MicroRNAs in cardiac disease

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MicroRNAs (miRs) are transcriptionally regulated single-strand RNAs that depress protein expression through posttranscriptional mRNA silencing. A host of recent studies have established essential roles for miRs in cardiac development and cardiac health. Regulated myocardial miR expression is observed in a variety of cardiac syndromes, and serum miR levels are being evaluated as disease biomarkers. The manipulation of miR levels in mouse hearts using genetic techniques or engineered miR mimetics and antagonists is elucidating the roles of specific cardiac miRs in cardiac development, and in the cardiac response to injury or stress, and heart disease. The ability to target multiple factors within a single biological response pathway by a given miR has prompted the development of small miR-targeting molecules that can be readily delivered and have sustained *in vivo* effects. These advances establish a foundation for novel diagnostics and new therapeutic approaches for myocardial infarction, cardiac hypertrophy, and heart failure. (Translational Research 2011;157:226–235)

Abbreviations: Dgcr8 = DiGeorge syndrome critical region 8; LVAD = left ventricular assist device; MHC = myosin heavy chain; PTU = propyl-thiouracil; miR = microRNA; pre-miR = precursor miR; pri-miR = primary nuclear miR; RISC = RNA-induced silencing complex

miRNAs (miRs) AND THEIR mRNA TARGETS

Transcriptional mechanisms regulating gene expression during cardiac development and in the adult heart after physiological stress or injury are well established.[1,2] Less progress has been made elucidating posttranscriptional control mechanisms, such as differential mRNA splicing and translational suppression. The concept that protein expression can be modulated at the stage in which protein is translated from mRNA derives in part from early work in *Drosophila* showing regulation of heat shock protein 70 by targeted deadenylation and destabilization of its mRNA transcript.[3] Ten years ago, studies in *Drosophila* and mammalian cells showed that the introduction of small RNA duplexes could induce posttranscriptional gene silencing via these same mechanisms.[4,5] Other studies identified endogenous microRNAs (miRs) performing similar functions in yeast and *Drosophila*.[6-8] Shortly thereafter, it was determined that miRs must be incorporated into Argonaute protein-containing RNA silencing complexes to target mRNAs for translational suppression.[9,10]

Now, miRs are a major investigative focus in cardiovascular research. In the 5 years since Srivastava et al began to describe roles for specific miRs in cardiac development[11] and the 4 years since van Rooij and coworkers published 2 seminal articles describing pathological roles for variably expressed miRs in cardiac hypertrophy and failure,[12,13] more than 350 articles have been published examining miRs in the heart;

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>120 of these during calendar year 2010 alone. Such excitement is engendered in part by the realization that miRs comprise a different type of biological control mechanism with unique pathological effects and therapeutic implications. Improved understanding of miR biology is prompting a reevaluation of older concepts that protein content is largely determined by transcriptional mechanisms that regulate RNA production from DNA templates (DNA > RNA > protein). Although it is true that tissue- and context-specific protein activators and suppressors of transcription (transcription factors) regulate gene expression (and therefore protein make-up) in health and disease, we now recognize these mechanisms also are modified by miRs that modulate protein translation from messenger RNAs. Because miRs themselves are transcribed from DNA and because their expression is subject to transcriptional control, miR effects on translation comprise a way to fine-tune protein expression via complex feed-back, feed-forward, and cross-talk regulatory pathways. A metaphor for the different roles of transcriptional and miR regulation is the modern propeller-driven airplane. The engine (like transcriptional control) is the overall determinant of plane function—idle when parked, full throttle for take-off, and cruise for normal flight. Varying the pitch of the propeller blades (like miR regulation of translation) is then used to provide fine control over airspeed while the engine continues to operate at its most efficient cruising RPM.

Here, major findings describing miR expression and function in adult cardiac disease are reviewed, emphasizing new data, existing challenges, and translational applications. For aspects of miR biology primarily related to cardiac development, the interested reader is referred to Liu and Olson's recent excellent review.[14]

A brief overview of miR biology. miRs are small (~18 to 25 nucleotide) noncoding single-strand RNAs that regulate protein expression of target mRNAs having complementary sequences, typically in their 3′ untranslated regions. Analysis of sequences within the human genome predicts more than 1000 different miR genes, located either in the introns of protein-coding genes (so-called "miRtrons") or as independent entities in the spaces between genes. The location of miR genes is an important determinant of their expression and regulation; when they are located within a parent gene, primary miR biogenesis is controlled by the same transcriptional mechanisms as the parent gene mRNA (although miR and parent mRNA splicing and stability may differ). In contrast, an independent miR gene will have its own transcriptional controls. In either case, the long primary nuclear miR transcript ("pri-miR") undergoes splicing by the RNase-III Drosha/Dgcr8 enzyme complex, generating a characteristically hair-pin folded precursor miR ("pre-miR") that is exported from the nucleus by exportin-5 (Fig, *A*).[15] Cytoplasmic pre-miRs are cleaved by a Dicer-containing complex[16] to generate a double-strand miR–miR* duplex containing the mature miR on one strand and a complementary nonfunctional miR* on the other strand. In some instances, the miR duplex contains 2 complementary functional miRs, designated 3p and 5p based on their positions within the pre-miR. Either case, the mature single-strand miR is loaded by an as-yet unidentified protein homologous to *Drosophila* R2D2 and *Caenorhabditis* RDE-4[17] into an Argonaut protein-containing RNA-induced silencing complex (RISC)—the site of translational repression—where it can be presented to target mRNAs with complementary sequences (Fig, *B*).

Because Drosha and Dicer nucleases specifically impact miR processing, some early studies examining the impact of miRs on the heart used the approach of ablating these miR-processing enzymes, thus generally diminishing all miR production. Interrupting miR formation at its proximal step by striated muscle-specific ablation of DiGeorge syndrome critical region 8 (Dgcr8), which participates with Drosha in intranuclear miR processing, induced left ventricular remodeling that progressed to lethal heart failure and suggests a broad requirement for miRs in cardiac development and homeostasis.[18] The first studies to interrupt miR processing at its penultimate step through *Dicer* gene ablation were germ-line knockouts, decreasing miR production in the entire organism and throughout development. The resulting embryonic lethality[19] proved that one or more miRs are necessary for normal fetal development but revealed little about miR functioning specific to the heart. To better address this issue mice were created in which *Dicer* was ablated only from cardiac myocytes, either in the early embryonic heart (directed by *Nkx2.5*-Cre), the fully developed heart within days of birth (directed by *MYH6*-Cre), or conditionally in juvenile and adult mouse hearts (directed by *MYH6*-driven tamoxifen activated MER-Cre-MER).[20-23] Each approach of interrupting cardiac myocyte miR processing generated a cardiomyopathy; *Dicer* deletion early in cardiac development produced lethal intrauterine cardiac hypoplasia, whereas postnatal and adult cardiac *Dicer* ablation produced lethal cardiomyopathies exhibiting pathological cardiac gene expression, abnormal sarcomeric structure, and cardiomyocyte hypertrophy and/or apoptosis. These results support an important role for miRs in cardiac development and health, but do not define specific critical events that are controlled by miRs or establish the important targets of cardiac-expressed miRs.