Cardiac Fibrosis Revisited by MicroRNA Therapeutics

Thomas Thum, MD, PhD; Johan M. Lorenzen, MD

Cardiac fibrosis is a result of a variety of injurious insults of different causes to cardiac tissue, which ultimately culminates in destruction of physiological tissue architecture and progressive organ dysfunction. Histologically, it is characterized by activation/proliferation of fibroblasts and excessive matrix deposition, including collagen. A major role in this process has been attributed to various growth factors, proteolytic enzymes, angiogenic factors, and fibrogenic cytokines. MicroRNAs (miRNAs/miRs) have recently come into focus as powerful regulators of gene expression, and they fundamentally influence the pathogenesis of different pathological events, including cardiac fibrosis. miRNAs are small noncoding RNAs (~22 nucleotides) that lead to silencing of genetic information through posttranscriptional degradation of mRNA and translational inhibition of protein expression. miRNAs are highly conserved in different species and are thought to regulate ≥50% of the genome. miRNAs are formed in a highly regulated process in the nucleus and are then transported into the cytosol, in which they are processed further. Numerous studies have underlined their critical importance for disease initiation and progression by influencing distinct disease-specific signal transduction pathways.

With respect to cardiac fibrosis, a number of miRNAs have been identified previously to critically affect fibrosis regulation (Figure). miR-29 was identified to target several collagens, including collagen type 1A1, collagen type 1A2, and collagen type 3A1, as well as fibrillin 1, thus promoting extracellular matrix deposition after myocardial infarction (MI) (Figure). Specifically, after MI, the miR-29 family is downregulated in vivo, thereby derepressing its targets and resulting in cardiac fibrosis. In addition, in cardiac fibrosis in response to cardiac hypertrophy and failure, miR-21 is specifically enriched in cardiac fibroblasts and regulates the extracellular signal-regulated kinase–mitogen-activated protein kinase signaling pathway through inhibition of sprouty homolog 1. By this mechanism, miR-21 promotes fibroblast survival and growth factor secretion. In contrast, silencing of miR-21 with a specific antagonist (antagomir) culminated in a reduction of cardiac extracellular signal-regulated kinase–mitogen-activated protein kinase activity, inhibition of interstitial fibrosis, and improvement of cardiac dysfunction. In angiotensin II–induced hypertension and cardiac fibrosis, miR-133a was shown to be downregulated, leading to a derepression of its target collagen, collagen type 1A1. This promoted the extent of fibrosis development. Additionally, another study found the expression of connective tissue growth factor, one of the major fibrosis-promoting factors, to be regulated by miR-30 and miR-133 in human and animal cardiac tissue.

In the current issue of Circulation, Pan et al add to the current knowledge of cardiac-important miRNAs by investigating a novel role of miR-101 in the context of cardiac fibrosis after MI. The expression of miR-101a and miR-101b in the peri-infarct area of rats was shown to be decreased 4 weeks after coronary artery ligation. In vitro, angiotensin II suppressed the expression of miR-101a and miR-101b in rat neonatal cardiac fibroblasts. Proliferation and collagen production in fibroblasts was abrogated by overexpression of synthetic miR-101a and miR-101b. These effects were prevented by cotransfection with a specific inhibitor of miR-101a/b. The investigators identified c-fos as a target of miR-101a by bioinformatic analysis and luciferase gene reporter assays. The expression of c-fos and transforming growth factor β1 (TGFβ1) was significantly increased in cardiac fibroblasts by angiotensin II treatment. This was prevented by simultaneous overexpression of miR-101a. Through gain- and loss-of-function studies, the role of c-fos was investigated further. Plasmid overexpression of c-fos in cardiac fibroblasts resulted in enhanced TGFβ1, collagen I, and collagen III expression, whereas silencing of c-fos by small interfering RNA reduced their expression, thus eliciting comparable antifibrotic effects as miR-101a mimics. Intriguingly, the cardiac performance 4 weeks after coronary artery ligation was improved by adenovirus-mediated overexpression of miR-101a as assessed by echocardiography and hemodynamic measurements. Interstitial fibrosis, myocyte apoptosis, as well as the expression of c-fos and TGFβ1 was also decreased.

A role of miR-101 and its targets has been demonstrated previously in several other disease contexts. For instance, miR-101 influences the epigenetic regulation of prostate cancer by targeting the histone methyltransferase enhancer of zeste homolog 2. In addition, miR-101 was shown to influence cellular self-renewal by targeting the autophagy-related genes, such as Stat1min 1, Ras-related protein Rab-5A, and autophagy-related 4 homolog B. Data on miR-101 and cardiac disease are also available, but scarce so far. In cardiac tissue of patients with dilated cardiomyopathy and aortic stenosis, the expression of miR-101 was shown to be significantly reduced. Van Rooij et al found the expression
of miR-101a and miR-101b to be diminished in the infarct border zone of mice after MI. In a previous study, Yang et al.\textsuperscript{13} reported miR-101 to be reduced in atrial tissue of patients with atrial fibrillation resulting from rheumatic heart disease and in a canine model of atrial fibrillation induced by atrial tachypacing for 8 weeks. In general, most studies so far identified cardiac miR-101 to be reduced on cardiovascular stress, suggesting enhancing miR-101 strategies to be of potential therapeutic interest. However, the regulatory mechanisms leading to miR-101 silencing during cardiac stress remain unexplored so far. The available studies about the role of miR-101 in cardiovascular disease are summarized in the Table.

c-fos together with the protein families of c-Jun, activating transcription factor, and Jun dimerization protein form the transcription factor activator protein 1 (AP-1).\textsuperscript{14} The expression of AP-1 is induced by various cytokines and growth factors and activates genes responsible for cellular differentiation and proliferation.\textsuperscript{14} This is partly mediated by activating cell-cycle regulators, such as cyclin D1, cyclin A, cyclin E, p53, p21\textsuperscript{Cip1}, p16\textsuperscript{Ink4a}, and p19\textsuperscript{ARF}.\textsuperscript{14} Interestingly, the transcription of fibrosis-associated miRNAs, such as miR-21, is also regulated by AP-1.\textsuperscript{15} In addition, the miR-29 promoter contains several putative binding sites for AP-1.\textsuperscript{16} In the case of miR-29, binding of AP-1 may result in transcriptional repression during fibrosis development. However, this has not been tested yet. A study in lung fibroblasts\textsuperscript{17} confirmed that tumor necrosis factor \(\alpha\) induces the expression of profibrotic TGF\(\beta\) via regulation of c-fos. Roy et al.\textsuperscript{18} investigated the regulation of TGF\(\beta\) in cardiac fibroblasts in response to oxygen to analyze the effect of reperfusion during ischemia/reperfusion injury. It was found that oxygen induced transcription of all 3 TGF\(\beta\) isoforms through activation of AP-1. Specifically, Fos-related AP-1 transcription factor and apoptosis signal-regulating kinase-1 were shown to be essential in the regulation of AP-1-dependent TGF\(\beta\) transcription in this setting. A recent study\textsuperscript{19} analyzed the contribution of miR-21 to cardiac fibrosis by stimulating endothelial-to-mesenchymal transition. Specifically, TGF\(\beta\) induced endothelial-to-mesenchymal transition in endothelial cells via upregulation of miR-21. Thus, the effects of miR-101 via c-fos regulation may affect additional fibrosis-related miRNAs to boost cardiac fibrosis.

The study by Pan et al.\textsuperscript{9} thus adds yet another dimension to the complex regulation of cardiac fibrosis by miRNAs (Figure). The current understanding suggests that, during the development of cardiac fibrosis, the AP-1 transcription factor is activated, which also turns on other profibrotic pathways, such as miR-21. Pan et al now present an additional team player in the miRNA game that directly interferes with the expression of c-fos, a component of the AP-1 transcription factor. Thus, altered expression of this transcription factor complex by miR-101 might directly interfere with downstream signaling cascades, including that of other miRNAs, thereby modulating cardiac fibrosis. This suggests an intricate feedback loop involving miR-21, miR-101, and the AP-1 transcription factor. However, Pan et al did not investigate effects of the other components of AP-1, namely the protein families of c-Jun, activating transcription factor, and Jun dimerization protein. Therefore, future studies have to elaborate on the possible regulation of these remaining protein families. It would also be interesting to investigate the direct effect of miR-101 modulation on the expression of other profibrotic miRNAs. The authors did not detect an effect of miR-101 on the rate of apoptosis of cardiomyocytes in vitro, and they explain the antiapoptotic effect of miR-101 in vivo

![Figure](https://example.com/image.png)  
**Figure.** miRNAs involved in the pathogenesis of cardiac fibrosis. AP-1 indicates activator-protein 1; ATF, activating transcription factor; CTGF, connective tissue growth factor; JDP, Jun dimerization protein; Spry 1, sprouty 1; and TGF\(\beta\), transforming growth factor \(\beta\).

### Table. miR-101 Deregulation in Cardiovascular Disease

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>Regulation</th>
<th>Target</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial fibrillation</td>
<td>Down</td>
<td>Not investigated</td>
<td>Human/dog</td>
<td>Lu et al.\textsuperscript{13}</td>
</tr>
<tr>
<td>Dilated cardiomyopathy/ aortic stenosis</td>
<td>Down</td>
<td>Not investigated</td>
<td>Human</td>
<td>Ikeda et al.\textsuperscript{12}</td>
</tr>
<tr>
<td>MI</td>
<td>Down</td>
<td>Not investigated</td>
<td>Mouse</td>
<td>van Rooij et al.\textsuperscript{6}</td>
</tr>
<tr>
<td>MI</td>
<td>Down</td>
<td>c-Fos</td>
<td>Rat</td>
<td>Pan et al.\textsuperscript{9}</td>
</tr>
</tbody>
</table>

MI indicates myocardial infarction.
by the observed overimprovement in cardiac function. In addition, their findings concerning apoptosis and miR-101 contradict a recent study,20 in which miR-101 enhanced the apoptosis rate in hepatoma cells. The authors attribute these varying results to the different cell types used in the respective studies. Unfortunately, the expression and effects of miR-101 on other cardiac cells, such as endothelial cells or smooth muscle cells, were not examined. It would be interesting to evaluate an effect of miR-101 modulation on postmyocardial neoangiogenesis and the underlying mechanisms in cardiac vascular cells.

Importantly, the authors found a positive effect of miR-101 mimics on cardiac function and hemodynamic measurements in rats subjected to MI. Still and in contrast to miRNA antagonists,21 the application and effectiveness of miR mimetics (with the exception of viral-based approaches) is difficult and needs to be improved in the future if we want to enrich cells with specific miRNAs using viral-free delivery methods.

In conclusion, Pan et al present a detailed and innovative analysis of miR-101 deregulation in cardiac fibrosis after MI. Unresolved points remain to be elucidated, but interfering with miR-101 expression in this setting carries future therapeutic potential.

Sources of Funding
This work was supported by Integrated Research and Treatment Center Transplantation Grant BMBF 01EO0802 (to Dr Thum) and Deutsche Forschungsgemeinschaft Grant TH903/10-1 (to Dr Thum).

Disclosures
Drs Thum and Lorenzen have filed several MicroRNA-based patent applications.

References
Cardiac Fibrosis Revisited by MicroRNA Therapeutics
Thomas Thum and Johan M. Lorenzen

_Circulation_. 2012;126:800-802; originally published online July 18, 2012;
doi: 10.1161/CIRCULATIONAHA.112.125013
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/126/7/800

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
_Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/