miR-33 Regulation of Adaptive Fibrotic Response in Cardiac Remodeling

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Despite substantial improvements in prevention, diagnosis, and therapeutic strategies, cardiovascular diseases remain the leading cause of mortality and morbidity worldwide. Cardiac fibrosis, a critical hallmark of maladaptive hypertrophy and heart failure (HF), is characterized by the excessive and uncontrolled accumulation of extracellular matrix (ECM) by cardiac fibroblasts (CFs) in the interstitial and perivascular space. CFs, primarily of embryonic epicardial and endothelial origins, are the predominant noncardiomyocyte population, accounting for 20% of total cell population in the adult murine heart. In addition to their traditional functions in regulating ECM synthesis and metabolism and controlling cardiac fibrosis, ECM remodeling, scar formation, and tissue repair, CFs serve as functionally pluralistic cells involved in inflammatory responses, cardiomyocyte survival, and vasculogenesis.

In the adult heart, CFs are present in a quiescent state with low proliferative capacity and are responsible for ECM homeostasis, providing a structural scaffold for cardiomyocytes. CFs also express gap junction protein connexin-43, which mediates electric coupling of cardiomyocytes and CFs. The adult mammalian heart has limited regenerative capacity after pathological injury. The repair of wounds in the heart consists of the removal of dead cardiomyocytes and replacement of myocytes loss by a collagen-based fibrotic scar to preserve myocardial integrity and cardiac function. The importance of CFs in myocardial remodeling and wound healing has been extensively studied and involves all of the 3 phases of the healing process: acute inflammatory response, proliferation, and late scar maturation. After an acute myocardial injury, CFs release proinflammatory cytokines, which promote their own proliferation in a feed-forward loop and trigger differentiation into a myofibroblast phenotype. This hyperproliferative cell type secretes high levels of proinflammatory and profibrotic factors and ECM proteins. This adaptive fibrosis can maintain structural integrity and prevent the injured heart from dysfunction and rupture; however, prolonged activation of CFs, particularly occurring in the sites remote from the primary injury, results in pathological cardiac remodeling characterized by fibroblasts accumulation and excessive deposition of ECM proteins.

Work over the last decade has identified the important role of noncoding RNAs, such as microRNAs (miRNAs), in regulating cardiac function and fibrosis. miRNAs are highly conserved small noncoding RNA molecules that regulate gene expression at the post-transcriptional level. Many reports have suggested an important role of miRNAs in heart development, cardiac fibrosis, and HF. In fact, cardiac-specific knockout of Dicer, an RNase III endonuclease essential for processing of pre-miRNAs into mature miRNAs, leads to rapidly progressive dilated cardiomyopathy, HF, and postnatal lethality. Moreover, conditional Dicer deletion in the postnatal myocardium induces spontaneous cardiac remodeling, myocyte hypertrophy, and cardiac fibrosis, indicating the crucial role of miRNAs in normal cardiac function and under pathological conditions.

In this issue of *Circulation Research*, Nishiga et al added miR-33a to the list of miRNAs that regulate cardiac fibrosis and remodeling by preserving lipid raft cholesterol content and controlling proliferation in CF. The miR-33 family of miRNAs is composed of 2 miRNAs, miR-33a and miR-33b, which are encoded within the intronic sequences of the SREBP-2 and SREBP-1 genes, respectively. Both miRNAs are cotranscribed with their host genes and regulate cholesterol and fatty acid metabolism. Of note, antagonism of miR-33 using miR-33 antisense oligonucleotides or miR-33 genetic deletion enhances cellular cholesterol efflux and high-density lipoprotein biogenesis by increasing the ATP-binding cassette transporter ABCA1 expression. As a result, several reports have demonstrated that silencing miR-33 in mice attenuates the progression and promotes the regression of atherosclerosis. Although the contribution of miR-33 in regulating of lipid metabolism is well established, its role in controlling cardiac function has not been elucidated. Nishiga et al provide the first evidence that the expression of miR-33a in human cardiac tissues is associated with cardiac function in patients with dilated cardiomyopathy. The authors found that miR-33a expression levels in patients with high-stage HF were significantly lower than in patients with low-stage HF. They further demonstrate that genetic ablation of miR-33 reduced chronic pressure overload-induced cardiac fibrosis. Moreover, they observed that the expression of miR-33a in the left ventricle was upregulated in response to pressure overload, confirming the relationship between miR-33a expression...
and cardiac fibrosis under pathological conditions. To further elucidate the mechanisms by which miR-33a regulates cardiac fibrosis, Nishiga et al. found that fibroblasts, instead of cardiomyocytes, were the predominant source of miR-33a in the heart. Most importantly, they observed that the expression of genes associated with fibrosis and remodeling of ECM was downregulated by pressure overload in miR-33−/− mice compared with wild-type mice. Considering the critical role of fibroblasts in ECM metabolism and cardiac fibrosis, the authors hypothesized that the loss of miR-33a in fibroblasts might be responsible for the reduced fibrosis observed in miR-33−/− mice. To test this idea, they generated a novel miR-33 conditional knockout mouse model that lacks the expression of miR-33 only in CFs. Interestingly, the authors found that the absence of miR-33 in cardiofibroblasts attenuates cardiac fibrosis in response to pressure overload. However, the effect was modest compared with the global miR-33−/− deficient mice, suggesting that other cell types such as macrophages may also be involved in the fibrotic phenotype observed in miR-33−/− mice. miR-33 deficiency in CFs impaired their proliferative capacity by upregulating the expression of ABCA1, which controls intracellular cholesterol levels and membrane cholesterol content. Cholesterol is an important component of lipid rafts and is the key factor in regulating raft stability and organization. Interestingly, miR-33 deficiency resulted in reduced lipid raft content in CFs. Further studies confirmed the specific role of ABCA1 in miR-33−/− associated fibroblast proliferation by inhibiting ABCA1 using specific siRNA in both wild-type and miR-33−/− fibroblasts. Mechanistically, the authors found that the absence of miR-33 in fibroblasts attenuates Akt activation, which has been associated with fibroblast proliferation and cardiac growth. In agreement with these in vitro observations, they further demonstrate that the proliferation rate was diminished in cardiofibroblasts from miR-33−/− mice compared with wild-type mice after transverse aortic constriction.

Another interesting finding of this study is the paradoxical observation that the marked attenuation in cardiac fibrosis observed in miR-33−/− mice was accompanied by a reduction in systolic function. This observation is surprising because increased cardiac fibrosis is commonly associated with failing hearts. Although there are some reports showing inconsistency between cardiac fibrosis and heart function, the molecular mechanisms by which absence of miR-33 reduces fibrosis and impairs heart function remain to be identified.

**Questions Remaining to Be Answered**

In this study, Nishiga et al. demonstrated that the deficiency of miR-33a inhibits CF proliferation by increasing ABCA1 expression and decreasing lipid raft content. These findings are consistent with the previous work, demonstrating that the lowering of intracellular cholesterol levels attenuates the proliferation of cancer cells in response to liver X receptor stimulation, and that the proliferation of bone marrow monocyte precursors is reduced after treatment with anti-miR-33 oligonucleotides. These results are further supported by Yvan-Charvet et al., who reported that ABCA1 and ABCG1 deficiency increases cellular cholesterol content, enhances proliferation of hematopoietic stem cells, and decreases growth factor–stimulated proliferation in response to liver X receptor agonist treatment. However, in this study, miR-33a overexpression or knockdown of ABCA1 also inhibited fibroblast proliferation, suggesting that the proliferation of CFs is inhibited by the alterations of intracellular cholesterol homeostasis, whether excess or insufficiency. Previous work has demonstrated that impaired intracellular cholesterol synthesis by simvastatin treatment induces cell cycle arrest and inhibits bladder cancer.

**Figure. Schematic of how microRNA-33a (miR-33a) regulates cardiac fibroblast proliferation and adaptive fibrotic response under pathological condition.** Pressure overload upregulates the expression of miR-33a in the heart. Deficiency of miR-33a in cardiac fibroblasts increases the expression of target gene ABCA1, which is responsible for the intracellular free cholesterol (FC) efflux to extracellular cholesterol acceptor apoAI. Increased cholesterol efflux from fibroblasts results in decreased intracellular cholesterol level and impaired cellular lipid raft content, which is involved in the activation of protein kinase B (AKT) signaling pathway and the expression of key proteins in cell cycle control. Impaired fibroblast proliferation leads to insufficient reactive fibrosis and adaptive remodeling and causes cardiac dysfunction and heart failure.
cell proliferation by downregulation of cyclin-dependent kinase 4/6 (CDK4/6) and cyclin D1. In addition, miR-33 regulates cell proliferation and cell cycle progression by targeting CDK6, cyclin D1, and p53 in different cell types. Although this study excluded the possible role of CDK6 in controlling CF proliferation, whether other target genes are directly or indirectly involved in miR-33–dependent regulation of fibroblast proliferation needs to be addressed. Moreover, it will be interesting to investigate whether the inhibition of CF proliferation by overexpression of miR-33a or knockdown of ABCA1 switches adaptive fibrotic response and cardiac fibrosis in the murine pressure overload model, as reported in this article.

Previous work has also established miR-33 as an important regulator of hepatic fibrosis. Activation of hepatic stellate cells, the liver-specific mesenchymal cells and primary source of liver fibroblasts, induces the expression of the SREBP-2/miR-33 locus. By contrast, in this study, the regulation of miR-33a expression in patients with dilated cardiomyopathy occurred primarily at the post-transcriptional level. The authors show that the deficiency of miR-33a in CFs did not influence SREBP-2 expression levels under normal conditions; however, this work does not address the regulation of SREBP-2 expression under pathological conditions. Further studies are required to investigate the underlying mechanisms by which fibroblasts activation in response to mechanical stress regulates miR-33 expression at a post-transcriptional level.

In conclusion, the study of Nishiga et al10 provides strong evidence that the deficiency of miR-33 in CFs impairs their proliferation capacity by depleting lipid raft cholesterol content and affecting ABCA1 function, which inhibits the adaptive fibrotic response in the remodeling heart and decreases cardiac fibrosis formation (Figure). Another important finding from this study is the correlation between miR-33 expression level and cardiac function, suggesting that the inhibition of miR-33 may cause cardiac dysfunction and HF: miR-33 inhibition is considered to be an effective therapeutic strategy for treating atherosclerosis in the mice.11 To promote protective effects on macrophage cholesterol homeostasis and to avoid adverse effects on cardiac dysfunction, effective strategies such as cell- or tissue-specific targeting of miR-33 may need to be explored in future studies.

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None.

References

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