miR-21 and cardiac fibrosis: another brick in the wall?

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Online publish-ahead-of-print 14 May 2015

This editorial refers to ‘Osteopontin is indispensible for AP1-mediated angiotensin II-related miR-21 transcription during cardiac fibrosis’, by J.M. Lorenzen et al., on page 2184.

Organ fibrosis is a common final pathway of long-lasting and iterative tissue fibrosis, and is present in several pathologies, including ischaemic heart disease, diabetes mellitus, hypertension, and chronic kidney disease. Thus, it represents a widespread cause of morbidity and mortality. Tissue fibrosis is characterized by an excessive and uncontrolled deposition of extracellular matrix (ECM) elements. The development of fibrosis requires: (i) increased synthesis by matrix metalloproteinases (MMPs) and decreased degradation of ECM due to down-regulation of MMP inhibitors; (ii) the stimulation of profibrotic mediators, such as transforming growth factor-β (TGF-β), α-smooth muscle actin (α-SMA), platelet-derived growth factor (PDGF), and cytokines; (iii) the differentiation of fibroblasts into myofibroblasts, which express features of smooth muscle differentiation; and (iv) the recruitment of cells of an endothelial origin for endothelial to mesenchymal transition (EndMT), generating cells that still express endothelial markers while gaining fibroblast-like characteristics.¹ In addition, innate and adaptive immune responses play an important role in development of fibrosis.² Despite recent advances in the understanding of the mechanisms underlying its development, therapeutic strategies specifically aimed at fibrosis remain limited.

microRNAs and fibrosis

The discovery of microRNAs (miRNAs or miRs)—small non-coding single-stranded RNAs that serve as key post-transcriptional regulators and that have been implicated in the pathogenesis of several cardiovascular diseases³—has added another layer of complexity to our understanding of the pathogenesis of myocardial fibrosis.

Among the most studied miRNAs directly involved in cardiac fibrosis are the miR-29 and the miR-21 families. Myocardial infarction has been found to be associated with down-regulation of miR-29a expression in cardiac fibroblasts, via the action of TGF-β.⁴ The miR-29 family, and in particular miR-29a, directly targets the mRNA of different types of collagens and ECM proteins, and has a strong antifibrotic effect in heart, kidney, and lung.⁵ miR-29b was shown through proteomics to target a large number of proteins involved in fibrosis.⁶ Moreover, miR-29a has been proposed as a circulating biomarker of fibrosis in hypertrophic cardiomyopathy,⁷ and plays an important role in inflammatory and immune cells.⁸ Strong evidence has also been obtained for miR-21 in cardiac fibrosis: it has been shown to promote fibroblast survival, growth factor secretion, and synthesis of collagens through regulation of the extracellular signal-regulated kinase (ERK–MAPK) signalling pathway, via the inhibition of sprouty homologue 1.⁹ In myocardial infarction, miR-21 activates the TGF-β/Smad pathway via suppression of TGF-β receptor III in the ischaemic area, producing an enhanced production of collagen, up-regulation of α-SMA expression, and facilitation of fibroblast differentiation into pathological myofibroblasts.¹⁰ miR-21 also promotes the progression of fibrosis upon ischaemia–reperfusion injury, regulating MMP-2 expression in fibroblasts of the infarct zone via inhibition of phosphatase and tensin homologue (PTEN), a direct target of miR-21.¹¹ In addition, up-regulation of miR-21 by TGF-β has been shown to promote cardiac fibrosis by stimulating EndMT of endothelial cells.¹² Another two miRNAs, miR-133 and miR-30, have also been found to be involved in fibrosis, directly down-regulating CTGF (connective tissue growth factor), a key profibrotic protein, and controlling structural changes in the ECM of human and mouse myocardium,¹³ whereas miR-133 was reported to regulate collagen 1A1 expression in an angiotensin II (Ang II)-dependent model of cardiac hypertrophy.¹⁴ Thus, the role of miRNAs, and in particular of miR-21, in cardiac fibrosis is being increasingly unravelled.

Osteopontin and cardiac fibrosis

In this issue, the article by Lorenzen et al.¹⁵ provides interesting and novel insight into the pivotal link between miR-21 induction, cardiac...
fibrosis, and dysregulated osteopontin (OPN) expression. In cardiac fibrosis induced by Ang II, OPN—a pleiotropic cytokine—was found to be essential in activating transcription factor activator protein-1 (AP-1), which subsequently induced miR-21 transcription in stimulated fibroblasts. In this setting, OPN activated ERK–MAPK as well as phosphoinositide 3-kinase–AKT signalling pathways. miR-21 reduced expression of the proapoptotic tumour suppressor gene PTEN, enhancing fibroblast survival, as well as of SMAD7, a component of the TGF-β1 signalling cascade, thereby promoting the fibrotic process. Finally, the authors demonstrate that silencing miR-21 in vivo, by targeting it with a locked nucleic acid (LNA), prevented the development of Ang II-induced cardiac fibrosis (Figure 1).

This last finding is particularly important. It is well established that myocardial miRNA expression can be blocked with the use of antisense RNAs. The development of anti-miRNA therapeutics aimed at arresting fibrosis at onset or during progression is of significant interest: miR-21, which is both dysregulated and pathogenetic in myocardial remodelling, seems to be target number one for such an approach.

On this point, an initial study by Ji et al. showed that inhibition of miR-21 expression via antisense oligonucleotide-mediated miRNA depletion significantly decreased neointima formation after angioplasty; this occurred via PTEN and Bcl-2, which are involved in miR-21-mediated cell proliferation and apoptosis. Thum et al. then demonstrated that silencing of miR-21 in vivo with the use of a specific cholesterol-conjugated anti-miRNA (or antagonor) could inhibit interstitial fibrosis and reduce cardiac dysfunction in a mouse model of pressure overload-induced heart disease. More recently, anti-miR-21 oligonucleotides were demonstrated to blunt chronic kidney disease in a murine model of Alport nephropathy, improving survival and reducing histological endpoints; in fact, inhibition of miR-21 protected glomerular and interstitial cells against TGF-β-induced fibrogenesis and inflammation. In contrast to the above, a study by Patrick et al. found that intravenous delivery of a short LNA-modified anti-miR-21 oligonucleotide failed to block the remodelling response of the heart to a variety of cardiac stresses. The factors involved in producing these conflicting results—such as the experimental protocol utilized or the performance of the LNA-based approach vs. the use of antagonors—may be speculated upon, but we are still simply too far from complete comprehension of the multifactorial mechanisms underlying fibrosis. The novel insight provided by the current study of Lorenzen et al. moves us in the right direction. Nevertheless, further studies are needed to unravel these complex mechanisms.

**Figure 1** Action of angiotensin II to promote cardiac hypertrophy, inducing overexpression of osteopontin. Secreted osteopontin initiates transcription of microRNA-21 in cardiomyocytes and fibroblasts, inducing cell hypertrophy, apoptosis, endothelial to mesenchymal transition, arterial remodelling, and myocardial fibrosis, through MAPK–SMAD7 and PDCD4–PTEN pathways. Antisense miRNA-21 (antagomir) could prevent these biological events. Ang II, angiotensin-II; MAPK, extracellular signal-regulated kinase–mitogen-activated protein kinase; PTEN, phosphatase and tensin homologue; PDCD4, programmed cell death 4; SMAD7, SMAD family member 7.
Funding

G.C. is supported by an Advanced Grant (CardioEpigen) from the European Research Council.

Conflict of interest: none declared.

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