

Cardiac fibrosis and miR-433

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Provenance: This is a Guest Editorial commissioned by Editor-in-Chief Jianxing He, MD, FACS (Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China).

Comment on: Tao L, Bei Y, Chen P, *et al.* Crucial role of miR-433 in regulating cardiac fibrosis. *Theranostics* 2016;6:2068-83.

Submitted Nov 02, 2016. Accepted for publication Nov 08, 2016.

doi: 10.21037/atm.2016.11.28

View this article at: <http://dx.doi.org/10.21037/atm.2016.11.28>

Heart failure (HF) is a major cause of morbidity and mortality (1). It affects approximately 5.4 million people in the United States with an incidence of 690,000 cases/year (2). Cardiac fibrosis (CF) has traditionally been regarded as a secondary phenomenon in the pathogenesis of HF. However, recent clinical studies suggest that CF is a major determinant of clinical outcomes in HF (3,4). Therefore, effective therapeutic modalities for CF are urgently needed. Fibroblasts are one of the main cell types in the myocardium and regulate the contractile functions of cardiomyocytes and homeostasis of surrounding connective tissues in myocardium (5,6). Under pathological conditions, fibroblasts differentiate into myofibroblasts that contain high levels of focal adhesion proteins and secrete excessive amounts of extracellular matrix proteins. The increased deposition of extracellular matrix proteins including collagens deteriorate diastolic function by increasing ventricular stiffness and reducing electrical connectivity (7,8). Myofibroblasts also secrete a variety of chemokines that increase inflammation through the recruitment of circulating lymphocytes (9,10).

Extensive efforts have been made to develop strategies that inhibit or reverse CF and its adverse consequences in the past 25 years. Current medical managements, including ACE inhibitors (11), aldosterone inhibitors (12) and statins (13), that aim at reversing cardiac remodeling in HF patients exhibit limited efficacies for CF. Recently, a matricellular protein, CCN5, was shown to reverse CF that had previously been established in a mouse model of HF (14). CCN5 induces apoptosis specifically in myofibroblasts but

not in other types of cells including cardiomyocytes and fibroblasts. This function, in addition to the inhibitory activity against endothelial-mesenchymal transition and fibroblast-to-myofibroblast transdifferentiation, is thought to be the major mechanism underlying the anti-CF activity of CCN5. The therapeutic efficacy of CCN5 is under intensive investigation in large animal models of CF and related pathologies.

MicroRNAs (miRNAs) are short (19–24 nt) single-stranded noncoding RNAs that fine-tune diverse signaling pathways through degradation or translational inhibition of target mRNAs. Several miRNAs are associated with CF and other cardiac disorders including cardiac hypertrophy, ischemia, and HF (15). For example, miRNAs that belong to the miR-29 family target the mRNA of several matrix proteins including collagens and are down-regulated by transforming growth factor- β (TGF- β) during myocardial infarction (MI) (16). Overexpression of miR-29 exhibits prominent anti-fibrotic effects in heart, kidney, and lung (17). In addition, miR-29a is known to play an important role in inflammatory and immune cells, and is regarded as a circulating biomarker of fibrosis in hypertrophic cardiomyopathy (18). MiR-21 was shown to promote survival of fibroblasts, secretion of growth factors, and synthesis of collagens in the hearts. Sprouty homologue 1 (SPRY1), TGF- β receptor III (TGFB3) and phosphatase and tensin homologue (PTEN) are known to be the targets of miR-21 (19–21). Antagomir-mediated silencing of miR-21 *in vivo* inhibits interstitial fibrosis and attenuates cardiac dysfunction in a mouse model of HF. Similarly, knockdown

of miR-21 by antisense RNA in the atria suppresses atrial fibrosis in a rat model of MI. MiR-133 and miR-30 were also shown to be involved in fibrosis through down-regulating the expression of connective tissue growth factor (CTGF), a key pro-fibrotic protein, and controlling structural changes in the ECM of human and mouse myocardium (22). A modified RNA that targets miR-21 (named RG012) is currently under clinical investigation in patients with kidney fibrosis (the ATHENA trial) (23).

In a recent *Theranostics* article, Dr. Tao's group reported that miR-433 is involved in the regulation of CF (24). In this study, they first identified miRNAs whose expression is dysregulated in post-MI ventricles by miRNA array. Among the identified mRNAs, miR-433 was of particular interest because it was known to be involved in kidney and liver fibrosis. The researchers found that miR-433 is consistently overexpressed in mouse models of MI and doxorubicin-induced cardiomyopathy and human dilated cardiomyopathy. Interestingly, miR-433 is expressed predominantly in cardiac fibroblasts but not in cardiomyocytes. To further understand the role of miR-433 in CF, mice were injected with an antagomir of miR-433 for 3 consecutive days and subjected to MI through ligation of the left anterior descending coronary artery (LAD). Three weeks later, examination of heart sections with Masson's trichrome staining revealed that the silencing of miR-433 markedly inhibited CF. Furthermore, the inhibition of CF is accompanied by the preservation of systolic function as shown by the increased left ventricular ejection fraction and fractional shortening. CF and ventricular dysfunction were also prevented when miR-433 was silenced by AAV9-mediated delivery of miR-433 sponge to the mouse hearts. These findings provide compelling evidence indicating that inhibition of miR-433 is anti-fibrotic. Overexpression of miR-433 in neonatal rat cardiac fibroblasts provoked proliferation and differentiation of the fibroblasts into myofibroblasts as determined by EdU incorporation, α -SMA staining, and expression profiles of fibrosis-associated genes, whereas inhibition of miR-433 attenuated these fibrotic responses upon treatment with TGF- β or angiotensin II. These data show the stimulatory effects of miR-433 on CF. Finally, the authors showed that antizyme inhibitor 1 (AZIN1) and c-Jun N-terminal kinases 1 (JNK1) are targets of miR-433. AZIN1 was previously shown to be a target of miR-433 in renal fibrosis; however, its role in cardiac fibroblasts was not elucidated. The AZIN1 level was significantly lowered in failing hearts and overexpression

of AZIN1 blunted the pro-fibrotic activity of miR-433. These data support the role of AZIN1 in the regulation of CF. Further investigation is warranted to understand how AZIN1 regulates CF. It is possible that the substrates of AZIN1, such as antizyme and ornithine decarboxylase, are somehow involved in CF (25,26). Another target gene is JNK and its role in cardiac remodeling is relatively well known. Deletion of JNK1 in the heart exacerbates fibrosis upon pressure overload (27). Chronic treatment with a JNK inhibitor, SP600125, increases apoptosis in cardiomyocytes and interstitial fibrosis in a hamster model of cardiomyopathy (28). In line with these findings, the suppression of JNK by miR-433 or siRNA was shown to trigger CF in the present study. In summary, the authors showed that miR-433 induces CF by suppressing the expression levels of AZIN1 and JNK1.

One interesting finding is that antagonizing miR-433 *in vivo* did not affect the post-MI infarct size in an acute phase (3 days). As the authors point out, miR-433 may be involved in CF in a chronic (remodeling) phase post-MI. Further data are needed to dissect the molecular mechanisms underlying the acute and chronic phases of CF. Another point is that antagonizing miR-433 leads to a preservation of left ventricular ejection fraction and fractional shortening even in cases where the same level of infarct are produced. Cardiac fibroblasts are known to express a wide array of paracrine factors and cytokines that exert complex biochemical and biophysical interactions with cardiomyocytes. Therefore, it would be interesting to see whether miR-433 affects the expression of genes involved in the regulation of contractile function in cardiomyocytes.

Overall, this work from Dr. Tao's group provides compelling evidence supporting miR-433 as a valid target for the development of therapeutic modalities for CF. The next step in this study would be to examine if antagonizing miR-433 is also efficient in a situation where CF is already established. In a clinical point of view, reversing and not merely preventing CF is more valuable.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Oh JG, Hajjar RJ, Park WJ. Cardiac fibrosis and miR-433. *Ann Transl Med* 2016;4(24):511. doi: 10.21037/atm.2016.11.28