Microrna Associated With Generation, Development, and Maintenance of Atrial Fibrillation

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Abstract

MicroRNAs (miRNAs) are a class of small non-coding RNAs, playing important roles in the regulation of the cardiovascular function. MiRNAs are associated with the generation, development, and maintenance of atrial fibrillation through the regulation of the gene expression on targets of ion channels, transporters, calcium binding proteins, extracellular matrix proteins, and other factors. Understanding the relationship between miRNAs and atrial fibrillation is critical to identifying the diagnostic markers, prognosis and therapeutic targets.

Keywords: MicroRNA; Atrial fibrillation; Gene regulation; Electrical remodeling; Structural remodeling

Abbreviations

AF: Atrial Fibrillation
Ang II: Angiotensin II
APD: Action Potential Duration
AT1-R: Angiotensin II Type 1 Receptor
CACNA1C: Calcium Channel Voltage-Dependent L Type Alpha 1C Subunit

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Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia which affects quality of life drastically [1]. The incidence of AF is 16.8% and 7.6% in men and women, respectively [2]. AF can cause and exacerbate heart failure. Also it is a significant risk factor for ischemic stroke [3,4] and therefore a major cause of cardiovascular morbidity and mortality [5]. Although a copious body of both basic and clinical research exists on AF, not all of the specific pathophysiological mechanisms of the generation, development and maintenance of AF are fully understood. Evidence shows that multiple factors are involved in the generation and development of AF. These factors include oxidative stress, systemic inflammation, autonomic imbalance, endocrine disorders, early electrical remodeling, such as shortened atrial effective refractory period (ERP) and late mechanical remodeling, such as cardiac hypertrophy and myocardial fibrosis [7]. Recently a few studies have unveiled that miRNAs expression and/or deregulation can influence the generation, development and maintenance of AF. This review
focuses on the current understanding about the role and function of miRNAs in AF.

**Biogenesis and Function of miRNAs**

miRNAs are highly conserved, non-coding endogenous and single-stranded RNAs, which bind to the 3'-untranslated regions (UTRs) of target mRNAs to regulate the expression of target genes [8]. miRNA-mediated post transcriptional gene regulation involves the degradation of the target mRNA or the suppression of its translation depending upon the sequence complementarity between the miRNA seed-sequence and the 3'-UTR of the target mRNA. Partial binding between seed sequence and target mRNA results in the repression of translation, whereas extensive pairing complementarity leads to the degradation of target mRNA [9-11].

The multiple-to-multiple relationship between miRNAs and their targets is recognised [12]. In such relationships one miRNA can target several genes and similarly several miRNAs or a miRNA cluster can target one single gene. Generally, the biological processes of miRNA biogenesis include four steps. First, generation/transcription of primary transcript called primary-miRNA (pri-miRNA) is mediated by RNA Polymerase II in the nucleus [9], which is then polyadenylated and capped by 7-methylguanosine [13]. Second, the pri-miRNA is cleaved by Drosha, a ribonuclease (RNase) III enzyme, with its co-activator partner, DGCR8 (DiGeorge Syndrome Critical Region 8, a microprocessor complex unit) to form precursor miRNA (pre-miRNA) [14,15]. The third step involves the cytosol export of pre-miRNA by the protein Exportin 5/Ran-GTP complex [16] and further cleavage into a mature miRNA by Dicer, another RNase III enzyme [14]. In the fourth and the last step, the double-stranded mature miRNA is dissociated and incorporated into the miRNA-induced silencing complex (RISC) which then targets complementary miRNAs [15,17] (Figure 1). Typically, miRNA expression is time and tissue type-specific [18].

![Figure 1: Biogenesis and function of miRNA.](Image)

The nucleus primary-miRNAs (pri-miRNA) are transcribed from long miRNA genes mediated by RNA polymerase II and cleaved by the ribonuclease III (RNase III) endonuclease Drosha with its partner DGCR8 to turn into precursor-miRNAs (pre-miRNA), which then move from the nucleus to the cytoplasm where the cytoplasmic enzyme Dicer further cleaves the pre-miRNA into double-stranded mature miRNA. The mature miRNAs are incorporated into the RNA-induced silencing complex (RISC). A mature miRNA binds to its target mRNA at 3'-UTR, leading to mRNA degradation or transcriptional repression.

miRNAs are associated with many physiological processes: embryonic development, cell proliferation, differentiation, apoptosis, autophagy, and tissue or organ formation and remodeling. Especially in the heart, miRNAs play a vital role in angiogenesis, fibrosis, myocardial cells regeneration, and cardiac remodeling and therefore considered to be strong potential tools for the diagnosis and treatment of heart related diseases [9,19-21]. MiRNAs can affect cardiomyocyte excitability and conductivity by the regulation of ion channels permeability, transporters and associated regulatory proteins which can lead to different forms of arrhythmias [22]. The expression of miRNA spectrum in atrial tissue is significantly different in mitral stenosis patients with AF compared with healthy subjects [23]. AF alters the miRNA expression profiles of the left atria of mitral stenosis patients with AF compared with healthy subjects [24], suggesting that miRNA may be involved in the generation and development of AF. In fact, AF is a complex pathology, involving both primary electrical and structural factors. Different miRNA families are associated with different aspects of AF (Table 1).

**Role of miRNAs in the Generation and Regulation of AF**

The mechanisms of AF are complex, but recent studies have shown that miRNAs contribute to the generation and regulation of AF in several ways (Figure 2). For example, miRNAs can induce cardiac immune/inflammatory response [38,39] and affect ion channels as well as structural and electrical remodeling including intracellular calcium overload [40]. These, in turn, can result in shortening of the action potential ERP and APD, which is conducive to the formation of reentry [4].

Two mechanisms generate and develop atrial fibrillation by making substrate more prone to reentry. MiR-1, miR-26, miR-328 and miR-499 regulate atrial electrical remodeling through the modulation of ion channel (Iion, Icalc) activity and expression. MiR-19, miR-21, miR-29, miR-30, miR-133, miR-155, miR-208 and miR-590 result in the increase of collagen secretion and extracellular matrix deposition which leads to atrial structural remodeling. Iion, inward rectifier potassium current. Icalc, intracellular calcium current.

**miR-29: Role in cardiac immune response**

MiR-29 has a crucial role in the generation and development of AF. The down-regulation of miR-29 can induce the fibrosis of the liver [41] and lung [42] and can also cause aneurysms [43]. The risk of AF prevalence is increased by 30% with celiac disease [44]. Overall, inflammation and chronic inflammatory diseases, such as psoriasis, also increase the risk of AF [45]. Therefore, AF is partially moderated by the humoral and systemic inflammatory response induced by local fibrosis. The miR-29 family (miR-29a, miR-29b and miR-29c) is expressed in T and B cells, dendritic cells and thymic epithelial cells, which play a regulatory role in the adaptive immune pathway [46]. Multiple biopsy specimens in left atria with AF have shown lymphocytic myocarditis in 66% of patients and noninflammatory localized cardiomyopathy in 17% of patients [47]. In the canine model of AF, miR-29b was found to target collagen-1A1 (COL1A1), collagen-3A1 (COL3A1) and fibrilin [30], which are the prime markers of tissue remodeling. Moreover, the serum level of miR-29b is reduced in patients with AF by 54%, strongly implicating...
miR-26, miR-328 and miR-499: Role in regulation of ion channels

The miR-26 family (miR-26a-1, miR-26a-2 and miR-26b) can increase susceptibility to AF by altering the inward rectifier potassium current (I_K1). I_K1 is the major potassium current of myocardial repolarization, which partially regulate cell excitability and APD. The lower expression of miR-26 in patients with AF and murine atrial tissue induces Kir2.1 and KCNJ2 expression and shortens APD, suggesting that miR-328 plays a central role in atrial electrical activity. MiR-499 has been recently shown to target KCNN3 which encodes the small-conductance Ca^{2+}-activated K^{+} channel 3 (SK3) and potentially contribute to the electrical remodeling event in AF. [37].

miR-1 and miR-133: Role in regulation of Ca^{2+} cycling

Mir-1 and miR-133 are two major muscle-specific miRNAs whose deregulation has been associated with the extrasystolic spontaneous Ca^{2+} release from sarcoplasmic reticulum and the generation of arrhythmogenesis after depolarization [22,56]. Increased occurrence of spontaneous Ca^{2+} release at the molecular level was found to be due the dissociation of ryanodine receptors (RyR2s) with the protein phosphatase (PP2A) resulting in increased phosphorylation of RyR2s. In canine model of heart failure, high levels of miR-1 and miR-133 were found to be correlated with the down regulation of the regulatory and catalytic subunits (B56α and B56β) of PP2A and the subsequent increase in calcium/calmodulin-dependent protein kinase II (CaMKII) mediated RyR2 hyperphosphorylation resulting in Ca^{2+} release [57].

Role of miRNAs in the Maintenance of AF

AF is maintained by a variety of factors. For example, local atrial structural remodeling (atrial dilatation, tissue fibrosis and scarring etc.) contributes to the formation of reentry substrate resulting in the abnormalities of the atrial internal electrical conduction. The miRNA-mediated regulation of the connective tissue growth factor (CTGF), lypol oxidase (LOX) and its contribution towards this ailment. The pro-inflammatory factors like transforming growth factor-β (TGF-β) [48] and nuclear factor-κB (NF-κB) are reported to be the upstream regulators of miR-29 [49].

Table 1: Different miRNAs with potential roles in atrial fibrillation.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target Function</th>
<th>Possible role in AF</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-1</td>
<td>KCNJ2, GJA1, RyR2</td>
<td>Abnormal Ca^{2+} handling</td>
<td>[25]</td>
</tr>
<tr>
<td>miR-19</td>
<td>CTGF</td>
<td>Extracellular matrix, myocardial fibrosis</td>
<td>[26]</td>
</tr>
<tr>
<td>miR-21</td>
<td>PDCD4, PTEN</td>
<td>MMP-2 expression increase, extracellular matrix, myocardial fibrosis</td>
<td>[27,28]</td>
</tr>
<tr>
<td>miR-26</td>
<td>KCNJ2</td>
<td>Increase</td>
<td>[29]</td>
</tr>
<tr>
<td>miR-29</td>
<td>COL1A1, COL3A1</td>
<td>Collagen secretion, inflammation</td>
<td>[30]</td>
</tr>
<tr>
<td>miR-30</td>
<td>Ang II</td>
<td>Myocardial hypertrophy</td>
<td>[31]</td>
</tr>
<tr>
<td>miR-133</td>
<td>ERK1/2 TGF-β1 TGF-β2 TGF-β3</td>
<td>Myoblast proliferation, myogenesis</td>
<td>[32,33]</td>
</tr>
<tr>
<td>miR-155</td>
<td>AT1-R</td>
<td>Endothelial dysfunction, remodeling, inflammation</td>
<td>[34]</td>
</tr>
<tr>
<td>miR-208</td>
<td>THRAP1 myostatin</td>
<td>Myocardial hypertrophy</td>
<td>[35]</td>
</tr>
<tr>
<td>miR-328</td>
<td>CACNA1C CACNB1</td>
<td>I_{Ca^{2+}} reduction</td>
<td>[36]</td>
</tr>
<tr>
<td>miR-499</td>
<td>KCNN3</td>
<td>Abnormal Ca^{2+} handling</td>
<td>[37]</td>
</tr>
<tr>
<td>miR-580</td>
<td>TGF-β1 TGF-β2 TGF-β3</td>
<td>Collagen content increase</td>
<td>[33]</td>
</tr>
</tbody>
</table>
miR-21 and miR-590: Role in collagen deposition

MiR-21 is involved in a variety of cancers and participates in tumor cell apoptosis, invasion and metastasis [59]. In the cardiovascular system, however, miR-21 leads to atrial fibrosis and structural remodeling and increases the risk and duration of AF [60]. MiR-21 has shown to increase the risk of AF. The phosphatase and tensin homolog gene (PTEN) has been identified as a miR-21 target gene. The PTEN-Akt pathway participates in extracellular matrix (ECM) remodeling in atria through MMP2 regulation which leads to atrial fibrosis [27,28]. A recent study has identified another target of miR-21 called sprouty homolog 1 (Spry1) jointly involved in the regulation of atrial fibrosis [58]. The lower Spry1 level activates the mitogen-activated protein kinase (MAPK) signaling pathway, increases the number of fibers, and promotes the secretion of growth factors, leading to myocardial fibrosis and tissue remodeling [61,62]. Angiotensin II (Ang II)-induced factors, such as Ras-related C3 botulinum toxin substrate 1 (Rac1), CTGF and LOX, decrease the level of miR-21 resulting in increased Spry1 level in atrial tissue which affects electrical conduction and atrial structure remodeling [26,58]. In mice with increasing age, transgenic cardiac overexpression of Rac1 results in spontaneous AF and fibrosis. However, the inhibition of the expression of miR-21 can effectively prevent atrial fibrosis, reducing associated risk factors for AF.

MiR-590 has recently been correlated with AF due to its role in reducing collagen production and deposition, which is a significant source of atrial fibrosis. In nicotine induced canine models, collagen content was found to be significantly increased, coincident with an increase in TGF-β/TGF-βRII and a decrease in miR-590 levels [33].

miR-155 and miR-19: Role in activation of renin-angiotensin-aldosterone system

MiR-155 can target the 3’-mRNA of Ang II type 1 receptor (AT1-R) and inhibit AT1-R activation. Endothelial dysfunction, vascular remodeling, and inflammation are prevented by miR-155, which slows down the changes in cardiac structure and reduces the progression of AF [34]. MiR-155 levels in atrial tissue may be reduced in patients with AF, causing AT1-R activation, and in turn leading to a series of changes in cardiac structure and myocardial tension.

It has been shown that in the newborn mouse cardiomyocyte, the high level of miR-19b significantly reduces Ang II-induced CTGF expression, decreases the accumulation of atrial ECM, and prevents atrial fibrosis [26].

miR-133 and miR-590: Role in regulation of myogenesis and atrial remodeling

MiR-133 (miR-133a and miR-133b) is involved in myoblast proliferation and differentiation. Through the next generation sequencing (Solexa), both miR133a and miR-133b were found to be upregulated during myogenesis. In C2C12 myoblasts, miR-133a and miR-133b were found to inhibit proliferation and promote differentiation by regulating ERK1/2 phosphorylation. Fibroblast growth factor 1 (FGF1) and PP2Ac (PP2Aca and PP2Acβ) are reported to be the potential targets of miR-133a/b [32]. Several studies have reported that the down-regulation of miR-133 was observed in chronic cases of AF [33,63]. Corresponding study of the upregulation of TGF-β and TGF-βRII in canine model of AF confirms that TGF-β and TGF-βRII are miR-133 targets in atrial fibrillators [33].

miR-30 and miR-208: Role in myocardial hypertrophy/atrial fibrosis

Atrial fibrosis is characterized by the structural alteration and hypertrophy of cardiac myocytes. Recently dysregulated autophagy has been implicated as a leading cause of myocardial hypertrophy. In the rat model of cardiac hypertrophy, a group has reported the upregulation of an autophagy related gene beclin-1 and a corresponding decrease in the expression of miR-30 in cardiac tissue [31]. Moreover CTGF, a key pro-fibrotic protein, was also found to be a target of miR-30 in rodent model of heart disease [64], implicating a negative regulatory role of miR-30 in the maintenance of AF.

MiR-208 is mainly expressed in cardiac tissue and this family consists of miR-208a and miR-208b encoded by the intron of a cardiac muscle myosin heavy chain gene (Myh6) and β-cardiac muscle myosin heavy chain gene (Myh7), respectively [65]. Thyroid hormone-associated protein 1 (THRAP1) and myostatin, both of which are negative regulators of muscle hypertrophy, are reported targets of miR-208 family. Transgenic expression of miR-208 in murine heart has proven hypertrophic to the cardiac tissue due to decreased expression of THRAP1 and myostatin and is sufficient to induce arrhythmias [35].

Summary and Perspective: miRNA in AF

AF is a common clinical arrhythmia, but the mechanisms are not yet fully understood. miRNAs assist in regulation, generation, development and maintenance of AF via modulations in ion channels, receptors and extracellular matrix proteins. Some miRNAs implicated in AF have potential for use as biomarkers and drug targets [66]. For example, serum miR-21 and miR-29 can be used as a noninvasive method for detecting AF and inhibition of miR-21 may prevent myocardial fibrosis to reduce the risk of AF. However, our current knowledge about miRNA and its contribution towards AF is still limited and therefore, learning more about miRNAs will provide new insights into their mechanisms of action and help us in determining effective treatment targets [67]. Data from different studies show that some miRNAs are causative while others have protective effect for AF depending upon their targets. For example, some are pro-fibrotic while others are anti-fibrotic. Similarly, some are pro-arrhythmic whereas others are anti-arrhythmic. Nevertheless, we can identify the deregulated ones to be associated with the disease development and/or maintenance and use them as biomarkers, at least, for that ailment. Future research in this direction is needed, including gathering of larger data sets from animal models and in-depth analysis of the role of miRNAs in the pathogenesis of AF. The definitive function of a particular miRNA can however be evaluated by the knockdown studies, after which the therapeutic potential could be assessed. The functional analysis through miRNA overexpression and their knockdown through anti-miRNA oligos can provide insight about their role in the pathogenesis and/or maintenance of a particular disease. This will help in the development of clinical trials to test miRNA-based therapeutic drugs. In case of AF there is substantial evidence regarding the role of miRNAs in the development and maintenance of the disease. Thus, the beneficial effect of using miRNA for AF seems to hold a lot of promise for future treatments and therapies.
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References


