The discovery of microRNAs (miRNAs) as important regulators of gene expression has greatly expanded our understanding of cardiac disease pathogenesis. These tiny molecules belong to a new class of small noncoding RNAs (typically ≈22 nucleotides in length), of which the principle function is to post-transcriptionally regulate target-gene expression. miRNAs are ubiquitously expressed in different organisms and are evolutionary conserved. Altered cardiac miRNA expression–profiles have been described in many pathological conditions. Distinct miRNA-signatures with discrete forms of cardiac pathology suggest potential value as biomarkers for heart disease. Causative roles of specific miRNAs in cardiac disease have been suggested by the observation that the experimental dysregulation of single miRNAs induces cardiac pathology consistent with conditions in which they are altered, and by the demonstration that normalizing miRNA-function reverses the pathological manifestations.

Intracellular Ca²⁺ regulation is crucial for intact cellular function. In cardiac cells, Ca²⁺ is a critical mediator of excitation–contraction coupling and is an important second messenger for Ca²⁺-dependent signaling that regulates vital cell functions. Thus, Ca²⁺ handling dysfunction is central to the pathophysiology of a broad range of cardiac diseases. Many gene-products contribute to cellular Ca²⁺ homeostasis: changes in their expression level are tightly linked to cardiac dysfunction. A range of miRNAs target genes encoding key control molecules for heart-cell Ca²⁺ homeostasis, thus playing a vital role in cardiac physiology and pathophysiology. Here, we review the present knowledge concerning miRNA regulation of Ca²⁺ handling processes, the participation of Ca²⁺-regulating miRNAs in the evolution of heart disease, the mutual relationship between Ca²⁺ signaling and miRNAs in the control of cardiac function, and the potential value of miRNA-control of Ca²⁺ handling as a therapeutic target.

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Key Words: gene expression ■ microRNA

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**Abstract:** MicroRNAs (miRNAs) are emerging as key control molecules in the regulation of gene expression, and their role in heart disease is becoming increasingly evident. Given the critical role of Ca²⁺ handling and signaling proteins in the maintenance of cardiac function, the targeting of such proteins by miRNAs would be expected to have important consequences. miRNAs have indeed been shown to control the expression of genes encoding important Ca²⁺ handling and signaling proteins, and are themselves regulated by Ca²⁺-dependent processes. Ca²⁺-related miRNAs have been found to be significant pathophysiological contributors in conditions like myocardial ischemic injury, cardiac hypertrophy, heart failure, ventricular arrhythmogenesis, and atrial fibrillation. This review is a comprehensive analysis of the present knowledge concerning miRNA regulation of Ca²⁺ handling processes, the participation of Ca²⁺-regulating miRNAs in the evolution of heart disease, the mutual relationship between Ca²⁺ signaling and miRNAs in the control of cardiac function, and the potential value of miRNA-control of Ca²⁺ handling as a therapeutic target.
provide a comprehensive review of the present understanding of miRNA-regulation of cardiac Ca\(^{2+}\) handling, the participation of Ca\(^{2+}\)-regulating miRNAs in the progression of heart disease, and the potential interest of miRNA-control of Ca\(^{2+}\) handling as a therapeutic target.

**Biogenesis of miRNAs**

The biogenesis of miRNAs starts from primary transcripts, primary miRNAs (pri-miRNAs), under the control of RNA polymerase II and conventional transcription factors (Figure 1). Depending on the genomic location of miRNA-encoding sequences, miRNAs are generally divided into 3 groups: intergenic, intronic, and exonic. The pri-miRNAs of intergenic miRNAs are transcribed under the control of distinct promoters. Pri-miRNAs, whereas the transcription of intronic and exonic miRNAs is mainly controlled by their host-gene promoters. Pri-miRNAs, typically hundreds to thousands of nucleotides in length, fold into imperfectly base-paired single or multiple stem-loop structures, which are further processed into ≈70- to 100-nucleotide hairpin-shaped precursor miRNAs (pre-miRNAs) with the participation of various endonucleases and regulatory proteins. Although the RNase-III endonuclease Drosha and the double-stranded RNA-binding protein DiGeorge syndrome critical region 8 are responsible for processing intergenic premiRNAs, both intronic and exonic premiRNAs require the additional help of splicing and debranching enzymes (Figure 1). Pre-miRNAs are translocated with the help of a nuclear export protein, Exportin 5, into the cytoplasm, where a final trimming step is performed by the RNase-III endonuclease Dicer, resulting in the short mature miRNA duplex.

The mature miRNA duplex is then assimilated into the RNA-induced silencing complex (RISC, Figure 1). On loading into the RISC, the miRNA duplex dissociates; one strand (the seed strand) remains, whereas the other (passenger) strand is generally degraded. The resultant miRNA–RISC complex binds to the 3′-untranslated region (3′UTR) of target mRNAs via Watson Crick base-pairing. Most such binding reactions involve imperfect complementarity; however, miRNAs must have perfect complementarity between the seed region (the first 2- to 8-nucleotides of the 5′-proximal end of a miRNA) and the target mRNA to properly exert their actions. The miRNA–RISC complex induces translation inhibition and mRNA degradation, resulting in post-transcriptional repression of gene expression. Multiple factors determine the relative efficiency of miRNA degradation and translation repression, including the overall complementarity between the miRNA and the mRNA, the number of target sites within the miRNA, and the secondary structure of the region surrounding the target site.

To date, ≈1000 human miRNAs have been identified; however, only some of these are expressed in the heart. Although most cardiac-expressed miRNAs are present in various cardiac cell-types, some miRNAs demonstrate cell-type selective expression. For example, miR-1 and miR-133a/b are muscle specific, preferentially expressed in cardiac and skeletal muscle cells. MiR-21 and miR-29a/b are primarily detected in fibroblasts. MiR-126, miR-24, and miR-92a are highly enriched in endothelial cells. The cell-type specificity of miRNAs is relevant to identifying miRNA-candidates that underlie conditions resulting from cell-selective dysfunction.

**General Principalsof Intracellular Ca\(^{2+}\) Regulation**

**Ca\(^{2+}\)-Induced Ca\(^{2+}\) Release in Cardiomyocytes**

A brief overview of the physiology and pathophysiology of cellular Ca\(^{2+}\) homeostasis is essential for understanding the potential targets for miRNAs. Cardiomyocyte excitation–contraction coupling is governed by Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR). Activation of voltage-gated L-type Ca\(^{2+}\) channels (LTCCs) by membrane depolarization leads to an influx of external Ca\(^{2+}\) into the cytoplasm, which triggers a much larger release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) Ca\(^{2+}\) storage complex (Figure 2). Within the SR, Ca\(^{2+}\) is bound to Ca\(^{2+}\)-buffering proteins, principally calsequestrin. The transmembrane SR-proteins, triadin and junction, anchor calsequestrin to the luminal membrane in close proximity to the SR–Ca\(^{2+}\) release channel, ryanodine receptor-2 (RyR2). Ca\(^{2+}\) binding to RyR2 activates the release of Ca\(^{2+}\) from the SR. Junctophilin-2 (JP-2) forms a complex between the plasma membrane transverse tubules (T-tubules) and the SR to maintain coordinated T-tubule–SR coupling, ensuring tight interaction between LTCCs and SR to activate efficient CICR.
The open probability of RyR2 is regulated by its phosphorylation-state, enhanced via kinases such as Ca2+-calmodulin–dependent kinase type-II (CaMKII) and protein-kinase A, and reduced by protein-phosphatases (PP) such as PP1 and PP type-2A (PP2A). These protein kinases and phosphatases also interact with LTCC-complexes and regulate LTCC activity, contributing to the control of Ca2+ current density and the activation of Ca2+ signaling.19,20

CICR produces a rapid rise in intracellular Ca2+ (the Ca2+ transient), initiating contraction when cytosolic Ca2+ binds to the Ca2+-sensitive myofilaments including troponin, actin, and myosin (Figure 2). Diastolic relaxation occurs as Ca2+ is released from the myofilaments, pumped back into the SR via the SR Ca2+-ATPase (SERCA2a) and extruded from the cell via the sarcolemmal Na+/Ca2+ exchanger (NCX). SERCA2a is under regulatory inhibition by the SERCA2a-binding protein phospholamban. Phospholamban-phosphorylation by CaMKII and protein-kinase A causes it to unbind from SERCA2a, enhancing SERCA2a-activity.19,20

**Ca2+ Release From Endoplasmic Reticulum**

Ca2+-dependent signaling is critical for regulation of cell survival, death, proliferation, and differentiation. Nonexcitable cells like fibroblasts lack voltage-gated Ca2+ channels. Two important intracellular Ca2+ regulatory mechanisms in such cells are inositol trisphosphate (IP3)–mediated Ca2+ release from the endoplasmic reticulum (ER, the primary cellular Ca2+ storage organelle in nonexcitable cells, analogous to SR in cardiomyocytes) and Ca2+ entry via voltage-independent Ca2+ permeable cell-membrane channels such as stretch-activated channels, receptor-operated channels, and store-operated channels (Figure 2). These mechanisms may also function in cardiomyocytes, but their importance is generally dwarfed by CICR.19

A major effect of growth factors and other ligands that bind to G-protein–coupled receptors is Ca2+ release from intracellular Ca2+ stores (ER/SR). G-protein–coupled receptor-dependent activation of phospholipase C cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into IP3 and diacylglycerol (DAG). IP3 diffuses to the ER/SR and binds to IP3 receptors, which act as ligand-gated Ca2+ channels, inducing channel-opening and Ca2+ release into the cytosol (Figure 2).22

Transient receptor potential (TRP) channels are Ca2+ permeable and act as multifunctional cellular sensors in both excitable and nonexcitable cells.23 TRP channels are activated by cell stretch, pathological stimuli, and Ca2+ store depletion in a voltage-independent manner and increase extracellular Ca2+ entry. Ca2+ influx via TRP channels activates
Ca²⁺-dependent signaling to modulate cell function. Of note, TRP canonical 3/6/7 channels are directly activated by DAG, a cleavage-product of PIP2, and therefore channel activation is coupled to IP3-dependent Ca²⁺ release from the ER. TRP subunits may play a significant role in store-operated channels, but recent work indicates that store-operated channels are principally composed of the proteins stromal interacting-protein 1 (STIM1) and Orai.24

Major Ca²⁺-Signaling Systems Relevant to Cardiac Disease

**Ca²⁺ Calmodulin/Nuclear Factor of Activated T Signaling**

Elevated cytoplasmic Ca²⁺ results in enhanced Ca²⁺ binding to calmodulin (CaM), which activates a downstream effector, calcineurin (Cn). Cn is a phosphatase that dephosphorylates a number of molecules, including nuclear factor of activated T cells (NFAT), a transcription factor. On dephosphorylation, NFAT translocates into the nucleus and regulates transcription of a wide range of genes involved in cardiac pathophysiology, including hypertrophy (Figure 2).25

**Ca²⁺–Cam-Dependent Kinase-II Signaling**

The Ca²⁺–CaM complex also activates CaMKII, a serine/threonine-specific protein kinase. Activated CaMKII phosphorylates itself (autophosphorylation), leading to persistent Ca²⁺-independent activation. CaMKII is a major regulator of key Ca²⁺-handling proteins and directly interacts with the molecules of downstream signaling cascades.26 Activated CaMKII phosphorylates class II histone deacetylase (HDAC), in particular HDAC4 and HDAC5, promoting myocyte enhancer factor-2-mediated gene expression involved in the development of cardiac hypertrophy (Figure 2).27 CaMKII

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**Figure 2. Cardiac cellular Ca²⁺ handling and Ca²⁺-dependent signaling.** The sarcolemma (cardiomyocyte plasma membrane) is represented by a brown hatched line with the extracellular space in pink above and the intracellular compartment below. AC indicates adenylate cyclase; β-AR, β-adrenergic receptor; CaM, calmodulin; CaMKII, Ca²⁺-calmodulin-dependent protein kinase II; CICR, Ca²⁺-induced Ca²⁺ release; Cn, calcineurin; CSQ, calsequestrin; DAG, diacylglycerol; ER, endoplasmic reticulum; ET-1/ATII-R, endothelin-1/angiotensin-II receptor; HDAC, histone deacetylase; IP3, inositol trisphosphate; IP3R, inositol trisphosphate receptor; JIP2, junctophilin-2; LTCC, L-type Ca²⁺ channel; mAKAP, muscle A-kinase anchoring protein; MEF-2, myocyte enhancer factor-2; MLC-2a, myosin light chain-2a; MyBP-C, myosin-binding protein-C; NCX, Na⁺/Ca²⁺ exchanger; NFAT, nuclear factor of activated T cells; NHE, Na⁺/H⁺ exchanger; P, phosphate; PP1, protein phosphatase-1; PP2A, protein phosphatase-2A; ROC, receptor operated channel; RyR2, ryanodine receptor type-2; SAC, stretch activated channel; SERCA2a, SR Ca²⁺-ATPase; SOC, store operated channel; SR, sarcoplasmic reticulum; SRF, serum response factor; T-tubule, transverse-tubule; Tn-I, troponin-I; and Tn-C, troponin-C.
activation also suppresses cardiomyocyte apoptosis through phosphorylation of heat shock factor 1 by induction of heat shock protein 70 (HSP 70) or through GATA-4–mediated induction of the antiapoptotic protein, B-cell lymphoma 2 (Bcl2). CaMKII also regulates transcription of proinflammatory genes via modulation of nuclear factor-κB (NFκB) signaling.

Protein Kinase C-Dependent Signaling
Agonist-ligand binding to G-protein–coupled receptors activates phospholipase C, which cleavesPIP2 into DAG and IP3; DAG in turn activates protein-kinase C (PKC). The PKC family is classified into 3 subfamilies based on the requirement for second messengers for activation: conventional PKCs (require DAG and Ca2+ for activation), novel PKCs (require DAG but not Ca2+), and atypical PKCs (require neither DAG nor Ca2+). Conventional PKCs comprise a regulatory domain responding to cellular Ca2+ and a conserved catalytic domain. On activation, the PKC phosphorylates downstream signaling molecules such as mitogen-activated protein kinase and NFXb, controlling diverse biological functions including cell survival/growth, hypertrophy, and inflammation.

miRNA Regulation of Ca2+ Signaling in Specific Forms of Cardiac Dysfunction
There is emerging evidence that miRNAs are actively involved during cardiac development and contribute importantly to cardiovascular diseases. A striking example of the involvement of miRNAs during cardiogenesis came from studies in a mouse model with conditional Dicer knockout (KO). Juvenile KO-animals had excess sudden death shortly after the induction of Dicer deletion in postnatal myocardium, whereas KO-induction in adult mice caused pathological hypertrophy without premature death. Extensive miRNA expression changes, predominantly downregulation, indicated major overall importance of miRNAs during cardiac development and disease. Numerous studies during the past 10 years have confirmed the roles of specific miRNAs in various aspects of cardiogenesis including myocardial differentiation and vasculogenesis and cardiac diseases including cardiac hypertrophy, heart failure, myocardial ischemia, and atrial fibrillation (AF). Given the substantial importance of cell Ca2+ in cardiac physiology and signaling, it is not surprising that Ca2+ regulating genes are significant miRNA targets in many of these processes, as discussed specifically below.

miRNAs and Altered Ca2+ Homeostasis in Myocardial Ischemia and Infarction
The expression of many miRNAs is altered in the ischemic myocardium. Dysregulation of these miRNAs produces fundamental elements of cardiac pathophysiology, including apoptosis, disturbed angiogenesis, fibrosis, and contractile dysfunction. For example, expression of miR-199a, miR-21, miR-494, and miR-24 is reduced in the infarcted region during cardiac ischemia/infarction. Restoration of these miRNAs improves cell viability and reduces infarct size. Overexpression of any of these miRNAs in cardiomyocytes is sufficient to prevent hypoxia-induced apoptosis. Their anti-apoptotic effect is likely because of the targeting of proapoptotic factors, like hypoxia-inducible factor 1 α (Hif-1α) (target of miR-199a), phosphatase and tensin homolog (PTEN, target of miR-21 and miR-494), Fas ligand (target of miR-21), programmed cell death 4 (PDCD4) (target of miR-21), Rho-associated coiled-coil containing protein kinase-1 (ROCK1) (target of miR-494), the CaMKII δ-subunit (CAMKIIδ, target of miR-494), and Bcl2 interacting mediator of cell death (BIM, target of miR-24).

Myocardial ischemia causes energy depletion and decreases ATP production, enhancing anaerobic glycolysis, generating lactic acid, and lowering intracellular pH. Protons are extruded from the cytoplasm via the Na+/H+ exchanger, leading to intracellular Na+ accumulation. Intracellular Na+ loading drives reverse mode Na+/Ca2+ exchange via the cardiac NCX, NCX1, causing intracellular Ca2+ overload. Decreased ATP production also suppresses ATP-dependent transport processes like the Na+/K+ ATPase and plasma membrane Ca2+ ATPase (PMCA), contributing to Na+ accumulation and the consequent increase in intracellular Ca2+. Metabolic disturbances also cause intracellular accumulation of reactive oxygen species. Ca2+ loading and reactive oxygen species accumulation activate Ca2+/calmodulin/CaMKII signaling and increase the permeability of mitochondrial membranes to small molecules (via the mitochondrial permeability transition pore [MPTP]), leading to mitochondrial swelling and cell death through apoptosis or necrosis. Energy depletion also changes the phosphorylation state of Ca2+-handling proteins and decreases the function of SERCA2a, altering Ca2+ handling and contractile properties. Recent studies have indicated roles for specific miRNAs in targeting Ca2+-handling proteins and Ca2+-signaling molecules in ischemic injury.

miRNA-1
MiR-1 has been implicated in cardiac development, apoptosis, and electrophysiology, and is dysregulated in patients with ischemic heart disease. MiR-1 targets B56δ, a regulatory subunit of PP2A, and miR-1 upregulation suppresses the function of PP2A (Figure 3). Decreased PP2A-activity increases phosphorylation and activation of RyR2 and LTCCs, without affecting phosphorylation of phospholamban, thus promoting spontaneous SR Ca2+ release and Ca2+ wave formation, facilitating the occurrence of delayed afterdepolarizations and associated spontaneous ectopic activity.

miRNA-214
MiR-214 is sensitive to cardiac stress and is upregulated in cardiac hypertrophy and failure. Aurora et al reported that miR-214 suppresses both NCX1 and proapoptotic effectors of Ca2+-signaling pathways like CaMKII, cyclophilin D (CypD), and BIM (Figure 3). On ischemia/reperfusion, the extent of myocardial loss, fibrosis, and impairment of cardiac performance are increased in the hearts of miR-214 KO-mice compared with wild-type mice. NCX1 miRNA (encoded by Slc8a1) has 3 conserved miR-214–binding sites in the 3′-UTR, and NCX1 protein expression significantly increases in the myocardium of miR-214 KO-mice versus wild-type mice. MiR-214 KO-mouse cardiomyocytes show increased Ca2+ transients under high extracellular Ca2+ concentrations (5.0 mmol/L) that mimic the environment of ischemia/reperfusion injury; mir-214–KO augments cardiomyocyte Ca2+ overload after ischemia/reperfusion injury. Moreover, miR-214 targets the proapoptotic proteins CaMKIIδ, BIM, and
CypD. BIM is a proapoptotic Bcl2 family member, and CypD is a major regulator of MPT opening. Thus, miR-214 protects the heart against ischemia/reperfusion injury by blunting Ca\textsuperscript{2+} overload and cell death through the repression of NCX1, CaMKII\textsubscript{δ}, CypD, and BIM (Figure 3).54

**miRNA-494**
Cardiac miR-494 is downregulated in human heart failure and experimental ischemic/hypertrophic models.55–57 Overexpression of miR-494 protects against ischemic cardiac injury.45 MiR-494 targets a variety of proapoptotic proteins, including CaMKII, ROCK1 and PTEN, as well as the antiapoptotic proteins fibroblast growth-factor receptor-2 (FGFR2) and leukemia-inhibitory factor (LIF) (an interleukin-6 class cytokine), upstream of the Akt-mitochondrial antiapoptotic pathway.45 The net result of their suppression is enhancement of Akt signaling.45 Thus, miR-494 provides cardioprotection via Akt-dependent antiapoptotic effects, and its downregulation in heart disease favors ischemic injury (Figure 3).45

**miRNA-145**
MiR-145 targets CaMKII\textsubscript{δ} and thus regulates proapoptotic reactive oxygen species–induced Ca\textsuperscript{2+} overload and Ca\textsuperscript{2+}-dependent signaling (Figure 3).58 MiR-145 overexpression and small interfering RNA–mediated CaMKII inhibition reduce H\textsubscript{2}O\textsubscript{2}-induced Ca\textsuperscript{2+} overload and apoptosis in rat ventricular cardiomyocytes, whereas suppressing H\textsubscript{2}O\textsubscript{2}-induced upregulation of the transcripts of PMCA-1, NCX, phospholamban, and RyR2.58 Whereas miR-145 is abundant in vascular smooth muscle, it is weakly expressed in cardiomyocytes. Therefore, miR-145 may be more relevant as a potential cardioprotective target than a direct pathophysiological contributor.

**miRNA-574-3p**
SERCA2a dysfunction decreases the removal of Ca\textsuperscript{2+} from the cytoplasm, potentially causing Ca\textsuperscript{2+} overload.59 In ventricular tissues from patients who have died of myocardial infarction, SERCA2a protein is downregulated in infarcted areas,60 whereas miR-574-3p is upregulated.60 MiR-574-3p has been...
shown computationally to preferentially bind the 3′-UTR of the SERCA2a gene. However, the effect of miR-574-3p on SERCA2a protein expression has not been tested directly.

**Cardiac Hypertrophy and Failure**

Cardiac hypertrophy is an adaptive response to increased wall stress. However, when stress is maintained under pathological conditions, maladaptive responses eventually develop, causing the transition to heart failure. Several miRNAs have been identified to play pathogenic roles in cardiac hypertrophy and failure (Figure 4). A pioneering study was performed in mice subjected to either transverse aortic constriction (TAC) or cardiac expression of activated calcineurin. A range of miRNAs was dysregulated in the cardiac tissues of these animals: miR-21, miR-23, miR-24, miR-125b, miR-195, miR-199a, and miR-214 were upregulated, and miR-29c, miR-93, miR-150, and miR-181b were downregulated. In terminally failing human hearts, the expression of miR-24, miR-125b, miR-195, miR-199a, and miR-214 is increased, indicating a common response pattern with hypertrophy. Cardiac overexpression of one of these dysregulated miRNAs, miR-195, recapitulated the phenotypic changes associated with cardiac hypertrophy and heart failure, whereas miR-214 overexpression had no phenotypic effect. Another cardiac-specific miRNA, miR-208a, encoded by an intron in the α-myosin heavy-chain gene, is an important mediator of hypertrophic responses. Mice with cardiac deletion of miR-208a fail to develop hypertrophy in response to TAC, whereas overexpression of the adult isoform miR-208a is sufficient to produce cardiac hypertrophy and arrhythmogenesis.

Figure 4. MicroRNA (miRNA)-mediated alteration of cellular Ca\(^{2+}\) handling/signaling during cardiac hypertrophy and failure. The sarcolemma (cardiomyocyte plasma membrane) is represented by a brown hatched line separating the extracellular space in pink (above and to the left) from the intracellular space in beige. A summary of the effects of key miRNA-mediated changes is provided in the blue box at the bottom left. β-AR indicates β-adrenergic receptor; CaM, calmodulin; CaMKII, Ca\(^{2+}\)/calmodulin-dependent protein kinase type-II; Cn, calcineurin; DAG, diacylglycerol; ERMK1/2, extracellular signal-regulated kinase 1/2; ET-1/ATII-R, endothelin-1/angiotensin-II receptor; FoxO3, forkhead box O3; Grb2, growth factor receptor bound 2; HDAC, histone deacetylase; IP3, inositol trisphosphate; IP3R, inositol trisphosphate receptor; JP2, juncophilin-2; LTCC, L-type Ca\(^{2+}\) channel; MEF-2, myocyte enhancer factor-2; MAPK, mitogen-activated protein kinase; MuRF1, muscle-specific ring finger protein 1; NCX, Na\(^{+}/\)Ca\(^{2+}\) exchanger; NFAT, nuclear factor of activated T cells; P, phosphate; PIP2, phosphatidylinositol bisphosphate; PKA, protein kinase A; PLB, phospholamban; PP1, protein phosphatase type-1; PP2A, protein phosphatase type-2A; PURB, purine-rich element-binding protein B; ROC, receptor operated channel; ROS, reactive oxygen species; RyR2, ryanodine receptor type-2; SAC, stretch activated channel; SERCA2a, SR Ca\(^{2+}\)-ATPase type-2a; SOC, store operated channel; SRF, serum response factor; and T-tubule, transverse-tubule.
and myostatin, both targets of miR-208a and negative regulators of muscle growth and hypertrophy. Other reports have shown that miRNAs like miR-1, miR-133, miR-23a, and miR-199b play important roles in cardiac hypertrophy and failure. 

**miR-1**

miR-1 targets various biological modulators involved in cardiac development, conduction/arrhythmogenesis, and pathological hypertrophy. Dicer downregulation causes a rapid decline in cardiac contractile function concurrent with reduced miR-1 expression. Knockdown of miR-1 with an antagomir caused marked cardiac dysfunction in wild-type mice, recapitulating changes in the conditional Dicer KO-mouse. Sorcin, a Ca\(^{2+}\)-subunit-binding protein, is a predicted target for miR-1 (Figure 4). Sorcin binds to RyR2, reducing its open probability, and accelerates LTCC inactivation, reducing cardiac contractility. Normalization of sorcin expression rescues cardiac dysfunction in both Dicer KO and miR-1 antagonist-treated mice. MiR-1 downregulation and sorcin upregulation are observed in mice with ischemic cardiomyopathy and humans with end-stage cardiomyopathy.

Diastolic Ca\(^{2+}\) increases in the failing heart cause CaMKII kinase-dependent activation of Akt signaling, which phosphorylates FoxO3a. FoxO3a is a nuclear transcription factor that regulates the host gene of premiR-1, and the export of FoxO3 from the nucleus because of Akt-dependent phosphorylation decreases miR-1 transactivation, reducing its expression (Figure 4). The NCX1 gene has conserved miR-1-binding sites in the 3′UTR, and miR-1 gene-silencing increases NCX1 protein expression. NCX1 upregulation during heart failure helps to extrude the increased diastolic Ca\(^{2+}\) resulting from reduced SR-uptake because of SERCA2a dysfunction. Adenovirus-mediated miR-1 restoration attenuates endothelin-1 induced hypertrophy in neonatal rat ventricular myocytes. The genes encoding calmodulin, Cam1 and Cam2, are predicted to have conserved miR-1 recognition sequences in their 3′UTRs. MiR-1 overexpression in neonatal rat ventricular myocytes decreases calmodulin expression, suppresses downstream signaling through calcineurin to NFAT, and suppresses myocyte enhancer factor-2a and GATA4 expression. These findings suggest that miR-1 regulates cardiac hypertrophy by controlling the Ca\(^{2+}\)-dependent signaling molecules calmodulin, myocyte enhancer factor-2, and GATA4.

**miRNA-24**

T-tubule–SR structural coupling, important for functional maintenance of CICR, occurs across an ≈15-nm junctional cleft, with JP2 ensuring proper subunit-localization. Defective excitation–contraction coupling during heart failure is accompanied by decreased expression of JP2. JP2 is a target of miR-24 (Figure 4). MiR-24 overexpression in rat ventricular cardiomyocytes causes T-tubule–SR ultrastructural remodeling, decreases Ca\(^{2+}\) transient amplitude, and induces defective excitation–contraction coupling without affecting I\(_{\text{CaL}}\), producing a heart-failure phenotype. The miR-23a/27a/24-2 gene cluster is upregulated by calcineurin–NFATc3 signaling during cardiac hypertrophy, and reversal of upregulation of these miRs is a potential therapeutic strategy. TAC-induced hypertrophy increases cardiac miR-24 levels and decreases JP2 protein expression; treatment with an antagonist to miR-24 prevents the transition from hypertrophy to failure while reversing functional and structural disruption of T-tubule–SR junctions.

**miRNA-22**

Gurha et al recently demonstrated that miR-22 deletion accelerates adverse cardiac remodeling and contractile dysfunction induced by TAC. Ca\(^{2+}\) transient decay and SR-load decreased in cardiomyocytes isolated from miR-22 KO-mice as a consequence of reduced SERCA2a expression and decreased phospholamban phosphorylation at Ser-16 and Thr-17. MiR-22 is predicted to target purine-rich element-binding protein B (PURB), a coactivator that represses SRF. MiR-22 is also implicated in the regulation of Ca\(^{2+}\)-dependent hypertrophic signaling. MiR-1 targets calmodulin and negatively regulates myocyte enhancer factor-2a and GATA4, transcription factors mediating hypertrophic gene expression (Figure 4). Adenovirus-mediated miR-1 restoration attenuates endothelin-1 induced hypertrophy in neonatal rat ventricular myocytes. The genes encoding calmodulin, Cam1 and Cam2, are predicted to have conserved miR-1 recognition sequences in their 3′UTRs. MiR-1 overexpression in neonatal rat ventricular myocytes decreases calmodulin expression, suppresses downstream signaling through calcineurin to NFAT, and suppresses myocyte enhancer factor-2a and GATA4 expression. These findings suggest that miR-1 regulates cardiac hypertrophy by controlling the Ca\(^{2+}\)-dependent signaling molecules calmodulin, myocyte enhancer factor-2, and GATA4.
miRNA-133
Carè et al. first reported that inhibition of miR-133a causes cardiac hypertrophy. Drawnel et al. reported that miR-133a is implicated in IP3-induced Ca++ release via cardiac IP3-receptors (IP3R2), which activates calcineurin/NFAT and CaMKII signaling. In failing hearts of TAC-rats, IP3R2 protein, but not mRNA, increases in cardiomyocytes, suggesting miR-dependent regulation. MiR-133a expression decreases in TAC-induced heart failure cardiomyocytes, and adenovirus-mediated miR-133 overexpression in neonatal rat ventricular myocytes downregulates IP3R2-protein; antagoniR-133a treatment of neonatal rat ventricular myocytes increases IP3R2-protein and elicits hypertrophic responses. These results suggest that IP3R2-related Ca++ mobilization is a target for miR-133a in the pathophysiology of heart failure (Figure 4).

SRF targets the miR-133a locus and negatively regulates miR-133a transactivation. SRF expression increases in endothelin-1–induced cardiac hypertrophy. Endothelin-1 and angiotensin-II binding to G-protein–coupled receptors activates phospholipase C and produces IP3. IP3-binding to IP3R2 activates calcineurin/NFAT and CaMKII signaling, which promotes hypertrophic gene transcription. Increased SRF expression causes miR-133a downregulation and thus IP3R2 upregulation, which increases Ca++ release, forming a positive feedback loop that promotes hypertrophy.

miRNA-23a
NFATc3 directly targets the gene encoding miR-23a; activation of calcineurin/NFATc3-signaling increases miR-23a gene transcription (Figure 4). MiR-23a induces hypertrophic signaling by targeting/downregulating the antihypertrophic protein muscle ring finger-1 (MuRF1). MuRF1 has a conserved miR-23a seed sequence in the 3′-UTR, and MuRF1 KO-mice show exaggerated cardiac hypertrophy in response to pressure overload. MuRF1 degrades troponin I via ubiquitination. MuRF1 suppression by miR-23a increases troponin I expression, contributing to hypertrophy.

miRNA-378
Nagalingam et al. demonstrated that phenylephrine and angiotensin II–induced hypertrophy in neonatal rat cardiomyocytes reduces miR-378 expression via negative regulation of rat sarcoma (Ras) activity. Conversely, overexpression of miR-378 mitigates phenylephrine- and angiotensin II–induced hypertrophy. Bioinformatic analysis revealed that several Ras-activators, including growth factor receptor bound 2 (Grb2), extracellular signal–regulating kinase 2, Ras-like without CAAX 1, kinase suppressor of ras 1 (KSR1), and rat sarcoma,guanine nucleotide-releasing protein-4 (RAS GRP4) are predicted targets of miR-378. The authors then went on to provide evidence directly implicating Grb2 upregulation attributable to miR-378 suppression in phenylephrine-induced hypertrophy.

Other miRNAs
MiR-21 and miR-132 are upregulated in hypertrophied cardiomyocytes, and the LTCC β2-subunit gene has seed sequences for these miRs in the 3′-UTR. Carrillo et al. demonstrated that adult rat cardiac hypertrophy caused by isoproterenol is associated with decreased LTCC β2-subunit protein but not miRNA expression, compatible with regulation by these miRNAs. Zhao et al. found that miR-210 and miR-30a are upregulated, reverting to fetal expression in human heart failure. They concluded that miR-210 dysregulation is likely related to Ca++-dependent signaling in heart failure.

Ventricular Tachyarrhythmias
Ventricular tachyarrhythmias are a major contributor to sudden cardiac death, and disordered Ca++ handling is an important underlying mechanism. Spontaneous SR-Ca++ release promotes triggered activity attributable to afterdepolarizations. Afterdepolarizations result from a net inward current within the plateau or repolarization phase of the action potential (AP) (early afterdepolarizations) or when repolarization has been completed (delayed afterdepolarizations [DADs]). DADs are induced by spontaneous Ca++ release from the SR into the cytosol through RyR2 (Figure 5). Ca++ that is released into the cytosol during diastole is exchanged for extracellular Na⁺ in a 1:3 ratio, generating a net inward (depolarizing) current called the transient inward current (Iₜₚ), which causes DADs. RyR2-opening is regulated by Ca++ at both the luminal and cytosolic side of the SR-membrane. SR-Ca++ increases during diastole because of SERCA-mediated uptake from the cytosol. Abnormal RyR2-opening results when diastolic SR-Ca++ reaches the threshold for opening, either because of Ca++ overload or because RyR2 Ca++ sensitivity is enhanced, allowing lower than usual Ca++ levels to open the channels. Hyperphosphorylation by either protein kinase A or CaMKII enhances RyR2 Ca++ sensitivity and can provoke DADs. Myocardial ischemia induces Ca++ overload (Figure 3), along with intense local catecholamine release that causes protein kinase A- and CaMKII-dependent RyR2-hyperphosphorylation. Early afterdepolarizations occur because of reactivation of Iₜₚ or Iₚ when the AP is excessively prolonged. Enhanced Ca++ release promotes Ca++ extrusion via NCX during the AP plateau, producing inward current that prolongs the AP. CaMKII hyperphosphorylation of LTCC enhances Ca++ window current during the AP plateau, also prolonging the AP and promoting early afterdepolarizations. In addition to directly promoting arrhythmias via changes in Ca++ handling, Ca++-related miRNAs can favor arrhythmia occurrence by causing arrhythmogenic tissue remodeling, as in the contexts of cardiac ischemia, hypertrophy, and failure detailed above.

miRNA-1 and miRNA-133
MiR-1 is upregulated in acute myocardial infarction, a context associated with a high risk of ventricular tachyarrhythmias and sudden cardiac death. MiR-1 targets the B56α regulatory subunit of PP2A, reducing PP2A function (Figure 5). Reduced PP2A dephosphorylation of LTCCs and RyR2 results in their hyperphosphorylation, increasing spontaneous Ca++ spark frequency and causing highly arrhythmogenic spontaneous diastolic Ca++ waves (Figure 5). MiR-1 expression is upregulated in patients with coronary
artery disease and animal models of ischemia/infarction. Therefore, miR-1 dysregulation of Ca\(^{2+}\) handling is likely to be implicated in ventricular arrhythmogenesis associated with ischemic heart disease.

Belevych et al.\(^9^6\) recently demonstrated that both miR-1 and miR-133 are significantly upregulated in a canine heart failure model. In addition to miR-1 targeting of B56\(^{\alpha}\), they demonstrated miR-133 targeting of PP2A. Decreased PP2A activity in failing cardiomyocytes was accompanied by increased frequency of diastolic Ca\(^{2+}\) waves and afterdepolarizations, along with RyR2 hyperphosphorylation at CaMKII sites, which were suppressed by CaMKII inhibitory peptide.\(^9^6\) Thus, miR-1 and miR-133 may contribute to ventricular arrhythmogenesis in heart failure.

**miRNAs and AF**

AF is a source of major cardiovascular morbidity and mortality.\(^9^7\) In the search for novel therapeutic approaches, disorders of cellular Ca\(^{2+}\) handling and signaling are emerging as important pathophysiological contributors and targets.\(^9^8\) AF can result from a wide range of cardiac conditions and, once initiated, AF induces remodeling changes that promote its own occurrence and maintenance (Figure 6).\(^9^8-^{10^0}\) AF-induced cardiomyocyte electric remodeling is characterized by AP-abbreviation attributable to reduced LTCC current and upregulation of \(I_{\text{K1}}\), as well as Ca\(^{2+}\) handling disturbances causing DAD-triggered activity.\(^9^8-^{10^0}\) In addition, structural remodeling involving atrial fibroblast activation and fibrosis plays an important role.\(^9^8,^{9^9,^{10^1}}\) Ca\(^{2+}\)-related processes are prominently involved in AF-related remodeling, both as signaling mechanisms initiating remodeling and as targets of remodeling.\(^9^9\) Recent evidence implicates miRNAs in AF as both targets of Ca\(^{2+}\) signal regulation and as determinants of Ca\(^{2+}\) handling abnormalities.\(^9^9,^{10^1}\)

**miRNA-328**

Lu et al.\(^4^0\) demonstrated that atrial miR-328 is upregulated in AF and contributes to AF-induced electric remodeling. Adenovirus-mediated miR-328 overexpression in dogs and cardiac-specific miR-328 overexpression in transgenic mice recapitulates the AF phenotype.\(^4^0\) The genes encoding Cav1.2
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CACNA1c, α1c subunits) and Cavβ1 proteins (CACNB1, β1 subunits) are targets of miR-328 (Figure 6), accounting for Cav1.2 and Cavβ1 downregulation in miR-328 transgenic mice. Knockdown of miR-328 in miR-328-transgenic mice restores Cav1.2 and Cavβ1 protein levels and reduces AF-vulnerability.

miRNA-26

MiR-26 is encoded as 3 isoforms (miR26a1, miR26a2, and miR26b) with the same seed sequence in 3 human gene locations, and all isoforms have multiple NFAT binding sites. NFAT-signaling is enhanced by AF conditions in both cardiomyocytes and fibroblasts, and negatively regulates miR-26 transcription. The KCNJ2-gene encoding Ik, is targeted/ negatively regulated by miR-26, and miR-26 knockdown with antimir-26 causes Ik upregulation and AF promotion in a mouse model. MiR-26 is also downregulated in canine and human models of AF, suggesting that Ca²⁺/calmodulin-induced NFAT translocation with consequent miR-26 downregulation is an important contributor to Ik upregulation and electric remodeling associated with AF (Figure 6).

MiR-26 may also be involved in atrial structural remodeling. MiR-26 targets transient receptor potential canonical (TRPC3), a nonselective cation channel implicated in cellular Ca²⁺-entry, particularly in the response of nonexcitable cells like fibroblasts to mechanical or chemical stimuli (Figure 6). TRPC3-dependent Ca²⁺ entry activates extracellular signal-regulating kinase-1/2 signaling that accelerates fibroblast proliferation and differentiation. Atrial fibroblast TRPC3-current is enhanced in AF dogs, and protein expression is upregulated in experimental and clinical AF. Atrial fibroblasts from AF dogs show increased proliferation, differentiation, and extracellular matrix-secretion; pharmacological TRPC3-inhibition or lentivirus-mediated TRPC3...
knockdown suppresses these profibrotic responses. In vivo TRPC3-blockade suppresses fibroblast activation and arrhythmia promotion in a canine AF model.

**miRNA-29, -133, and -590**

Transforming growth factor-β1 (TGF-β1) regulates connective tissue growth factor (CTGF) and collagen production in cardiac fibroblasts through activation of several pathways, including Smads and Ca²⁺-related PKC/αs/mitogen-activated protein/extracellular signal–regulating kinase-1/2 signaling. TGF-β1 and TGF-β receptor type II are putative targets for miR-133 and miR-590 (Figure 6). Chronic administration of nicotine to dogs produces a fibrotic AF-maintaining substrate. Nicotine treatment of cultured atrial fibroblasts downregulates miR-133 and miR-590 and causes upregulation of TGF-β1 and TGF-β receptor type II, increasing collagen production. TGF-β1 also suppresses miR-29 expression; miR-29 targets extracellular matrix proteins such as collagen-1 and fibrillin-1. Thus, TGF-β1–downregulation of miR-29 promotes fibrosis. MiR-133 is also downregulated in cardiac fibroblasts isolated from dogs with 1 week of electrically maintained AF. Castoldi et al recently demonstrated that collagen-1 is a putative target of miR-133, and miR-133 downregulation increases collagen secretion in cardiac fibroblasts.

**Areas for Future Research**

**Unexplored Ca²⁺-Handling Targets**

Although several miRNAs have been shown to affect cellular Ca²⁺ homeostasis, these effects are probably only the tip of the iceberg. Bioinformatic analysis suggests that many previously explored miRNAs can target multiple additional components and regulators of Ca²⁺-handling (Table 1). Although bioinformatic methods can only point to potential regulatory pathways, and experimental validation is essential, this analysis points to many additional potential targets for further research.

**MiR-1** is widely reported to be downregulated during clinical and experimental heart failure. In addition to its known targets of PPP2R5A, SERCA2a, NCX1, SR, Annexin V, CaM, Mef2a, and GATA4, miR-1 is also predicted to target NFATc3, suggesting that miR-1 may influence Ca²⁺-calmodulin/NFAT signaling. Our bioinformatic analysis also suggests that miR-1 may affect the activity of PP1 via a regulatory subunit, PPP1R3B. MiR-133 is also known to be downregulated in heart failure and to indirectly activate CaMKII signaling via IP3R2. However, CaMKII is also predicted to be a target of miR-133, so decreased miR-133 expression could enhance CaMKII expression. In addition to CaMKII, another key component of Ca²⁺ handling, SERCA2a is predicted to be a target of miR-133, along with an array of regulatory and catalytic subunits of PP1 (PPP1R9B) and PP2 (PPP2CA, PPP2CB, PPP2R2D, PPP2R4, PPP2R5D, PPP2R5E).

**MiR-214** plays a cardioprotective role by preventing Ca²⁺ overload and cell death through its direct repression of NCX1, CaMMIIb, CypD, and BIM. Based on computational prediction, miR-214 can also potentially target other key Ca²⁺-related genes, including SERCA2a, NFATc3, and NFATc4, as well as a catalytic subunit of PP2 (PPP2CB). Whether miR-214–dependent regulation of these different Ca²⁺-handling proteins also contributes to the beneficial role of miR-214 during ischemic injury is of potential interest. Cardiac miR-494 is downregulated in patients with heart failure and ischemic/hypertrophic animal models, and targets both proapoptotic (CaMKII, ROCK1, and PTEN) and antiapoptotic (FGFR2 and LIF).

**Table 1. Summary of Currently Unexplored Ca²⁺-Related miRNAs and Their Validated and Predicted Ca²⁺ Handling and Signaling-Related Targets in Various Cardiac Pathological Conditions**

<table>
<thead>
<tr>
<th>Explored miRNAs</th>
<th>miRNA Alteration in Cardiac Diseases</th>
<th>Validated Targets</th>
<th>Predicted Targets Related to Ca²⁺ Handling</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-1</td>
<td>Up (IHD)</td>
<td>PPP2R5A (B56α, PP2 regulatory subunit)</td>
<td>NFATc3, PP1 (PPP1R3B)</td>
</tr>
<tr>
<td>miR-133</td>
<td>Down (CH/HF)</td>
<td>SERCA2a, NCX1, SRI, Annexin V, CaM, Mef2a, and GATA4</td>
<td>IP3R2</td>
</tr>
<tr>
<td>miR-145</td>
<td>? (MI)</td>
<td>CaMKIIβ</td>
<td>PP1 (PPP1R9B), PP2 (PPP2R3A)</td>
</tr>
<tr>
<td>miR-22</td>
<td>? (CH/HF)</td>
<td>PURB</td>
<td>PPP1 (PPP1R3B, PPP1R9A)</td>
</tr>
<tr>
<td>miR-23a</td>
<td>Up (CH/HF)</td>
<td>MuRF1</td>
<td>PPP2 (PPP2R5E, PPP2R3A)</td>
</tr>
<tr>
<td>miR-24</td>
<td>Up (CH/HF)</td>
<td>J2P</td>
<td></td>
</tr>
<tr>
<td>miR-214</td>
<td>Up (MI)</td>
<td>NCX1, CaMMIIb, CypD, BIM</td>
<td>NFATc3/c4, PP2 (PPP2CB), SERCA2a</td>
</tr>
<tr>
<td>miR-494</td>
<td>Down (IHD)</td>
<td>CaMKII, ROCK1, PTEN, FGFR2, and LIF</td>
<td>CaCN1a/c, PP2 (PPP2R2B, PPP2R5E)</td>
</tr>
<tr>
<td>miR-574-3p</td>
<td>Up (MI)</td>
<td>SERCA2a</td>
<td></td>
</tr>
</tbody>
</table>

All miRNA targets are predicted by TargetScan (http://www.targetscan.org/). B56α indicates regulatory subunit of PP2; BIM, proapoptotic Bcl2-like protein 11; CaM, calmodulin; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II β; CaMIIb, Ca²⁺/calmodulin-dependent kinase type-II β; CH, cardiac hypertrophy; CypD, cyclophilin D; FGFR2, fibroblast growth-factor receptor-2; HF, heart failure; IHD, ischemic heart disease; J2P, junctophilin-2; MI, myocardial infarction; MuRF1, muscle ring finger 1; NFAT, nuclear factor of activated T-cells; PP1, protein phosphatase type-1; PP2, protein phosphatase type-2; PPP2CB, protein phosphatase 2, catalytic subunit ε; PPP2R5A, protein phosphatase 2, catalytic subunit δ; PPP2R5B, protein phosphatase 2 regulatory subunit B; PPP2R5D, protein phosphatase 2A activator, regulatory subunit 4; PPP2R5E, protein phosphatase 2, regulatory subunit B; PPP2R2D, PPP2R4, PPP2R5D, PPP2R5E, SERCA2a. Change in the corresponding miRNA in the given cardiac pathological conditions remains unclear because of conflicting data.
Table 2. Summary of Currently Unexplored Ca\(^{2+}\)-Related miRNAs and Their Validated and Predicted Ca\(^{2+}\)-Handling and Signaling-Related Targets in Various Cardiac Pathological Conditions

<table>
<thead>
<tr>
<th>Unexplored miRNAs</th>
<th>Alteration in Cardiac Diseases</th>
<th>Predicted Targets Related to Ca(^{2+}) Handling</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-15b</td>
<td>Up (MI)</td>
<td>NFATc3</td>
</tr>
<tr>
<td>miR-195</td>
<td>Up (CH/HF)</td>
<td>NFATc3</td>
</tr>
<tr>
<td>miR-29b</td>
<td>Down (CH/HF,MI)</td>
<td>CaMKII (CAMK2G, CAMKK2), NFATc3/c4, CaM (CALM3)</td>
</tr>
<tr>
<td>miR-320</td>
<td>Down (MI)</td>
<td>CACNA1c, CaM (CALM3), PP1 (PPP1R9B), PP2 (PPP2R2C)</td>
</tr>
<tr>
<td>miR-92a</td>
<td>Down (MI)</td>
<td>CACNA1c, CaMKII (CAMKK2), CaM (CALM3), PP1 (PPP1R9A, PPP1R12C)</td>
</tr>
</tbody>
</table>

All miRNA targets are predicted by TargetScan (http://www.targetscan.org). CALM3 indicates phosphorylase kinase δ/calmodulin 3; CAMKII, Ca\(^{2+}\)/calmodulin-dependent kinase type-II γ; CaMKK2, Ca\(^{2+}\)/calmodulin-dependent kinase type-II; NFATc, nuclear factor of activated T-cells; PP1, protein phosphatase type-1; PP2, protein phosphatase type-2; PPP1R9A, protein phosphatase 1, regulatory subunit 9A; PPP1R9B, protein phosphatase 1, regulatory subunit 9B/spinophilin; PPP1R12C, protein phosphatase 1, regulatory subunit 12C; and PPP2R2C, protein phosphatase 2, regulatory subunit B, γ.

In addition to obtaining a more complete perspective of miRNA regulation of Ca\(^{2+}\) handling and signaling genes, we will need to understand better the complex phenomena that result from the combined effects of the multiple miRNAs known to be dysregulated in any given cardiac condition. Systems approaches will be needed to analyze the detailed interplay between miRNAs and their targets in complex physiological systems.

\textit{miRNA Therapeutics}

Given the emerging importance of miRNA-mediated changes in Ca\(^{2+}\) handling and signaling in heart disease, in vivo manipulation of a dysregulated miRNA could be therapeutically beneficial. Ongoing clinical and preclinical trials have shown promise for miRNA-based therapeutic approaches in the treatment of various diseases, including hepatitis-C virus liver injury,\(^{113}\) cancer,\(^{114}\) and cardiovascular diseases (chronic heart failure and postmyocardial infarction).\(^{115,116}\)

The ultimate goal of miRNA therapeutics is to specifically correct disease-causing aberrant miRNA expression in affected tissue(s) without causing adverse effects in other systems. Figure 7 illustrates potential approaches to correcting disease-causing overexpression or underexpression of miRNAs. miRNA antisense oligonucleotides (anti-miRs) represent the most widely used knockdown approach with superior in vivo efficacy. Anti-miRs are chemically modified oligonucleotides that contain reverse complementary sequences to a mature miRNA. Their principle action is to either degrade or functionally block the targeted endogenous miRNA.\(^{117}\) Chemical modification strategies include 2′-O-methyl (2′-OMe)-modification locked nucleic acid (LNA) modification of oligonucleotides.\(^{118,119}\) For LNA oligonucleotides, the 2′-O-oxygen is connected to the 4′ position by a methylene linker to form a tight linkage and is locked into the C3′-endo (RNA) sugar conformation, favoring the formation of a thermodynamically strong duplex with the complementary RNA.\(^{119}\) These modifications enhance oligonucleotide cellular uptake and in vivo stability, thus optimizing in vivo effectiveness.\(^{118,119}\) The anti-miR technique is currently being used in a clinical trial for the treatment of hepatitis-C virus, as well as in several animal model studies of cardiovascular diseases.\(^{115,118,119}\) One major concern about anti-miRs is tissue-selectivity after systemic delivery. One way to circumvent the problem is to use vector-based knockdown techniques, such as have been applied with miRNA sponges and erasers.
(harboring a tandem repeat of the antisense or binding site for a given miRNA),\textsuperscript{120,121} with the vector constructed to be expressed under the control of a cardiac-specific promoter.\textsuperscript{120,121} Nonetheless, each of these techniques has potential limitations. For example, antimiRs may have a lack of specificity because they are generally designed to be shorter than target miRNAs to allow better cellular uptake and knockdown efficacy. On the contrary, although miRNA sponge/eraser may have better specificity for miRNA-targeting, they may be less effective in knocking down their targets because of unexpected secondary structure limitations, as well as unfavorable cellular environments for construct expression.

In addition to miRNA knockdown, miRNA overexpression may also be of therapeutic interest. One experimentally proven way to enhance miRNA expression is by using an miRNA mimic. MiRNA mimics are synthetic RNA duplexes in which 1 strand (guide strand) is identical to the sequence of a mature miRNA and the other (passenger strand) is deliberately designed to be partially complementary, thus mimicking the function of the endogenous miRNA. Like endogenous miRNAs, miRNA mimics need to be recognized by argonaute protein and incorporated into RISCs to exert their action; therefore, the chemical modifications possible for these RNA duplexes are very limited. The only applicable modification is the conjugation of cholesterol to the passenger strand, which improves the efficiency of cellular uptake. Although miRNA mimics might be a powerful tool to compensate for disease-promoting miRNA downregulation, unwanted extracardiac effects could be a problem. One way to overcome this problem is to deliver the miRNA mimic via cardiotropic AAVs. For example, AAV9 preferentially targets cardiac tissue. Tail-vein injection of AAV9-delivered miR-1 has recently been shown to restore the cardiac function of TAC rats.\textsuperscript{77}

Although knockdown or overexpression techniques for miRNA intervention offer exciting potential for miRNA therapeutics of cardiovascular diseases, there are important practical concerns. First, because a single miRNA may have multiple targets, it is important to understand the functional interactions between these targets as well as the contribution of each to the observed phenotype. Second, miRNAs may produce discrepant effects in different cardiac regions because of
regional differences in cellular environment. Many cardiac disease conditions involve the dysregulation of multiple miRNAs, hence targeting a single miRNA may be insufficient to correct the phenotype. Thus, although miRNA therapeutics are a promising possibility, the challenges to effective application should not be underestimated.

Conclusions

miRNA regulation of Ca\(^{2+}\) handling and signaling genes is proving to be an important contributor to the pathophysiology of a wide range of cardiac disease processes. Although much work is needed to obtain a more complete understanding of this important area, it has great potential for improving our understanding and management of heart disease.

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Disclosures

S. Nattel is listed on patents-pending belonging to the Montreal Heart Institute: “TRPC3 channels are critical for regulating fibroblast proliferation in the heart” and “MiR21 as a target in prevention of atrial fibrillation.” The other authors report no conflicts.

References


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