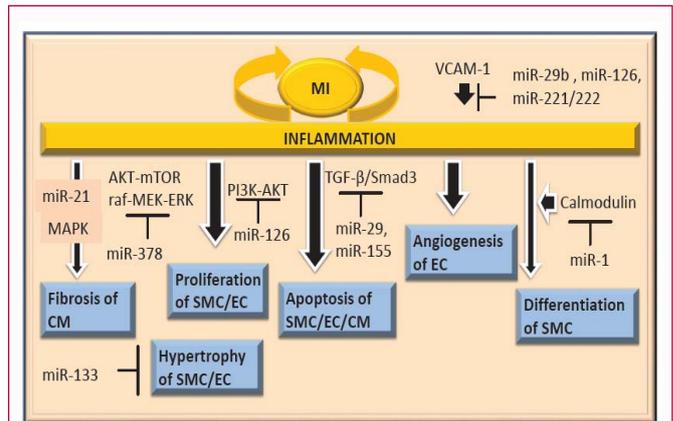
mediated though the down regulation of expression of calmodulin, main mediator of calcium signaling. This will finally limit the calmodulin activity within cardiomyocytes and, thereby, the calcium mediated cardiomyocyte growth and function. miR-21 regulates the fibrosis in fibroblasts and apoptosis in cardiomyocytes [14]. It can involve in the proliferation and migration of endothelial cells. The fibrotic effect was mediated through the up-regulation of mitogen-activated protein kinase phosphorylation [15]. This was supported by the observation that an increased miR-21 level in the fibroblasts was evidenced in the failing heart. Therefore, knockdown of miR-21 can prevent the fibrosis of myocardium [14]. Members of miR-29 family such as miR-29a, b, and c may degrade certain genes relating to fibrosis such as collagens, elastin, fibrillins and can thus able to suppress TGF-β/Smad3 signaling pathway which finally produce the fibrotic changes in cardiac myocytes. miR-29b showed as a specific inhibitor of angiotensin II-induced cardiac fibrosis [16]. Despite the beneficial role, these miR members were found to be down-regulated during the myocardial infarction [17]. Similarly, miR-133 family such as miR-133a-1 and a-2 were expressed in cardiac smooth muscle cells and are able to regulate their differentiation and proliferation. miR-133 modulates the hypertrophy during heart failure probably mediated by a signal transduction kinase, Cdc42 [18]. Hence, the inhibition of miR-133 can regulate the sustained cardiac hypertrophy. Some of the miRs were involved in the inflammatory cascade. miR-126 can regulate the angiogenesis during the stages of embryonic development and inhibits the inflammation mediated *via* direct inhibition of expression of vascular cell adhesion molecule-1 and, thereby, prevents the adhesion of leukocyte to endothelial cells. miR-126 targets the genes of proteins involved in the phosphoinositol-3 kinase (PI3K)-AKT and ras-MEK-ERK signaling pathways [19]. miR-155 is expressed in Smooth Muscle Cells (SMCs), Endothelial Cells (ECs), and macrophages and can able to inhibit the TGF-beta-induced phosphorylation of SMAD2 [20]. During the early lesion, LDL and mildly-oxidized LDL facilitate the endogenous miR-155-mediated macrophage activation and foam cell formation [21]. The regulation of miR 126 and 155 may suppress the inflammatory cascade associated with the atherosclerosis and further leading to the inhibition of cardiomyocytes hypertrophy. miR-146 a/b can up

regulate the components downstream to the Toll-like receptor 4 signal pathway such as interleukin-1-receptor-associated kinase 1 and tumor-necrosis-factor-receptor-associated factor 6 and, thereby, pays an important role in CAD [22]. miR-221/222 aggravated the intimal growth in SMCs, but mitigated the angiogenesis, inflammation or atherosclerosis in ECs. miR-221/222 decreased the inflammation through inhibition of the target gene, vascular cell adhesion molecule [23]. Over expression of miR-378 can inhibit the epinephrine induced cardiac hypertrophy through the down regulation of growth factor receptor-bound protein2, and inhibit the raf-MEK-ERK or PI3K-AKT signaling pathway through ras [24]. miR-378 can also inhibit ras-related signaling pathway to prevent from cardiac hypertrophy. *In vitro* studies revealed that miR-486 aggravate the cholesterol accumulation in cells by targeting the 3'UTR of histone acetyltransferase-1 which eventually down regulated mRNA and thereby, the protein expression [25]. miR-486 directly suppresses NF-κB-negative regulators and, thereby, exhibit anti-inflammatory activity [26]. Furthermore, miR-486-5p can target the gene of SMAD2, a crucial mediator of fibrosis was identified to be one of target genes of miR-486-5p [27].

Role of miRNA as Novel Biomarkers in Cardiovascular Diseases

Evidences suggest that miRs are found to be altered in many pathophysiological conditions of CVD [28]. They finely regulate lipid metabolism and the progression of atherosclerosis. However, the exact role played by them has not yet been elucidated completely. The expression of a large number of miRs has described in ECs and cardiomyocytes. Some of them were demonstrated as novel biomarkers in CVDs. According to Wang et al. [29] the plasma levels of miR-1, miR-133 family, miR-208a and miR499 potential value as biomarkers in CVD [29]. The muscle-enriched miR-1, miR-133 (a and b) and miR-499-5p were elevated in patients with Acute Myocardial Infarction (AMI). The heart and skeletal muscle expressed miR-208 showed a superior ROC curve when compared with miR-1, miR-133a, and miR-499. Furthermore, miR-208 level is not detected in healthy control and showed well correlation with troponin I and creatinine kinase. This suggested miR-208 as a novel potential biomarker for early diagnosis of AMI [29]. D'Alessandra et al. [30] demonstrated that high *Area Under the* Receiver Operating Characteristic *curve (AUC)* value for miR-1 and miR-126 instable angina (SA) and for miR-1, miR-126 and miR-133a in Unstable Angina (UA) patients when compared to controls. However, no discrimination could evidence between SA and UA patients found in the AUC values either alone or in combination [30]. miR-33 (miR-33a and -33b) and miR-122 represent one of the most interesting and attractive targets for metabolic-related disorders [31]. miRs were found to be transported in low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Therefore, HDL- and LDL-miR may represent a novel class of disease biomarkers [32]. Highest level of serum miR-486 and miR-92a were associated mainly with HDL lipoprotein. Hence, their level can discriminate between stable and vulnerable CAD patients [33]. Michell and Vickers were also reported that the HDL-miR-92a and miR-486 levels were increased in AMI and unstable angina [34]. A group of miR such as miR-486, miR-92a, miR-122, miR-125a, miR-146a and miR-33a were significantly increased in patients with SA/UA when compared to the healthy controls even one month after the onset of AMI [22,35]. miR-150 level with AUC of 0.845 was reported in AMI patients, especially in ST-segment elevation [36,37]. Cardiac muscle-enriched miR such as

miR-133a, miR-208a level were found to be elevated in patients with CAD while the irculating levels of miR-126, miR-26a-5p, miR-191-5p,miR-17, miR-92a, miR-155 and smooth muscle-enriched miR-145 were significantly reduced when compared to the healthy controls [38,39]. Among the family of miR-208, miR-208b was highly elevated in some AMI patients than the patients with chest pain with normal angiograms. A good correlation was found with the level of troponin-T (TnT) [40]. miR-208 a has a higher sensitivity (100%) and specificity (90.9%) for diagnosing AMI within 4 h of the onset of symptoms. It was undetectable in non-AMI patients [40]. Circulating miR-499-5p also showed a diagnostic accuracy comparable with TnT and superior to miR-208 [41]. Discrimination of AMI was accurate for miR-208b (AUC=0.82) and miR-499-5p (AUC=0.79) but considerably lower than for Tn T (AUC=0.95) [42]. Though increased miR-208b and miR-499-5p levels were strongly associated with increased risk of mortality or heart failure within 30 days, but found poor association when adjusting for TnT [42]. Found that plasma level of miR-499 was below the limit of detection in AMI patients [43]. Reported that circulating miR-24 may predict CHD in patients with type 2 diabetes [44]. It can also discriminate the type 2 diabetes patients with CHD from CHD patients (AUC: 0.953).

Conclusion and Future Perspectives

Common methods for detecting miR included the extremely sensitive and accurate quantitative real-time polymerase chain reaction [45]. Since miR has central role in the regulation of gene expression, they can be possibly developed as reliable diagnostic, predictive or prognostic biomarkers in CVD. Their stability in extracellular fluids, measurement of multiple miR simultaneously and tissues specific expression might improve the accuracy of the diagnostic test. Further, the plasma level of miR were not affected by age, body mass index, gender, white blood cell count, kidney function or by, systolic blood pressure. However, a quality control assisted rapid, simple, efficient, sensitive and reproducible detection method has to be developed for their wide application. Most of the available data derived from a small numbers of patients and the prognostic impact of miRs has been examined in few studies. A detailed mechanism of each miR in the pathology of atherosclerosis also required to explore them as biomarkers. Therefore, larger prospective population based studies are mandatory to elucidate the contribution of circulating miRs to recommend them as validated biomarkers.

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