MicroRNAs: Novel Regulators of the Heart

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ABSTRACT MicroRNAs are approximately 22 nucleotides in length, and they play central roles in the regulation of gene expression. MicroRNAs participate in many essential biological processes, such as cell proliferation, differentiation, apoptosis and stress. Emerging evidence has indicated that microRNAs are novel regulators involved in cardiac physiology and pathophysiology, including the regulation of cardiac physiological function and participation in the genesis of cardiac diseases. Although several challenges remain, microRNAs might have a promising diagnostic and therapeutic potential in cardiac diseases.

KeyWords: MicroRNAs; cardiac pathophysiology; cardiac disease; diagnosis; treatment.

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MicroRNAs are endogenous single-stranded non-coding RNAs consisting of approximately 22 nucleotides. These small RNAs regulate target gene expression by base pairing with specific binding sites located in the 3' untranslated region of target mRNAs (1, 2). As negative regulators of targeted gene expression, microRNAs inhibit mRNA translation and promote mRNA degradation (3, 4). However, microRNAs can also up-regulate gene expression, likely via the suppression of transcriptional repressors (3, 5). Intriguingly, individual microRNAs can target multiple genes, and a single gene can be regulated by several microRNAs (6, 7). Being the central players in gene expression regulation, microRNAs participate in many essential biological processes, such as cell proliferation, differentiation, apoptosis and stress (8, 9).

Thus far, at least 700 human and 500 mouse microRNAs have been catalogued in the miRBase online database (http://microrna.sanger.ac.uk) (8, 10). Among these microRNAs, there are many that are enriched in a tissue- or cell-specific manner (11, 12). MicroRNA-1, microRNA-133 and microRNA-208 are muscle specific and are primarily expressed in cardiac and skeletal muscles (13). The microRNA-1 family, representing over 40% of all microRNAs expressed in the heart, consists of the microRNA-1 subfamily (microRNA-1-1 and microRNA-1-2) and microRNA-206 (14). The microRNA-133 family consists of microRNA-133a-1, microRNA-133a-2 and microRNA-133b (14). The microRNA-208 family, microRNAs unique to the heart, is composed of microRNA-208a and microRNA-208b, the sequences of which are located within the cardiac-restricted α- and β-myosin heavy chain (MHC) genes, respectively (13, 15). Emerging evidence has indicated that microRNAs are novel regulators of cardiac pathophysiology (13, 16).

MicroRNAs and cardiac physiology

Dicer and microRNAs

Dicer is the only known essential enzyme for the maturation of microRNAs (17, 18). In zebrafish, maternal-zygotic Dicer mutants display abnormal morphogenesis during gastrulation, brain formation, heart development and somitogenesis (16). A cardiac-specific knockout of Dicer, using -MHC promoter-driven Cre-recombinase, does not affect the specification or patterning of the heart but leads to progressive dilated cardiomyopathy, heart failure and postnatal lethality (19). Therefore, it is speculated that Dicer and microRNAs are essential for cardiac development and function.

MicroRNAs and cardiac development

The heart is one of the first organs to function in a developing embryo (8). Currently, although our understanding of microRNA function in embryogenesis is rudimentary, the emerging role of the biogenesis and activity of microRNAs as key regulatory mechanisms in controlling developmental timing, tissue differentiation, and maintenance of tissue identity during embryogenesis has been revealed (20-22).

MicroRNA-1 plays a major role in cardiac development. Hand2, a transcription factor controlling the proliferation of cardiac

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myocytes, is one target of microRNA-1 during cardiac development. During development, microRNA-1 levels increase, causing Hand2 protein levels to decrease, eventually reaching the levels found in mature cardiac myocytes. Excess microRNA-1 expression during the development period causes a reduced pool of proliferating ventricular myocytes. In short, microRNA-1 controls the balance between proliferation and differentiation during cardiogenesis via targeting critical cardiac regulatory proteins (23). Histone deacetylase 4 (HDAC4) down-regulates the expression of GATA4 and Nkx2.5 in P19 embryonic carcinoma stem cells, thereby inhibiting cardiomyogenesis. MicroRNA-1 can promote myogenesis by targeting another target, HDAC4 (5).

Similar to microRNA-1, microRNA-133 also plays roles in cardiac development, especially the development of the atrioventricular canal. MicroRNA-133 deletion results in severe cardiac malformations together with embryonic and postnatal lethality due to the insufficient number of cardioblasts (16).

However, the pivotal roles of the microRNA unique to the heart, microRNA-208, remain unknown. Additionally, the requirement for microRNAs at different developmental time points should also be explored using knockout or conditional-knockout strains (17).

MicroRNAs and cardiac ion channels

MicroRNAs can regulate cardiac ion channel genes, including GJA1 (which encodes connexin 43), CACNB2 (dihydropyridine-sensitive L-type calcium channel β 2 subunit), KCNJ3 (Kir3.1 or GIRK1, a subunit of ACh-sensitive K^+ channel), SCN5A (encoding cardiac Na^+ channel α-subunit), KCNJ2 (encoding Kir2.1, a pore-forming -subunit of the inward rectifier K^+ channel) and KCNAB1 (β1-subunit of Shaker-type voltage-gated K^+ channels). Interestingly, the distribution of microRNA-133 and microRNA-1 transcripts within the heart is spatially heterogeneous, with the patterns corresponding to the spatial distribution of the KCNQ1 and KCNE1 proteins and of I_{Ks} (14).

MicroRNAs and cardiac mitochondrial function

Mitochondria are highly abundant and constitute approximately 40% of the total volume of cardiac myocytes in the heart (24). Mitochondria in the heart play two roles essential for cell survival: ATP synthesis and maintenance of Ca^{2+} homeostasis. Adenine nucleotide transporter (ANT) plays a central role in mitochondrial oxidative phosphorylation via exchanging matrix ATP for cytosolic ADP across the mitochondrial inner membrane. ADP-ribosylation factor-like 2 (Arl2) colocalizes with ANT1, forming a complex with an Arl2-specific effector named Binder of Arl2. The interaction between Arl2 and ANT1 can regulate cellular ATP levels. MicroRNA-15b, microRNA-16, microRNA-195 and microRNA-424, all of which have the same seed sequence—the most critical determinant of miRNA targeting—can specifically down-regulate Arl2 and decrease cellular ATP levels, indicating that microRNAs can affect the ATP synthesis in mitochondria (24). However, whether microRNAs can affect mitochondrial Ca^{2+} homeostasis remains unknown.

MicroRNAs and cardiac diseases

MicroRNAs and myocardial hypertrophy

MicroRNA-133 and microRNA-1 have been found to be down-regulated in three different animal models of cardiac hypertrophy, including pressure overload-induced hypertrophy, Akt overexpression-induced hypertrophy, and adaptive cardiac hypertrophy. RhoA is a GDP-GTP exchange protein that regulates hypertrophy, whereas Cdc42 is a signal transduction kinase implicated in cardiac hypertrophy. These proteins are responsible for the rearrangements of cytoskeletal and myofibrillar proteins during cardiac hypertrophy. Nelf-A/WHSC2 is a nuclear factor involved in cardiogenesis; however, its exact role in hypertrophy remains unclear. MicroRNA-133 controls cardiac hypertrophy by targeting RhoA, Cdc42 and Nelf-A/WHSC2 (25). MicroRNA-1 regulates the growth responses of cardiac myocytes by negatively regulating the calcium signaling components calmodulin, Mef2a and Gata4, which are key transcription factors that mediate Ca2+-dependent changes in gene expression (26).

The expression of microRNA-195 is up-regulated in hypertrophic human hearts. The role of microRNA-195 in promoting cardiac growth is in contrast with that of microRNA-1, a muscle-specific microRNA that inhibits cardiac growth by suppressing the expres sion of Hand2. Although microRNA-1 is highly expressed in the heart, microRNA-195 is obviously able to override its inhibitory influence on cardiac growth. Cardiac-specific overexpression of microRNA-195 results in dilated cardiomyopathy and heart failure in mice as early as two weeks of age, implying that the up-regulation of microRNA-195 during cardiac hypertrophy actively contributes to the disease process (27).

MicroRNA-1, microRNA-133, microRNA-29, microRNA-30 and microRNA-150 are often down-regulated during myocardial hypertrophy, whereas microRNA-21, microRNA-23a, microRNA-125, microRNA-195 and microRNA-199 are up-regulated (3, 28). The forced expression of any one of the above up-regulated miRNAs is sometimes sufficient to induce hypertrophy in cultured cardiac myocytes, whereas inhibition of one of the down-regulated microRNAs can blunt the increase in cardiac myocyte size (29).

MicroRNAs and heart failure

A shift toward a fetal microRNA profile seems to be an important basis of part of the modification of the cardiac transcriptome that occurs with heart failure (30, 31). Thum et al. revealed that 67 microRNAs were up-regulated by over 1.5-fold in failing human left ventricles versus normal human hearts, whereas 43 microRNAs

were down-regulated by over 1.5-fold, as shown by microRNA microarray analysis. The microRNAs up-regulated in a failing heart contain binding sites mainly for the down-regulated mRNAs and vice versa (30). In addition, Ikeda et al. also showed divergent microRNA expression patterns, which pointed to microRNAs as active participants in the disease processes of heart failure (32). Dgcr8 is a gene required for microRNA biogenesis. Normally, the levels of Myh7 (a fetal myosin) and Tnni (a slow skeletal muscle-specific troponin-complex subunit) are down-regulated in the heart after birth. Cardiomyocyte-specific deletion of dgcr8 revealed a phenotype of heart failure. The drastic loss of cardiac function is due to the continuous expression of Myh7 and Tnni in the failing heart (31).

MicroRNAs and myocardial infarction

Myocardial infarction in mice and humans results in the dysregulation of specific microRNAs that are distinct from those involved in hypertrophy and heart failure (33, 34). The microRNA-29 family, targeting a cadre of mRNAs that encode proteins involved in fibrosis, comprises the myocardial infarction-regulated microRNAs. The microRNA-29 family is down-regulated in the region of the heart adjacent to the infarct, and this down-regulation is responsible for the induction of collagens and additional extracellular matrix genes that contribute to cardiac fibrosis in response to myocardial infarction (34).

MicroRNAs and cardiac ischemia/reperfusion (I/R) injury

Ren XP et al. detected the expression pattern of microRNAs in murine hearts subjected to I/R and found that only miR-320 expression was significantly decreased. Overexpression of microRNA-320 in cardiac myocytes results in increased sensitivity to I/R injury, whereas knock-down of endogenous microRNA-320 is cytoprotective via antithetical regulation of Hsp20 (35). Another study found that I/R rapidly elevated microRNA-21 levels in the heart. I/R-induced microRNA-21 limits phosphatase and tensin homologue function and, therefore, causes activation of the Akt pathway and increases matrix metalloprotease-2 expression in cardiac fibroblasts of the infarct region of the I/R heart (16).

MicroRNAs and arrhythmia

Ventricular arrhythmias, a major public health problem, are common events leading to sudden death. MicroRNA-1 overexpression exacerbates arrhythmogenesis via direct repression of KCNJ2 and GJA1 (36). KCNJ2 encodes Kir2.1, the main K^+ channel subunit responsible for regulating the resting cardiac membrane potential (37). GJA1 encodes connexin 43, the main cardiac gap junction channel responsible for intercellular conductance in the ventricle (38). In addition, a recent study found that miR-1 enhances cardiac excitation-contraction coupling by selectively increasing phosphorylation of the L-type Ca^{2+} channels and ryanodine receptors (RyR2) by disrupting the localization of the protein phosphatase PP2A to these channels. Through translational inhibition of the PP2A regulatory subunit B56, miR-1 causes CaMKII-dependent hyperphosphorylation of RyR2, enhances RyR2 activity, and promotes arrhythmogenic sarcoplasmic reticulum Ca^{2+} release (39). However, down-regulation of microRNA-1 and microRNA-133 in hypertrophied rat hearts has been shown to be associated with arrhythmias via the pacemaker channel genes HCN2 and HCN4, respectively (40). Additionally, microRNA-1-2 knockout mice that survive until birth have a high incidence of electrophysiological abnormalities that often result in sudden death by repressing KCND2, a potassium channel subunit involved in the transient outward K^+ current (41).

Atrial fibrillation (AF), the most prevalent arrhythmia, displays an age-dependent prevalence that exceeds 10% in elderly populations, affecting more than 5 million people worldwide. MicroRNA-1 levels have been found to be greatly reduced during human AF, possibly contributing to the up-regulation of Kir2.1 subunits and leading to increased I_{K1} (42). Another study found that the expression of microRNA-328 was up-regulated in a rat model and in human tissues with AF, which might cause AF by decreasing the expression of caveolin-3. Caveolin-3 is muscle specific and participates in regulating many ion channels in the heart (43).

MicroRNAs and congenital heart defects

The heart is more susceptible to congenital defects than any other organ (29). When microRNA-133a-1 or microRNA-133a-2 is individually deleted in mice, no obvious cardiac abnormalities in either morphology or function occur. However, combined targeted deletion of microRNA-133a-1 and microRNA-133a-2 can result in severe cardiac malformations, including ventricular septal defects. Additionally, the targeted deletion of microRNA-1-2 in mice results in 50% lethality, largely attributed to ventricular septal defects (VSDs). VSDs result from the dysregulation of myriad events during cardiogenesis, and it is likely that microRNA-1-2 regulates numerous genes, including Hand2, during the genesis of VSDs (41). Moreover, mice deficient in microRNA-17-92 die shortly after birth because of lung hypoplasia and ventricular septal defects via targeting Bim. Bim belongs to the BH3-only family of proapoptotic genes, and its overexpression leads to apoptosis (44). However, whether and which microRNAs actively contribute to congenital heart defects in humans remain unknown.

Potential applications of microRNAs in clinical practice

Diagnostic potential of microRNAs

MicroRNA expression profiles are highly accurate for the prediction of outcomes in cancer. Serum microRNAs have been found to be good diagnostic markers for cancer (45). An increasingly im-

portant question is whether microRNAs can function as diagnostic indicators in cardiac diseases.

Distinctive signature patterns of miRNA expression exist within different cardiac disease states, making new diagnostics possible (32). Moreover, the huge potential of serum microRNAs to diagnose cardiac diseases, in particular, presymptomatic screening of complications of hypertensive heart disease and heart failure, is also obvious.

In the plasma of healthy people, microRNA-1 and microRNA-133 are present in low abundance, and microRNA-208a is absent (46). Circulating microRNA-1 may be a novel, independent biomarker for the diagnosis of acute myocardial infarction (AMI). Compared with non-AMI patients, the plasma level of microRNA-1 in AMI is significantly higher and drops to normal on discharge following medication. The area under the ROC curve, a predictive method for AMI, is 0.7740 for the separation between non-AMI and AMI patients and 0.8522 for the separation between AMI patients that are hospitalized and those that can be discharged. However, the microRNA-133 level in the plasma between AMI and non-AMI subjects is not different (47). MicroRNA-208a also reveals a high sensitivity and specificity for diagnosing AMI. In AMI rats, microRNA-208a is undetected in the plasma at 0 h but is significantly increased to a detectable level within 1 h after coronary artery occlusion. Although microRNA-208a is undetectable in non-AMI patients, it can be easily detected in 90.9% of AMI patients and in 100% of AMI patients within 4 h of the onset of symptoms (47).

Therapeutic potential of microRNAs

As the changes in microRNA expression play important roles in the genesis of cardiac diseases, microRNA-based therapeutics may have promising potential.

Individual microRNA inhibition can be achieved by antisense microRNA oligonucleotides (AMOs), which are fully complementary to target microRNAs. The silencing of miRNAs using antagomirs is dose-dependent and long lasting, being detectable for as long as 23 days after injection (48). Moreover, AMOs cannot cross the blood-placental barrier, making them feasible for use even in pregnant women (49). A microRNA family can be inhibited by microRNA sponges via the introduction into the 3'UTR of a reporter gene of a series of arrayed binding sites for a specific seed in tandem (50).

The action of mature endogenous microRNAs can be mimicked with synthetic double-stranded RNA (3). For example, the administration of a microRNA mimic of microRNA-29 is capable of blunting fibrosis during hypertrophy and after myocardial infarction by targeting collagen I, II, and the gene translation (33).

Perspectives

Thus far, microRNA expression profiles for human cardiac diseases are mainly for heart failure (6). More microRNA expression studies with myocardial specimens from patients with different cardiac diseases are required, including studies that focus on the effects of the underlying etiology and the effects of treatment, age, gender, and other potential regulators (5). More importantly, the present collection of microRNAs implicated in cardiac diseases is likely to be incomplete as new microRNAs are being continuously discovered (41).

Although microRNAs have a promising diagnostic and therapeutic potential in cardiac diseases, several challenges remain. First, as gene therapy, modes of delivery, specificity, potential toxicity, reversibility and regulation of microRNA modulators are problems faced in this field. Additionally, the broad and poorly understood consequences of modulating microRNA function also pose challenges with respect to specificity and possible off-target effects (1). Second, the therapeutic window for any microRNA-directed therapeutic may be narrow. As microRNAs have numerous molecular targets, the modulation of one microRNA may perturb multiple cellular functions (6). Some of the perturbations are beneficial, while others are pathological. For example, strategies to up-regulate microRNA-133 levels in vivo might serve as a therapy for preventing pathological cardiac growth. However, microRNA-133 overexpression causes abnormalities in cardiac electrical activity (1). As another example, overexpression of microRNA-1 can be used to treat cardiac hypertrophy. However, similar to the case with miR-133a, increased levels of microRNA-1 are also accompanied by electrophysiological abnormalities (8).

In conclusion, what we have learned about microRNAs to date is just the tip of the iceberg. However, rarely has an opportunity arisen to advance such new biology for the diagnosis and treatment of cardiac diseases.

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