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STATE-OF-THE-ART PAPERS

microRNAs in Cardiovascular Diseases

Current Knowledge and the Road Ahead

Gianluigi Condorelli, MD, PhD,*†‡ Michael V. G. Latronico,* Elena Cavarretta, MD, PhD§

Rozzano, Rome, and Latina, Italy

Over the last few years, the field of microribonucleic acid (miRNA) in cardiovascular biology and disease has expanded at an incredible pace. miRNAs are themselves part of a larger family, that of non-coding RNAs, the importance of which for biological processes is starting to emerge. miRNAs are ~22-nucleotide-long RNA sequences that can legate messenger (m)RNAs at partially complementary binding sites, and hence regulate the rate of protein synthesis by altering the stability of the targeted mRNAs. In the cardiovascular system, miRNAs have been shown to be critical regulators of development and physiology. They control basic functions in virtually all cell types relevant to the cardiovascular system (such as endothelial cells, cardiac muscle, smooth muscle, inflammatory cells, and fibroblasts) and, thus, are directly involved in the pathophysiology of many cardiovascular diseases. As a result of their role in disease, they are being studied for exploitation in diagnostics, prognostics, and therapeutics. However, there are still significant obstacles that need to be overcome before they enter the clinical arena. We present here a review of the literature and outline the directions toward their use in the clinic.
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Although protein-coding and transcription-regulating sequences occupy <3% of our genome, it seems that at least 75% is transcribed (1). A certain amount of the genome has been known for some time to be dedicated also to encoding infrastructural ribonucleic acid (RNA)—such as transfer, ribosomal, and small nuclear and nucleolar RNAs (2)—but the fact that most of the genome did not seem to be functional was a mystery to be resolved. It is now understood not only that much of a cell's transcriptome is involved in the production of regulatory RNA species, but also that these RNAs rival proteins in importance for the control of biological processes (3). Therefore, a vast part of our genome is not dedicated to proteins but, rather, to the production of non–protein coding RNAs (ncRNAs) with regulatory functions. The first eukaryotic ncRNA was reported in the late 1980s (4), and since then their number has grown steadily, with reports demonstrating novel roles in many biological processes (5). A hint of the vital importance of ncRNA for higher organisms is conveyed by the hypothesis that eukaryotic complexity and phenotypic variation is engendered by the degree of intricacy of the regulatory network—of which ncRNA are a chief component—rather than merely the size of the protein repertoire (3).

Among the different types of ncRNA, the microRNA (miRNA/miR) family has a central role in pathophysiological response to stress (6), regulating at least half of the transcriptome and constituting a layer of regulation that works in concert with more conventional protein-mediated mechanisms (7–9). miRNAs form an abundant ncRNA species, with nearly 2,000 human miRNA sequences catalogued hitherto (10), although it is not clear if all are bona fide. Many of these miRNAs have tissue-specific and developmental stage–specific patterns of expression (11).

microRNAs exert their function by regulating gene expression at the level of messenger RNA (mRNA) translation: It is solidly established that they regulate specific cellular processes through mRNA target recognition leading to the inhibition of protein synthesis. In particular, a specific miRNA may target multiple mRNAs (divergent miRNA pathway), a given mRNA may harbor binding sites for different miRNA (leading to combinatorial control by miRNAs), and sets of related miRNAs may affect a given pathway at different levels (convergent miRNA pathway) (Fig. 1). These characteristics create a 3-dimensional miRNA–mRNA interactome (the set of interactions occurring within a cell) that changes in relation to developmental stage, age, and pathophysiological state of the cell (12,13). A list of cardiovascular miRNAs and validated mRNA targets has been reported in a recent extensive review (14). It is possible, moreover, that they work also through other regulatory mechanisms, such as within the nucleus to regulate gene expression (15,16).

From the *Cardiovascular Research Center, Humanitas Research Hospital, Rozzano, Italy; †Department of Medical Biotechnologies and Translational Medicine, University of Milan, Rozzano, Italy; ‡Institute of Genetics and Biomedical Research, National Research Council of Italy, Rome, Italy; and the §Department of Medical–Surgical Sciences and Biotechnologies, University of Rome "La Sapienza", Latina, Italy. Dr. Condorelli has received funding from the European Research Council (Advanced Grant 'CardioEpigen' 294609), Fondation LeDucq, and Fondazione CARIPLO (12-4-5157157-31). All other authors have reported they have no relationships relevant to the contents of this paper to disclose.

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Abbreviations and Acronyms

CAM = cell adhesion molecule
cTNT = cardiac troponin T
DM = diabetes mellitus
EC = endothelial cell
HF = heart failure
MI = myocardial infarction
mRNA = messenger ribonucleic acid
miRNA/miR = microribonucleic acid
ncRNA = non-protein coding ribonucleic acid
RNA = ribonucleic acid
VSMC = vascular smooth muscle cell

Once bound to the target, miRNAs can proceed to organelles called P-bodies, which fuse with endolysosomes. As a result, miRNAs can either enter a degradative pathway or, via the formation of multivesicular bodies, can be released into the extracellular space and circulation within small vesicles called exosomes (17). miRNAs can also be secreted from cells as vesicular bodies arising from the plasma membrane or can be simply extruded by cells through membrane shedding. Moreover, some cells, such as endothelial cells (ECs), can release miRNAs under the form of apoptotic bodies (17). Thus, miRNAs can be found within the circulation either in a "free" form—that is, complexed with proteins, such as argonaute 2 or plasmatic proteins—or within membrane-bound bodies.

miRNAs in Myocardial Development and Disease

In 2002, 9 years after the description of the first miRNA in the nematode *Caenorhabditis elegans* by Lee et al. (18) and Wightman et al. (19), Calin et al. (20) published the first report describing a pathogenic role for an miRNA, specifically implicating deletion of the miR-15a/miR-16 cluster in the development of chronic leukemia. Since then, many other diseases have been linked with miRNA dysregulation, and today the importance of miRNA-mediated post-transcriptional regulation for the proper functioning of cardiovascular homeostasis, and the implications for heart disease pathogenesis, diagnosis, and prognosis are well established for the scientific community (21).

The role of miRNAs in myocardial development was first assessed in the fruit fly *Drosophila*, in which it was determining that miR-1—one of the most expressed miRNA in cardiac muscle—controlled the Notch 1 receptor (22). A causal link was established between cardiac hypertrophy and miRNAs by studying those preferentially expressed in the heart (23). In particular, miR-208a was found to be encoded by a gene residing within an intron of alpha-myosin heavy chain, to be regulated during cardiac hypertrophy, and to regulate this process itself by targeting a protein interacting with thyroid hormone receptor (24). At the same time, miR-1 and miR-133—which are encoded together in a bicistronic unit—were found to be inversely related to cardiac hypertrophy and to regulate cardiomyocyte size and function (25). miR-1 was found to modulate the insulin-like growth factor-1 pathway either directly, inhibiting insulin-like growth factor-1 and its receptor (26), or by down-regulating secreted targets related to this pathway (27). The manipulation of miRNA levels with specific anti-sense molecules—called antagomiRs —was proven to work efficiently in the myocardium in vivo and to induce significant cardiac effects (25). A direct link between miR-21 and the miR-29 family with myocardial fibrosis during hypertrophy was also demonstrated (28,29), suggesting that miRNAs control different components of myocardial remodeling. Indeed, further studies demonstrated that miRNAs control fundamentally all critical aspects of cardiovascular biology, such as angiogenesis (30), metabolism (31–34), aging (35), and also the inflammatory component of myocardial remodeling: for instance, miR-155 was found to control macrophage activity, thereby regulating cardiac hypertrophy through an indirect, inflammation-dependent mechanism (36).

The involvement of miRNAs in human cardiomyopathies has also been suggested. For example, a rare mutation of miR-499—a muscle-specific miRNA—was found at its 3′ end, outside the seed region thought to determine target recognition; this mutation was able to modify mRNA targeting and end-organ function, leading to heart failure (HF) in mice (37).

Very recently, exogenous administration of miRNAs, in particular miR-590-3p and miR-199a-3p, was found to enhance cardiomyocyte proliferation in newborn pups and in adult mice within the peri-infarct area (38). These results, if confirmed, imply that miRNAs could restore left ventricular mass and promote functional recovery after myocardial infarction (MI).

miRNAs in Vascular Diseases

As for the field of cardiology, that of vascular pathophysiology has seen an explosion of studies on miRNAs. miRNA were clearly demonstrated to play a fundamental role in controlling smooth muscle cell proliferation and maturation, vasculogenesis, neoangiogenesis, bone marrow cells, and endothelial function. We will briefly review some of the most critical discoveries in vascular biology.

In EC dysfunction, the differential expression of miR-10a was found to contribute to the regulation of the proinflammatory EC phenotype in atherosusceptible regions by inhibiting proinflammatory adhesion molecules, such as vascular cell adhesion molecule (CAM)-1, E-selectin, or the NF-κB pathway; similarly, miR-181, miR-126, miR-31, and miR-17-3p control vascular inflammation, acting on the expression of vascular CAM-1, intracellular CAM-1, and E-selectin (39–41). Cholesterol is a pivotal player not only in atherosclerosis development but also in metabolic syndrome and diabetes mellitus (DM). All the processes involved in the maintenance of cholesterol levels, such as de novo biosynthesis, internalization of exogenous cholesterol, and removal of its excessive cellular levels by high-density lipoproteins, are controlled at least partially by miRNAs. Two miRNAs have been implicated the most