Searching for MiR-acles in Cardiac Fibrosis

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Tissue fibrosis is a major cause of organ dysfunction in diseases of the cardiovascular, pulmonary, renal, and hepatic systems. Despite a relatively sophisticated understanding of the cellular processes underlying fibrosis, there are few effective therapies, emphasizing the need for new insights into this disorder. A series of recent articles, including one by Creemers and colleagues in this issue of Circulation Research, reveal central roles for microRNAs (miRNAs) in the control of cardiac fibrosis and provide not only a new dimension to our understanding of this disease but also point to novel therapeutic opportunities. In addition, although most attention has been focused on abnormalities in cardiac myocytes that lead to cardiac dysfunction, these recent studies on the involvement of miRNAs in heart disease emphasize the importance of a much less understood cell type, the cardiac fibroblast, in pathological cardiac remodeling.

Fibrosis is a pathological feature common to numerous forms of heart disease, including myocardial infarction; ischemic, dilated, and hypertrophic cardiomyopathies; and heart failure. The cellular basis of fibrosis is the adverse accumulation of collagens and other extracellular matrix (ECM) proteins, which impairs ventricular function and predisposes the heart to arrhythmias. Transforming growth factor (TGF)-β, a potent stimulator of collagen production by cardiac fibroblasts, is induced in response to cardiovascular injury. Binding of TGF-β to its cell surface receptors activates the Smad pathway, which regulates the transcription of several key fibrotic genes, including those encoding connective tissue growth factor (CTGF), fibronectin, collagens, and plasminogen activator inhibitor-1. TGF-β reduces collagenase production and stimulates the expression of TIMP (tissue inhibitor of metalloproteinases), resulting in an overall inhibition of ECM degradation and leading to excessive matrix accumulation. Although targeting TGF-β has shown promise as an antifibrotic therapy, the effect on the cardiovascular system is ambiguous. Besides its profibrotic actions, TGF-β plays a significant role in postangioplasty restenosis, post--myocardial infarction remodeling (resulting in the development of heart failure), and numerous other fibrotic pathologies. In contrast to the pleiotropic effects of TGF-β, CTGF regulates the fibrosis pathway more specifically by promoting fibroblast proliferation and ECM production in fibrotic disorders. However, cardiac fibrosis is multifactorial and many aspects of this disorder remain to be elucidated and additional levels of regulation should be considered.

In the last few years, it has become clear that microRNAs (miRNAs) are dominant players in different aspects of cardiac remodeling, including fibrosis (Figure 1). MiRNAs are ~22-nucleotides long and act as negative regulators of gene expression by inhibiting mRNA translation or promoting mRNA degradation. There are estimated to be more than 1000 miRNAs encoded by the human genome, many of which are expressed in a tissue- and cell-specific manner. Individual miRNAs are capable of targeting multiple miRNAs, which are often functionally related, enabling them to modulate complex physiological or disease phenotypes by regulating entire functional networks. The possibility that miRNAs might participate in heart disease was first suggested by the discovery of distinctive patterns of miRNA expression in healthy and diseased hearts from mice and humans (reviewed elsewhere). Based on more elaborate genetic studies in mice, it is now apparent that miRNAs are actively involved in all aspects of cardiac remodeling, growth, proliferation, apoptosis, conductance, and contractility.

Several methods are available to selectively target miRNA pathways in vivo. miRNAs can be inhibited by antisense oligonucleotides, which can be chemically modified and conjugated to cholesterol to enhance their stability and uptake by tissues, thereby facilitating the knockdown of potentially pathogenic or aberrantly expressed miRNAs. Because miRNAs typically act as inhibitors of gene expression, the effect of inhibitors of specific miRNAs is to relieve the inhibition of the genes normally targeted by the miRNA. Conversely, miRNA mimics can serve to elevate the levels of beneficial miRNAs, so as to decrease the expression of their target mRNAs. Although the exact mechanisms of oligonucleotide-mediated miRNA silencing and overexpression are still unknown, it is clear that intravenous delivery leads to highly efficient and specific miRNA regulation in vivo. The ability of miRNAs to influence entire networks of genes involved in common cellular processes provides enormous therapeutic potential and differs from the specificity of classical drugs, which act on specific cellular targets.

In this issue of Circulation Research, Duisters et al describe an essential function of miRNAs in regulating CTGF expression and fibrosis in pathological left ventricular hypertrophy. In their article, the authors show that 2 major cardiac miRNAs, miR-30 and miR-133, are downregulated during cardiac disease, which inversely correlates with the upregulation of CTGF, collagen production, and fibrosis (Figure 1). Cell culture experiments designed to overexpress or inhibit these miRNAs indicate that both miRNAs can effectively repress CTGF expression by directly interacting with the 3' untranslated region of CTGF mRNA (Figure 2). Whereas...
miR-133 is expressed specifically in cardiomyocytes, miR-30 is expressed in cardiac fibroblasts, as well as cardiomyocytes. Consistent with the postulated role of miR-133 as a negative regulator of fibrosis, we reported that knockout mice lacking miR-133-a1 and miR-133-a2 develop severe fibrosis and heart failure, resulting in cardiomyopathy and sudden death. These phenotypes were ascribed to the upregulation of serum response factor, a known transcriptional activator of heart failure. However, given the many predicted targets of miR-133, it is conceivable that elevation in CTGF expression could also contribute to the excessive fibrosis seen in miR-133 knockout mice (and SRF upregulation could contribute to the effects seen by Duisters et al). MiR-133 knockout mice do not show evidence of cardiac hypertrophy despite the finding that knockdown of miR-133 expression through systemic delivery of an antagonist in mice caused marked and sustained hypertrophy with impaired cardiac function. Although fibrosis was not assayed in that study, the hypertrophy in these mice would appear to contrast with the phenotype resulting from miR-133 knockout. The basis for these potential differences remains to be resolved and underscores how much remains to be understood about the biology of miRNA actions.

Another study from our group showed that the miR-29 family, which is fibroblast-enriched, targets mRNAs encoding a multitude of ECM-related proteins involved in fibrosis, including multiple collagens, fibrillins, and elastin (Figure 1). In response to myocardial infarction, members of the miR-29 family are downregulated in the region of the heart adjacent to the infarct. Downregulation of miR-29 would be predicted to derepress the expression of these mRNAs and enhance the fibrotic response (Figure 2). Indeed, downregulation of miR-29 with antimiRs in vitro and in vivo induces the expression of collagens, whereas overexpression of miR-29 in fibroblasts reduces collagen expression. We conclude that miR-29 acts as a regulator of cardiac fibrosis and, as such, miR-29 mimics represent a potential therapeutic approach for tissue fibrosis in the heart and other tissues.

miR-21 is among the most strongly upregulated miRNAs in response to a variety of forms of cardiac stress. Recently, Thum et al showed that miR-21 is upregulated in cardiac fibroblasts in the failing heart, where it represses the expression of Sprouty homolog 1 (Spry1), a negative regulator of the extracellular signal-regulated kinase/mitogen-activated protein (ERK-MAP) kinase signaling pathway (Figures 1 and 2). Upregulation of miR-21 in response to cardiac injury was shown to enhance ERK-MAP kinase signaling, leading to fibroblast proliferation and fibrosis. Conversely, in vivo silencing of miR-21 using an antagonist in a mouse model of pressure-overload, induced by thoracic aortic constriction, reduced cardiac ERK-MAP kinase activity, inhibited interstitial fibrosis, and attenuated cardiac dysfunction. Remarkably, miR-21 inhibition in fibroblasts not only prevented cardiomyocyte hypertrophy in response to thoracic aortic constriction but was capable of reversing cardiac fibrosis. TGF-β also promotes fibrosis by repressing expression of miR-29, -30, and -133.
remodeling in response to stress. Thus, it was proposed that Spry1 upregulation in response to inhibition of miR-21 expression suppresses pathological ERK-MAP kinase signaling and prevents cardiac dysfunction. Given the multitude of predicted mRNA targets of miR-21, as a further test of this hypothesis, it will be important to determine whether elevated expression of Spry1 to the level seen in the presence of miR-21 antagonist is sufficient to block pathological cardiac remodeling and, conversely, whether inhibition of Spry1 expression causes heart disease.

These findings indicate that miR-21, like miR-29, can contribute to myocardial remodeling by affecting cardiac fibroblasts. Downregulation of miR-21 might be a beneficial approach to block fibroblast proliferation in heart disease, thereby inhibiting secondary cardiac remodeling. Given the disparity between the phenotypes of miR-133 knockout mice and the effects of miR-133 antagonist treatment, it will be interesting to see whether miR-21 knockout mice display the same resistance to cardiac hypertrophy and fibrosis seen with miR-21 antagonist. Together, these data indicate that miRNAs are important regulators of cardiac biology and the different aspects of cardiac disease, like fibrosis. Especially intriguing are the findings that modulation of a single miRNA can apparently disrupt the processes of fibrosis and pathological cardiac remodeling. Exploiting this new biology of disease regulation by manipulating miRNA levels in vivo by oligonucleotide-based approaches provides an exciting, albeit challenging, therapeutic opportunity for heart failure and pathological cardiac remodeling. Developing miRNAs into therapeutics will also, undoubtedly, pose significant challenges, such as modes of delivery and duration of action. Optimizing oligonucleotide design and methods for local delivery to the heart, through direct injection, catheters, or conjugations that allow for specific cardiac uptake, should obviate these challenges and should make it possible to avoid off-target effects on noncardiac tissues.

Although the roles of miRNAs in cardiac fibrosis are rapidly being illuminated, most of the miRNAs that control fibrosis in the heart are also expressed in other tissues. Thus, it is reasonable to anticipate that these miRNA-based mechanisms are operative in numerous tissue disorders leading to fibrosis. Given the pace of this field and the power of these miRNA-based mechanisms, it is reasonable to anticipate that these miRNA-based mechanisms are operative in numerous tissue disorders leading to fibrosis. Given the pace of this field and the power of these miRNA-based mechanisms, it is reasonable to anticipate that these miRNA-based mechanisms are operative in numerous tissue disorders leading to fibrosis. Given the pace of this field and the power of these miRNA-based mechanisms, it is reasonable to anticipate that these miRNA-based mechanisms are operative in numerous tissue disorders leading to fibrosis.

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**Disclosures**

The authors are cofounders of miRagen Therapeutics Inc, a company based on the therapeutic application of miRNAs in heart disease.

**References**


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