Is altered atrial microRNA-ome a critical contributor to the pathophysiology of atrial fibrillation?

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Atrial fibrillation (AF) is the most frequent arrhythmia in the clinical setting and is associated with substantial cardiac morbidity and mortality [19]. Current drug interventions have limited efficacy and are accompanied by significant risk of proarrhythmic events [9]. The generally poor outcome with current antiarrhythmic drugs likely reflects the fact that most of the used agents were developed in the absence of precise understanding of pro- and anti-arrhythmic drug actions and the arrhythmogenic disease-specific cardiac substrate. Conceptually, AF induction requires a vulnerable substrate and a trigger that acts on the substrate to initiate the arrhythmia [25, 35]. Once AF is initiated, the rapid-atrial rate creates progressive AF-related changes in atrial electrical and structural properties (atrial remodeling). Electrical remodeling involves a shortening of the atrial effective refractory period (AERP) and abnormal atrial Ca^{2+} signaling, whereas increased atrial fibrosis and heterogeneous conduction slowing are typical characteristics of structural remodeling. Atrial remodeling can promote ectopic (triggered) activity and facilitate reentry, thereby contributing to AF perpetuation and the progression from short-lasting (paroxysmal) to long-standing persistent AF [7, 10, 25, 26, 35], which makes AF more resistant to both pharmacological and non-pharmacological therapeutic approaches. Therefore, it is assumed that a better understanding of the molecular mechanisms underlying AF maintenance will help to

design novel drugs with improved efficacy and safety profiles [8].

The mechanisms contributing to ectopic activity and reentry during AF are incompletely understood. Recent studies have provided compelling evidence that alterations in atrial microRNA (miR) expression could contribute to the basic mechanisms of AF [36]. MiRs are small (20–25 nucleotides) non-coding RNAs that bind to complementary sequences in the 3' untranslated regions of target mRNA in a sequence-specific manner, usually resulting in gene silencing by either blocking translation or directing degradation [20, 27]. They act in a complex functional network in which individual miRs can control hundreds of genes and a single gene can be regulated by multiple miRs. Because miRs are central players in the regulation of gene expression, they participate in many physiological processes, including differentiation, proliferation, migration, metabolism, apoptosis and cell death. Therefore, it is not surprising that miR dysfunction plays a critical role in the pathogenesis of cardiovascular diseases [24].

In this issue of the journal, Adam et al. [2] show that miR-21, which was previously shown to play a crucial role in cardiac remodeling by affecting ERK-MAP kinase signaling in ventricular fibroblasts [31], is a critical regulator of atrial fibrosis formation and a potential contributor to the evolution of structural remodeling in AF patients. They reported that miR-21 is increased in left atria of AF patients and is associated with reduced expression of the cysteine-rich signaling protein Sprouty1, which is an endogenous suppressor of fibroblasts ERK-MAP signaling. Using cardiac fibroblasts they also demonstrated that the increase in miR-21 and the decrease in Sprouty1 could be reproduced by activation of a small GTPase of the Rho family (Rac1), connective-tissue growth-factor (CTGF) and lysyl oxidase with angiotensin-II. They also showed that overexpression

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of lysyl oxidase resulted in enhanced expression of Droscha and Dicer, two important endonucleases for miR formation, mechanistically linking lysyl oxidase and miR-21 formation. Moreover, Rac1 overexpressing mice, which develop spontaneous AF at older age [1], exhibited increased atrial levels of miR-21, decreased Sprouty1 expression and elevated CTGF, lysyl oxidase and atrial fibrosis. Most importantly, inhibition of Rac1 with statins reduced atrial fibrosis, likely because of decreased miR-21 levels with concomitant increases in Sprouty1 expression. Finally, they showed that inhibition of miR-21 with chemically modified antisense oligonucleotides specific for miR-21 (antagomir-21) prevented atrial fibrosis secondary to myocardial infarction, suggesting that the direct silencing of miR-21 by antagomirs might be a potential therapeutic option to target atrial fibrosis in AF patients.

The elegant study of Adam et al. [2] adds miR-21 to the growing list of miRs implicated in the fundamental AF mechanisms (Fig. 1). The reentry-promoting shortening of the action potential duration is largely due to reduced L-type Ca^{2+} current and enhanced inward-rectifier (I_{K1} and a constitutive form of the acetylcholine-gated $I_{K,ACh}$) and slow delayed-rectifier (I_{Ks}) K^{+} currents [3, 7, 10]. Although the molecular basis of these ion current abnormalities is multifactorial [35], there is evidence for involvement of altered miR expression in all of these current abnormalities (Fig. 1). Repressed translation of α_{1C} (Cav1.2) and β_1 (Cav β 1) $I_{Ca,L}$ subunits by enhanced miR-328 is one potential mechanism of reduced $I_{Ca,L}$ in human AF [21]. The KCNQ1 gene encoding the major I_{Ks} channel

subunit contains putative binding sites for miR-1, which potentially links reduced miR-1 [13] levels with enhanced I_{Ks} current in AF patients [3]. Reduced levels of miR-1 [13], miR-26 and miR-101 [22], which repress expression of the principal I_{K1} subunit Kir2.1 (KCNJ2), enhance Kir2.1 proteins levels contributing to the larger I_{K1} in human AF [34]. Recent work identified $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger 1 (NCX1) as a novel target of miR-1 [17], suggesting that deregulated miR-1 may contribute to the higher NCX expression and function in AF patients [14]. However, reduced miR-1 is not a consistent finding in AF, because unaltered [21] and enhanced miR-1 levels [4] have also been reported. The reason for these inconsistent findings in atrial miR levels among studies is not known, but might reflect use of right versus left atrial tissue and differences in age, concomitant diseases and medication in the studied patient populations [5, 12, 29]. Interestingly, overexpression of miR-1 enhances the incidence of spontaneous sarcoplasmic reticulum (SR) Ca^{2+} -release events by increasing steady-state S2814 phosphorylation of type-2 ryanodine receptor channels (RyR2) through a selective miR-1-induced decrease in B56 α expression, which targets the protein phosphatase 2A (PP2A) to specific subcellular microdomains [30]. Myocytes from AF patients show S2814-hyperphosphorylated RyR2 and a higher incidence of spontaneous SR Ca^{2+} -release events and delayed afterdepolarizations leading to triggered activity/abnormal automaticity [11, 33]. This points to the possibility that the hyperphosphorylation-mediated RyR2 dysfunction in AF patients may at least in part result from increased miR-1

Fig. 1 Schematic highlighting putative contribution of altered atrial miR expression to established fundamental mechanisms of AF. $I_{Ca,L}$ L-type Ca^{2+} current, I_{K1} inward-rectifier K^{+} current, I_{Ks} slow delayed-rectifier K^{+} current, TRPC3 transient-receptor potential C type-3 channel, RyR2 ryanodine-receptor type-2, NCX1 $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger type-1, PP2A protein phosphatase 2A, B56 α 56 kDa regulatory subunit alpha of PP2A, ERP effective refractory period, DADs delayed afterdepolarizations. The miR identified by Adam et al. [2] as a critical regulator of atrial fibrosis formation (miR-21) is underlined

levels, which may decrease PP2A activity within the RyR2 macromolecular complex despite the global increase of PP2A activity in AF patients [15].

In addition to electrical and Ca^{2+} -handling remodeling, miRs also promote structural remodeling in AF. Shan et al. [28] showed that downregulation of miR-133 and miR-590 contributes to nicotine-induced atrial structural remodeling in canines. The downregulation of these two antifibrotic miRs significantly increased the protein levels of transforming growth-factor β_1 (TGF- β_1) and its type-II receptor, and enhanced collagen production. The profibrotic mediator TGF- β_1 is secreted by both fibroblasts and cardiomyocytes and cardiac-overexpression of constitutively-active TGF- β_1 causes selective atrial fibrosis and conduction heterogeneity, increasing AF susceptibility [32]. miR-29 inhibits collagen genes and miR-29 downregulation likely contributes to atrial fibrosis in heart failure [6]. CTGF is a downstream mediator of TGF- β_1 profibrotic signaling and miR-30 and miR-133, which suppress CTGF production, are downregulated in chronic AF [18]. In addition, recent work suggests that Ca^{2+} -entry through non-selective transient-receptor potential (TRP) C type-3 (TRPC3) channels is central to AF-related fibroblast activation [16]. Fibroblast TRPC3 expression is increased in AF, mediates angiotensin-II-induced Ca^{2+} influx and promotes fibroblast proliferation and differentiation [16]. Moreover, TRPC3-expression is controlled by miR-26 [16], creating a mechanistic link between signaling pathways affecting electrical and structural remodeling. The present study of Adam et al. [2] extends these findings by being the first to show that miR-21 is an important contributor to atrial fibrosis and structural remodeling in AF patients. This paper also points to new therapeutic options to prevent structural remodeling by targeting miR-21 and the related signaling involving activation of TGF- β_1 , CTGF, Rac1 and lysyl oxidase pathways.

Taken together, the emerging role of miRs in AF-promoting atrial remodeling presents potentially exciting therapeutic opportunities. miRs are stable in blood and interventions have been developed to enhance or suppress the expression of miRs involved in disease progression [27]. The apparent participation of miRs in the fundamental AF mechanisms, including APD reduction, abnormal SR Ca^{2+} release, and tissue fibrosis points to potential novel mechanism-based therapeutic targets. Finally, better insights into the molecular basis of AF may allow to identify AF biomarkers [23], thus helping to develop personalized therapeutic approaches.

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