MicroRNAs (miRNAs) are recently discovered regulatory RNA molecules consisting of 21 to 24 non-coding nucleotides that regulate gene expression by hybridizing to messenger RNAs (mRNAs) and causing mRNA degradation or translational inhibition. The importance of miRNAs has been demonstrated in model organisms, such as Caenorhabditis elegans and Drosophila, where they control key steps in development. However, recent evidence suggests that miRNAs participate in the control of heart development, the pathogenesis of cardiovascular disease, and the differentiation of embryonic stem cells into cardiomyocytes.

MiRNAs are processed from precursor molecules (pri-miRNAs), which are either transcribed from independent miRNA genes or are portions of introns of protein-coding RNA polymerase II transcripts. A single pri-miRNA often contains sequences for several different miRNAs. Pri-miRNAs fold into hairpin structures containing imperfectly base-paired stems and are processed in 2 steps, catalyzed by the RNase III type endonucleases, Drosha and Dicer. Both Drosha and Dicer function in complexes with proteins containing dsRNA-binding domains (dsRBDs). The Drosha partner is DiGeorge syndrome critical region gene 8 (DGCR8) in mammals. The Drosha-DGCR8 complex processes pri-miRNAs into ~70-nucleotide hairpin structures known as pre-miRNAs. Some spliced-out introns correspond precisely to pre-miRNAs (mirtrons), thus circumventing the requirement for Drosha-DGCR8. In animals, pre-miRNAs are transported to the cytoplasm by exportin5, where they are cleaved by Dicer [complexed with TAR RNA binding protein (TRBP) in mammals] to yield ~20-bp miRNA duplexes. One strand is then selected to function as a mature miRNA, whereas the other strand is degraded. Occasionally, both arms of the pre-miRNA hairpin give rise to mature miRNAs. Following their processing, miRNAs are assembled into ribonucleoprotein (RNP) complexes called micro-RNPs (miRNPs) or miRNA-induced silencing complexes (miRISCs). The key components of miRNPs are proteins of the Argonaute (AGO) family.

The miRISCs help mediate miRNA:mRNA interactions according to a set of rules. One rule for miRNA-target base pairing is perfect and contiguous base pairing of miRNA nucleotides 2 to 8, representing the ‘seed region’, which nucleates the miRNA-mRNA association. GU pairs or mismatches and bulges in the seed region greatly affect repression. However, an A residue across position 1 of the miRNA, and an A or U across position 9, improve the site efficiency, although they do not need to base pair with miRNA nucleotides. Another rule is that there must be reasonable complementarity to the miRNA 3’ half to stabilize the interaction. Mismatches and bulges are generally tolerated in this region, although good base pairing, particularly to residues 13–16 of the miRNA, becomes important when matching in the seed region is suboptimal. When miRISCs bind to miRNAs, they can repress their expression by several mechanisms: inhibition of translation elongation, co-translational protein translation, proteolysis, competition for the cap structure, inhibition of ribosomal subunit joining, inhibition of mRNA circularization, deadenylation, and decapping. Anti-miRs (antagomir, LNA and 2’-MOE) enhance translation of the target gene. Conversely, miRNA mimics enhance the repression of the target gene.
degradation, competition for the cap structure, inhibition of ribosomal subunit joining, and inhibition of mRNA circularization through deadenylation and decapping.\textsuperscript{11} In the field of cardiovascular disease or cardiomyogenesis during development, the function of the miRNA-1 (miR-1) and miRNA-133 (miR-133) families, which are specifically expressed in both skeletal muscle and the heart, have been investigated intensively. The vertebrate genome contains 2 distinct loci for the miR-1 and miR-133 bicistronic clusters that give rise to identical mature miRNA sequences. Although miR-133a is expressed in the same bicistronic unit as miR-1-1 or miR-1-2, miR-133b is expressed as a separate gene transcript. During skeletal myogenesis and in the developing heart, their tissue-specific expression is largely controlled by myogenic transcriptional networks involving transcriptional regulators, such as myocyte enhancer factor 2 (MEF2), serum response factor (SRF), MyoD, and myocardin.\textsuperscript{12-14} In cardiac myocytes, SRF induces the expression of miR-133, which in turn inhibits SRF itself in a feedback regulatory loop that affects differentiation and promotes progenitor proliferation. In skeletal muscle progenitors, miR-133 enhances myoblast proliferation by targeting SRF, whereas miR-1 promotes myogenesis by targeting the HDAC4. HDAC4 acts as a transcriptional repressor of MEF2. Thus, miR-1 and miR-133 participate in key negative feedback regulatory loops controlling the proliferation and differentiation status of skeletal and cardiac muscle progenitors.\textsuperscript{15}

Targeted deletion of the muscle-specific miRNA, miR-1-2, revealed numerous functions in the heart, including regulation of cardiac morphogenesis, electrical conduction, and cell-cycle control, which are processes controlled by this miRNA targeting the genes encoding Hand2 and Irf5, as well as those regulating the cell-cycle, and tumor suppressors? MiR-1 and miR-133 also regulate hypertrophic responses in the heart.\textsuperscript{16}

Takaya et al investigated the role of miR-1 and miR-133 during spontaneous myocardial differentiation of ES cells by 2-dimensional culture? The levels of miR-1 and miR-133 were increased during spontaneous differentiation but reduced during forced myocardial differentiation by the HDAC inhibitor, trichostatin A. The overexpression of miR-1 in ES cells by lentiviral infection reduces cardiomyogenesis via post-transcriptional inhibition of cyclin-dependent kinase-9 (CdK9). In contrast, Ivey et al\textsuperscript{18} reported that miR-1 promotes myocardial differentiation during the embryoid body-based culture of mouse and human ES cells by targeting the Notch ligand Delta-like1. Thus, muscle-specific miRNAs precisely regulate the spatiotemporal expression of target genes in different situations including myocardial differentiation, cardiovascular development, and adult cardiac disease.

Although the understanding of miRNA function is rapidly increasing, many challenges remain to utilize modulation of miRNA levels as a novel therapeutic strategy. First, the establishment of methods to accurately and efficiently determine the miRNA targets is essential. Current bioinformatic approaches have provided useful information, but precise targets must be identified among the many predicted targets. The identification of specific target genes would further our understanding of the mechanisms underlying cardiovascular disease pathogenesis. Second, the function of low expressed miRNAs including novel ones or other types of non-coding RNAs, which could be identified by high-throughput sequencing, should be analyzed as well as those expressed at higher levels in the heart. Third, proteins such as HuR, an AU-rich-element binding protein, that interact with the 3' untranslated region (3'UTR) of miRNA molecules, should be explored as they might act as modifiers to alter the potential of miRNAs to repress gene expression under stress conditions.\textsuperscript{17}

Furthermore, RNA-interference-based technologies have potential as a therapeutic strategy. Modified antisense oligonucleotides targeting the mature miRNA sequence, miRNA inhibitors (anti-miRs), including cholesterol-conjugated 2'-O-methyl oligonucleotide (antagomir), locked-nucleic-acid-modified oligonucleotide (LNA), and 2'-O-methoxyethyl phosphorothioate (2'-MOE), can reduce the levels of pathogenic or aberrantly expressed miRNAs.\textsuperscript{17} Conversely, miRNA mimics can serve to elevate the levels of miRNAs.\textsuperscript{18,19} Aberrant miRNA expression might become a novel strategy for the treatment of cardiovascular disease.

References


Circulation Journal Vol. 73, August 2009