miR-21: a star player in cardiac hypertrophy

Burcu Duygu and Paula A. Da Costa Martins*

Department of Cardiology, CARIM School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

This editorial refers to ‘miR-21-3p regulates cardiac hypertrophic response by targeting histone deacetylase-8’ by M. Yan et al., pp. 340–352, this issue.

1. Introduction

In recent years microRNAs (miRNAs) have been identified as important molecular players in the process of pathological cardiac remodelling. Indeed, modulation of miRNA levels in vivo reveals their causative role in cardiac remodelling and shows that the therapeutic impact of miRNA-based interventions can have in the treatment of various cardiac pathologies.

2. Biogenesis of miRNAs

MicroRNAs (miRNAs), small non-coding RNA molecules, are derived from intergenic, intronic, or exonic regions in the genome and are transcribed by RNA polymerases into primary transcripts (pri-miRNAs) as single genes or clusters. Pri-miRNAs, which contain stem-loop structures harbouring the miRNAs in the 5′ or 3′ half of the stem, are recognized by the endonuclease Drosha in the nucleus to produce miRNA precursors that are then exported to the cytosol. Once in the cytosol, the precursors are recognized by the endonuclease Dicer at specific positions and released as short double-stranded RNA duplexes (~22 nucleotides in length; Figure 1). Both strands of the duplex are produced in essential equivalent amounts by transcription, but their accumulation is unequal at steady state. Because one of the strands usually accumulates more than its partner, it is generally assumed that the ill expressed products, termed miRNA* (miRNA star), represent functionally inactive and, therefore, irrelevant carrier strands. In agreement, it was assumed that miRNA* is degraded, whereas the mature strand is taken up into the RNA-inducing silencing complex (RISC) that directs mature miRNAs to its target miRNAs leading to either blockage of protein synthesis or mRNA degradation.

However, miRNA/miRNA* ratios vary dramatically during development and some miRNA* sequences are abundantly expressed and functional or secreted in exosomes to take part in cell–cell communication. Interestingly, some hairpin sequences produce miRNAs from both strands at equal rates and because the miRNA* will be physiologically abundant they will be able to associate with Argonaute proteins. The fact that specific miRNA precursors can generate two distinct but functional miRNAs from different arms suggests evolutionary implication across miRNA gene evolution but limited evolutionary information of miRNA/miRNA* has been generated mainly across different animal species.

miRNAs take part in the development of various pathological conditions among which cardiovascular diseases through their ability to regulate post-transcriptional gene expression. Moreover, preclinical studies revealed the therapeutic value of manipulating miRNA expression and function through the administration of synthetic miRNA inhibitors or mimics to several animal models. In this regard, the clinical trial targeting miR-122 to suppress hepatitis C virus (HCV) replication is the first successful evidence of the therapeutic strength of targeting miRNAs in humans, reinforcing its potential use against various human diseases.

3. miR-21 in adverse cardiac remodelling

Several expression profiling studies identified miR-21 as one of the most abundantly expressed and dysregulated miRNAs in murine and human hypertrophic and failing hearts, which attracted the attention of many researchers to unveil the mechanistic function of miR-21 during adverse cardiac remodelling. Controversial reports on the function and mechanism of action of miR-21 in heart disease made very soon clear that this miRNA works in complex and, to some extent, mysterious ways.

On the one hand, increased miR-21 expression seems to be detrimental during pathological cardiac remodelling by either inducing cardiac fibroblast proliferation/survival or enhancing metalloprotease (MMP) activity in those cells. Apart from partly trigger endothelial–mesenchymal transition (EndMT), an important process in generation of fibrosis in response to stress, miR-21 may also be involved in angiogenesis as it is responsible for angiogenic progenitor cell dysfunction in patients with coronary artery disease by affecting the expression of superoxide dismutase (SOD), an antioxidant enzyme. Remarkably, inhibition of miR-21 by systemic delivery of synthetic cholesterol-conjugated antagonist in a mouse model of pressure overload-induced cardiac disease attenuated fibrosis and preserved cardiac function. On the other hand, a protective effect of miR-21 was also reported and attributed to its ability to regulate cardiac myocyte apoptosis via targeting PDCD4, an anti-apoptotic gene, in a rat model of ischaemia/reperfusion and a mouse model of myocardial infarction (MI). In agreement, adeno-virus-mediated overexpression of miR-21 in a mouse model of MI leads to decreased infarct size due to reduced apoptosis in cardiomyocytes. Other study has, however, disputed that an abrupt increase in miR-21

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* Corresponding author. Email: p.dacostamartins@maastrichtuniversity.nl

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expression levels in response to cardiac stress does not implicate an essential role for this miRNA in adverse cardiac remodelling. Here, inhibition of miR-21 either by genetic deletion or by systemic administration of locked nucleic acid-modified (LNA) oligonucleotides did not attenuate pathological myocardial remodelling nor prevented cardiac dysfunction in different mouse models of heart failure.\textsuperscript{15}

Although the different groups mainly suggested the discrepancies between the different studies to be technical, one must take them into consideration when drawing conclusions while studying the function of this particular miRNA and also other individual miRNAs by using different approaches such as synthetic therapeutic tools and/or genetic modifications.

4. The fate of miR-21\textsuperscript{*}

Recently, Bang et al.\textsuperscript{16} evaluated the miRNA content of cardiac fibroblast-derived exosomes and revealed the abundance of many miRNA\textsuperscript{*}, previously believed to undergo intracellular degradation. One such identified miRNAs\textsuperscript{*} was miR-21-3p (miR-21\textsuperscript{*}) which was shown to induce hypertrophy of cardiomyocytes in a paracrine fashion by mediating crosstalk between cardiac fibroblasts and cardiomyocytes (Figure 1).\textsuperscript{16} Moreover, inhibition of miR-21\textsuperscript{*} in a mouse model of Angiotensin II-induced heart failure blunted cardiac hypertrophy.\textsuperscript{16} In this study, the technical limitations of studying cell–cell communication in vivo makes it difficult to identify the cellular source of miR21\textsuperscript{*} and exclude possible endogenous regulation of miR-21\textsuperscript{*} expression in cardiomyocytes.

Others showed distinct expression pattern and function for miR-21\textsuperscript{*} on different cell types and animal models such as its key immunopathological function in controlling apoptosis in eosinophils in vitro,\textsuperscript{17} its differential expression with age\textsuperscript{18} and its increased expression levels in human failing hearts.\textsuperscript{19} Of note, extensive deep RNA sequencing in human hearts identified miR-21\textsuperscript{*} to be expressed and increased during heart failure.\textsuperscript{19} The exact mechanisms of how circulating star-miRNAs are protected from RNAse activity and loaded into exosomes were not yet addressed and remain to be clarified.

Yan et al.\textsuperscript{20} draw attention to the intrinsic anti-hypertrophic function of miR-21\textsuperscript{*} in cardiomyocytes. Adeno-associated virus-9 (AAV-9)-mediated overexpression of miR-21\textsuperscript{*} in mice, 2 weeks after TAC-induced cardiac pressure overload, attenuated cardiomyocyte hypertrophic growth and preserved cardiac function. miR-21\textsuperscript{*} exerts its function via directly targeting the histone deacetylase HDAC8, a transcriptional co-repressor. HDAC8 further regulates the levels of different proteins known to be involved in cardiac hypertrophy, and thus linking histone modification with miRNA biology.

An end-point of 2 weeks may have limited the study to reveal the long-term therapeutic effect of miR-21\textsuperscript{*} overexpression, but this time

Figure 1 Fate of miR-21 duplex in heart failure. Unwinding of the miR-21 duplex generates miR-21 and miR-21\textsuperscript{*}, both of which with distinct regulatory functions within the different cardiac cell types, fibroblasts and cardiomyocytes. While mir-21 up-regulation in cardiac fibroblasts results in enhanced fibroblast proliferation and survival, fibroblasts are also a source of miR-21\textsuperscript{*} that will be delivered to cardiomyocytes through fibroblast-derived exosomes. Once in cardiomyocytes, and depending on its origin, miR-21\textsuperscript{*} seems to exert both anti- and pro-hypertrophic roles. Mir-21, on its turn, seems to promote apoptosis in cardiomyocytes.
window was based on the expression pattern observed for miR21*: down-regulation at 2 weeks after TAC followed by up-regulation up to 4 weeks after TAC. Although at first glance miR21* could be considered a potential therapeutic target in heart failure, the dynamic expression pattern of this miRNA during disease development should not be neglected and more attention should be dedicated to understand miRNA behaviour before decisions are made regarding miRNA-based therapeutic approaches and timing of treatment. Specifically for the study of Yan et al.,20 questions remain to be addressed, whether overexpression of miR-21* has a beneficial long-term effect or only plays crucial roles during early pathological remodelling, and how expression of one strand (guide or star) affects the expression of the other.

5. Conclusion and future perspectives

The impact of miRNA* in disease development is generally underestimated. Star and guide strands have fundamentally distinct target genes and therefore exert distinct functions in the development of a disease. Studies showing that miRNA* are functional and have great potential as diagnostic and therapeutic tools in heart failure have now emerged and will hopefully provide clarification on the biogenesis, mechanisms of action, and mobility of miRNAs in both physiological and pathological conditions. Such studies may also answer the continuing controversies on the role of miR-21 and miR-21* in heart failure.

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