Connexin and fibrosis related microRNAs in complex fractionated atrial electrograms

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Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia encountered in clinical practice. There are two main factors facilitating the formation and persistence of AF: enhanced automaticity in some rapidly depolarizing foci and abnormal conduction substrates [1]. Fractionated or fragmented electrograms during AF were initially reported by Konings *et al.* [2] Then, other authors defined complex fractionated atrial electrograms (CFAEs) as fractionated electrograms with two or more deflections or with continuous activity over a 10-s period; electrograms with a very short cycle length (mean cycle length of < 120 ms over a 10-s period) [3]. These electrograms were recorded in regions of slow conduction or in which the wavelets pivot, and were attributed to heterogeneous local activation, due to either spatial dispersion in refractory periods, tissue anisotropy or underlying fibrosis. Liu *et al.* [4] found that the expression of connexin-43 (Cx43) at CFAE sites was significantly decreased while myocardial fibrosis was enhanced compared with other sites. These discoveries suggested that the decreased expression of Cx43 and enhanced myocardial fibrosis of the left atrium may be the structural abnormalities underlying CFAE. The existing research has proved the ability of microRNAs to target genes associated with fibrosis and Cx. In this regard, certain microRNAs may contribute to the formation of CFAEs. Here, we review the latest research on microRNAs which are associated with either Cx or cardiac fibrosis.

We identified references from Medline and current contents using the search terms microRNA, complex fractionated atrial electrograms, atrial fibrillation, connexin, fibrosis. We included those reports published up to June 16, 2013 in English, or with an abstract in English, that provided data for microRNA, complex fractionated atrial electrograms, atrial fibrillation, connexin and myocardial fibrosis. A total of 14 studies were included in this article.

The roles of microRNAs in AF have been discussed in several reviews. Briefly, different kinds of microRNAs are associated with AF, either in the heart or in the plasmid, such as microRNA-21, microRNA-1, microRNA-150 and so on [5, 6].

MicroRNA-1: Among the over 300 microRNAs found in humans, microRNA-1 and microRNA-133 are considered to be muscle specific. Yang *et al.* revealed that microRNA-1 is overexpressed in patients with coronary artery disease, and that it could exacerbate arrhythmogenesis when overexpressed in normal or infarcted rat hearts. Conversely, when the microRNA-1 was depressed in infarcted rat hearts, the arrhythmogenesis could be relieved. A possible mechanism is that microRNA-1 overexpres-

sion slowed conduction by post-transcriptionally repressing GJA1 (which encodes connexin 43) [7]. In a mouse model of viral myocarditis, Xu *et al.* also showed that overexpression of microRNA-1 causes post-transcriptional repression of Cx43 synthesis in cardiac myocytes [8]. Interestingly, in a clinical study, in tissues isolated from the left atrium (LA) of AF patients, expression of microRNA-1 was reduced by approximately 86%, while the Cx protein expression remained unchanged [9]. These differences indicate that the expression of microRNA-1 and its effects on Cx expression may differ between species, or have spatial and temporal changes or be associated with other mechanisms.

MicroRNA-21: In cardiac fibroblast, microRNA-21, which is increased in heart failure, was capable of augmenting extracellular regulated protein kinases (ERK)-mitogen-activated protein kinases (MAPK) activation through inhibition of sprouty homologue 1 (Spry1), and controlled the extent of interstitial fibrosis. In a mouse pressure-overload-induced disease model, silencing of microRNA-21 by a specific antagonist to microRNA-21 inhibited interstitial fibrosis and attenuated cardiac dysfunction through the ERK-MAP kinase pathway [10]. Moreover, in isolated cardiac fibroblasts, Roy *et al.* [11] demonstrated that phosphatase and tensin homologue (PTEN) was

a direct target of microRNA-21 and modulation of microRNA-21 regulated expression of matrix metalloprotease-2 (MMP-2) via a PTEN pathway.

MicroRNA-25: Divakaran *et al.* [12] also proved that among the microRNAs related to the drosophila mothers against decapentaplegic protein 3 (SMAD3) pathway, microRNA-25 was sufficient to decrease collagen gene expression when transfected into isolated cardiac fibroblasts *in vitro*.

MicroRNA-29 family: During the process of fibrosis replacement after myocardial infarction in a mice model, van Rooij *et al.* [13] revealed that the microRNA-29 family targets a number of mRNAs that encode various proteins involved in fibrosis, including multiple collagens, fibrillins, and elastin. They also found that the microRNA-29 family members were downregulated in the region of the heart adjacent to the infarct, and when microRNA-29s were down-regulated with anti-microRNAs *in vitro* and *in vivo*, the expression of collagens was induced, whereas over-expression of microRNA-29 in fibroblasts reduced collagen expression. The authors also pointed out that transforming growth factor β (TGF-β), a major regulator of cardiac fibrosis, can repress microRNA-29 expression. These results were similar to another work, which reported that suppression of microRNA-29 expression by TGF-β1 promotes collagen expression and renal fibrosis [14]. Using SMAD3 null mice, Divakaran

Table I. MicroRNAs related to CFAEs

Reference	MicroRNA (MiR)	Expressing cell type	Impact on CFAE formation	Possible mechanism
[7, 8]	MiR-1	Cardiac myocyte	↑	Post-transcriptionally repressing Cx43
[10, 11]	MiR-21	Cardiac fibroblast	↑	Augmenting ERK–MAP kinase activity via inhibition of Spry1; downregulating PTEN and subsequently decreasing MMP-2 synthesis via an AKT-phosphorylation-dependent pathway
[12]	MiR-25	Cardiac fibroblast	↓	Decreasing expression of various collagens including type I and type III
[12, 13]	MiR-29 family	Cardiac fibroblast	↓	Decreasing expression of various proteins involved in fibrosis such as type I and type III collagen
[15]	MiR-30	Cardiac fibroblast and myocyte	↓	Post-transcriptionally repressing CTGF
[16]	MiR-132	SVP cell	↓	Directly repressing profibrotic MeCP2 expression
[15, 17, 19]	MiR-133	Cardiac myocyte	↓	Directly downregulating CTGF expression; indirectly protecting against myocardial fibrosis without affecting hypertrophy; repressing TGF-β1 and TGF-βRII expression
[18]	MiR-208	Cardiac myocyte	↑	Enhancing β-MHC expression in response to stress and hypothyroidism through repressing THRAP1
[19]	MiR-590	Cardiac fibroblast	↓	Post-transcriptionally repressing TGF-β1 and TGF-βRII

MeCP2 – methyl-CpG-binding protein 2, β-MHC – β-myosin heavy chain, THRAP1 – thyroid hormone receptor associated protein 1.

et al. [12] determined a significant 60% decrease in myocardial fibrosis, and this effect was related to a number of microRNAs, including microRNA-29a, which were sufficient to decrease collagen gene expression when transfected into isolated cardiac fibroblasts *in vitro*.

MicroRNA-30: Interestingly, the microRNA-30 seems to have the same effects as microRNA-133 in the CTGF pathway to regulate interstitial matrix through directly binding to connective tissue growth factor (CTGF) [15].

MicroRNA-132: Among the factors secreted by saphenous vein-derived pericyte progenitor cells (SVP) after being transplanted to the peri-infarct zone of immunodeficient CD1/Foxn-1(nu/nu) or immunocompetent CD1 mice, microRNA-132 is remarkable because it acts as a paracrine activator of cardiac healing. An *in vitro* study showed that the targets of microRNA-132 (Ras-GTPase activating protein and methyl-CpG-binding protein 2) were inhibited. Meanwhile endothelial tube formation was stimulated and myofibroblast differentiation was reduced when treated with SVP conditioned medium. Furthermore, microRNA-132 inhibition by anti-microRNA-132 decreased SVP capacity to reduce interstitial fibrosis in infarcted hearts [16].

MicroRNA-133: MicroRNA-133a is downregulated in transverse aortic constriction (TAC) and isoproterenol-induced hypertrophy in mice, and transgenic expression of microRNA-133a prevented TAC-associated microRNA-133a downregulation and improved myocardial fibrosis [17]. MicroRNA-133 could also directly downregulate connective tissue growth factor (CTGF), a key molecule in the process of fibrosis, and therefore took part in the control of structural changes in the extracellular matrix of the myocardium [15].

MicroRNA-208: MicroRNA-208 is a cardiac-specific microRNA, which was encoded by an intron of the α-myosin heavy chain gene, and it was required for cardiac fibrosis in response to stress and hypothyroidism [18].

MicroRNA-590: Shan *et al.* found that TGF-β receptor type II (TGF-βRII) is a target for microRNA-590 repression. Transfection of microRNA-590 into cultured atrial fibroblasts decreased TGF-βRII levels and collagen content. These effects could be abolished by the antisense oligonucleotides against microRNA-590. The authors concluded that the profibrotic response to nicotine in the canine atrium is critically dependent upon downregulation of microRNA-590 [19].

In conclusion, as connexin and fibrosis (which was also related to atherosclerosis [20]) are the pathologic basis of CFAE, and microRNAs are associated with both fibrosis and connexin (Table I), it is reasonable to speculate that the abnormal distribution of certain microRNAs facilitates the formation of CFAE, which contributes to the initiation and maintenance of AF. Though there is some literature accumulating on adjunctive use of CFAE-guided ablation for AF, use in paroxysmal AF is still controversial [21]. Also, their specificity in localizing the areas of origin of AF have been questioned [22–24]. Improving conductibility in CFAE sites through interfering with certain microRNAs seems to be an alternative method in AF management. However, there has been little research associated with microRNAs and CFAEs as yet. As we know, the expression of microRNAs varies greatly with physical and pathologic conditions. For example, microRNA-590 is expressed at relatively low levels in the myocardium, but has been shown to have an important role in regulating cardiac fibrosis. Further explorations on the directly relevant factors between microRNAs and CFAEs in AF patients are required.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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