Editorial

Mir-33: miR-acles in cardiac fibrosis?

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Heart diseases (HDs) are the primary cause of death in the world (1). An estimated 17.3 million people died from HDs in 2015 (1), representing about 30% of all global deaths. Heart failure is the clinical manifestation of numerous forms of HDs. It is a destructive disorder characterized by ventricular remodeling and reduced compliance. In nearly all etiologies of HDs, the progression toward failure is accelerated by fibrosis, i.e., the improper deposition of extracellular matrix (ECM) proteins by cardiac fibroblasts (CFs) resulting in the reduction of tissue compliance. Physiologically, fibroblasts are the major cell type implicated in the construction and maintenance of connective tissue. The ECM, a highly organized collagenrich meshwork, provides a structural and flexible scaffold for cardiac cells populations, dispenses mechanical forces through the myocardium, and mediates mechanical conduction of cells in the environment (2-4). CFs are fundamentally involved into the heart response to injury and tackle the limited regenerative capacity of the heart after injury. Fibrotic scar tissues preserve cardiac tissue structure and function. Upon injury, CFs within the connective tissue are activated, and secrete high levels of ECM to generate a pro-fibrotic environment. This environment enhances stiffness of the cardiac tissue and inhibits ventricular contraction and relaxation, which lead to abnormal heart architecture and function. Excessive ECM deposition and fibrosis have been clearly associated with myocardial diastolic and systolic dysfunctions (5). Inhibiting or reversing fibrosis and its damaging repercussions is an established strategy used in many clinical interventions aiming to treat HDs.

Recently, Nishiga *et al.* (*Circulation Research*) found, in clinical samples, that the cardiac level of miR-33a was modestly but significantly correlated with ejection fraction (EF) and inversely correlated with pulmonary capillary wedge pressure, obtained by catheterization in 33 patients with dilated cardiomyopathy. miR-33a levels in patients with high-stage HF were significantly lower than in patients with low-stage HF. Therefore, miR-33 was considered to worsen cardiac function and to play a role in the development of HF. In genetic models, miR-33 deficiency resulted in reduced fibrotic response to transverse aortic constriction (TAC) induced-pressure overload in vivo, but despite the reduction in fibrosis, cardiac function deteriorated in miR-33KO hearts. This observation underline the fact that the association between increased fibrosis and decreased cardiac function might not be as linear as originally thought. Several others reports are in keeping with these findings. Inhibition of miR-15b by subcutaneous injections of LNA-based anti-miRs in C57BL/6 mice subjected to transverse aorta constriction seems to aggravate fibrosis but to have less effect on hypertrophy (6). Treatment with antagomiRs to miR-29b has been shown to induce excess fibrosis after aortic constriction without overt deterioration in cardiac function (7). miR-33 adds up to the long list of miRNA described to target cardiac fibrosis (see *Table 1* for a non-exhaustive list of miRNAs that have been implicated in cardiac fibrosis), increasing the insight into the pathophysiology of this syndrome. miR-29, miR-133, miR-26a, miR-24, miR-19a-3p/19b-3p, and miR-101a are major antifibrotic miRs. However, the main profibrogenic miRs include miR-21, miR-15 and miR-1. Loss- and gain-of-function experiments revealed an important role for

Table 1 Summary of miRNA involved in cardiac fibrosis

miRNA	Heart disease	Cell type	Expression	Signalling	Reference
miIR-29	Acute myocardial infarction	Cardiac fibroblasts	Downregulated	Increases collagens expression	(1)
miR-21	Heart failure	Cardiac fibroblasts	Upregulated	Augments ERK-MAP kinase activity through inhibition of sprouty homologue 1 (Spry1)This mechanism regulates fibroblast survival and growth factor secretion	(2)
miR-133 and miR-30	Left ventricular hypertrophy	Cardiomyocytes and fibroblasts	Downregulated	Allows connective tissue growth factor levels to increase, which contributes to collagen synthesis	(3)
miR-18/19	Age-related heart failure	Cardiomyocytes	Downregulated	Increases connective tissue growth factor and thrombospondin-1, which contributes to collagen1 and 3 synthesis	(4)
miR-24	Myocardial infarction	Unknown	Downregulated	Increases the level of furin, a protease controlling latent TGF-β activation processing, increasing TGF-β secretion and Smad2/3 phosphorylation in cardiac fibroblasts	(5)
miR-22	Cardiac aging	Cardiac fibroblasts and smooth muscle cells	Upregulated	Silences mimecan (osteoglycin), induces cellular senescence and promotes migratory activity of cardiac fibroblasts	(6)
miR-101	Chronic myocardial infarction	Cardiac fibroblasts	Decreased in the peri-infarct area	Increases the levels of c-Fos and its downstream protein transforming growth factor-β1	(7)
miR-122	Myocardial fibrosis in aortic valve stenosis	Cardiac fibroblasts	Downregulated	Induces TGF-β1 up-regulation	(8)
miR-15	Overloaded heart		Upregulated	Inhibits the TGFβ-pathway by targeting TGFBR1 and several other genes within this pathway directly or indirectly, including p38, SMAD3, SMAD7, and endoglin	(9)
miR-34a	Myocardial infarction	Cardiac fibroblasts	Upregulated	Inhibits Smad4 expression	(10)
miR-125b	Heart failure	Cardiac fibroblasts	Upregulated	Induces the fibroblast-to-myofibroblast transition by functionally targeting apelin, a critical repressor of fibrogenesis, and inhibits p53 to induce fibroblast proliferation	(11)
miR-503	Left ventricular hypertrophy	Cardiac fibroblasts	Upregulated	Decreases the expression levels of Apelin-13	(12)
miR-433	Myocardial infarction and dilated cardiomyopathy	Cardiac fibroblasts	Upregulated	Decreases the level of AZIN1, activates TGF-β1 while downregulates JNK1 resulting in activation of ERK and p38 kinase, Smad3 activation and ultimately cardiac fibrosis	(13)
miR-33	Dilated cardiomyopathy, Left ventricular hypertrophy	Cardiac fibroblasts	Upregulated	Preserves lipid raft cholesterol content in fibroblasts and maintains adaptive fibrotic responses	(14)

miR mimics and inhibitors for patients with HF. In vivo silencing of miR-21 by a specific antagomir in a mouse pressure-overload-induced disease model reduces cardiac ERK-MAP kinase activity, inhibits interstitial fibrosis and attenuates cardiac dysfunction (8). Four weeks after adenovirus-mediated overexpression of miR-101a in rats with chronic myocardial infarction, echocardiography and hemodynamic measurements indicated remarkable improvement of the cardiac performance (9). Furthermore, the interstitial fibrosis was alleviated and the expression of c-Fos and transforming growth factor-β1 was inhibited (9). Intramyocardial injection of Lentiviruses expressing miR-24 improves heart function and attenuates fibrosis in the infarct border zone of the heart two weeks after MI (10). Systemic neutralization of miR-433 or adeno-associated virus 9 (AAV9)-mediated cardiac transfer of a miR-433 sponge attenuates also cardiac fibrosis and ventricular dysfunction following myocardial infarction (11). On the other hand, cardiac fibrosis is significantly reduced in infarcted heart when treated with miR-328 antisense (12). It is unclear whether the dissociation between fibrosis and heart function is due to different methodologies, to specific pathways studied but it will be important to clarify this in future studies and to determine whether reversing fibrosis is still a clinical intervention of interest.

miR-33 in the heart was predominantly expressed in CFs, and miR-33 deficiency impaired cell proliferation. miR-33 was found to preserve the lipid raft cholesterol content in fibroblasts by regulating genes involved in cholesterol metabolism, including ABCA1, ABCG1, and NPC1. This is a well-known and not surprising role for miR-33 as miR-33a and miR-33b are expressed as intronic miRNAs along with *SREBF2* and *SREBF1*, their host genes coding for transcription factors that regulate the synthesis/uptake of cholesterol and fatty acid (13-15). miR-33a/b have been shown to inhibit genes implicated in pathways opposed to functions driven by SREBP, including cholesterol efflux (*ABCA1*, *ABCG1*, *NPC1*) (13-15) and FAO (*HADHB*, *CROT*, *CPT1A*, *PRKAA1*) (16-18). Several other properties have been attributed to miR-33. Interestingly, several mitochondrial genes [i.e., PGC-1α (19), PDK4 and SLC25A (20)] has been determined as direct and specific miR-33 targets, with conserved binding sites in the 3'UTR of both human and mouse transcripts. Anti-miR33, by de-repressing PGC-1α levels and enhancing downstream factors, can indirectly stimulate NRF1 and OXPHOS complexes expressions, promoting respectively mitochondrial biogenesis and efficient production of ATP (21,22). Therefore, miR-33 inhibition promotes mitochondrial biogenesis, aerobic respiration and activity. In addition, miR-33a and miR-33b have also been shown to control the expression of *Ampkα1* and sirtuin 6 (*Sirt6*), altering the balance of cellular glycolysis/FAO. AMPK increases FAO via phosphorylation and inhibition of acetyl-CoA carboxylase (22,23), and via SIRT1 and PGC-1α expression (24,25). This is of great importance because the failing heart normally adapts using changes in enzymatic and signaling pathways, ultimately resulting in a metabolic switch, away from FAO toward greater glucose metabolism to maximize efficiency (26,27). This is in keeping with the results presented by Nishiga *et al.* showing that the expression level of miR-33 was significantly upregulated in response to TAC and suggesting a metabolic switch toward glycolysis during the hypertrophic phase in this model.

Given its broad range of implications, including in metabolism, miR-33 cannot be only considered as an anti-fibrotic miR. The dissociation between improvement of cardiac fibrosis and still the deterioration in cardiac function in miR-33 KO mice might be explained by the fact that miR-33 is possibly needed for a proper metabolic adaptation of the heart during the compensatory phase. It will be interesting to further investigate the role of miR-33 in cardiac failure. Moreover, although being an aberrant wound healing process, fibrosis is an adaptive response to injury and attempts to inhibit it may not be beneficial at certain stages of the disease.

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