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The Role of miR-1 in the Heart: From Cardiac Morphogenesis to Physiological Function

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Abstract

Heart is pumping throughout life of animals ever since embryogenesis. The mammalian heart consists of four chambers along with specialized myocardial system for electrical conduction and force production, which guarantees the heart to pump efficiently. miR-1, the cardiac specific mi RNA, is suggested to play essential roles in cardiogenesis and in regulating physiological function of heart. During early stage of cardio genesis, miR-1 promotes cardiac mesoderm induction. In the subsequent morphogenesis, miR-1 controls cell fate of various lineages and the balance between proliferation and differentiation so that cardiac chambers could develop normally. In postnatal life, miR-1 modulates atrioventricular and ventricular conduction at multiple levels and contributes to the formation of organized sarcomere. Advance in the role miR-1 plays in cardiac biological processes have put new perspectives on mechanisms of heart diseases.

Keywords: MicroRNA-1; Cardiogenesis; Electrophysiology

Introduction

The heart which acquires its delicate structure from embryonic development pumps uninterruptedly throughout the life of all animals. Morphogenesis as well as maintenance of cardiac function requires precise regulation of various gene expressions. During cardiogenesis, signaling pathways and transcription factors exert spatiotemporal control of downstream gene transcription to guarantees cardiac morphogenesis progresses properly. In postnatal period, this regulatory network which is further joined by large quantity of enzymes and other participant's maintains rhythmic contraction. Some regulators in this network are effective in specific stage of life. While others, like GATA4, have shown to be essential both for embryonic development and postnatal heart function [1,2]. Recently, a novel regulatory mechanism involving post-transcriptional regulation by microRNAs (miRNAs) have been shown to function in both life stages [3].

MiRNAs are classes of small, non-coding RNAs that regulate target mRNAs by interacting with their 3'UTR in a sequence-specific manner [4]. Advances in miRNA analysis have made it possible to identify the role of miRNAs in heart. Disruption of Dicer allele, which is essential for processing of pre-miRNAs into mature form, in early stage of cardiogenesis results in profound heart defects, and the embryos die from cardiac failure by E12.5 [5]. Cardiac-specific deletion of Dicer allele in later stage of development causes enlargement of chambers and myocyte hypertrophy that leads to functional defects resemble dilated cardiomyopathy [6,7]. These studies indicate that modifications in miRNA biogenesis affect both embryonic and adult myocardial morphology and function [6].

MiR-1 is most abundantly and specifically expressed in heart [5]. Expression of miR-1 can be detected in developing mouse heart as early as E8.5, and the expression becomes even more robust after birth [8]. MiR-1 null mice manifest morphological, electrophysiological and functional defects. mRNA expression microarray analysis of mutant and wide-type hearts reveals dysregulation of numerous cardiac transcription factor genes. These genes cluster into several categories, including regulators of cell cycle, cardiac differentiation and conductive system [5]. Here we review the role miR-1 plays in morphogenesis during cardiac development and in regulating physiological function in postnatal period.

MiR-1 Modulates Cell Fate Decision and Controls Chamber Development during Cardiogenesis

Cell fate decision refers to the process that pluripotent progenitors or stem cells adopt characteristics which belong to a specific lineage while suppress the potential of other lineages [9]. MiR-1 is suggested to regulate lineage-specific determination both in embryonic stem cell (ESC) and cardiac progenitors. Over expression of miR-1 in mouse ESC promotes mesodermal induction and early cardiac markers expression. On the other hand, down regulation of miR-1 in mESCs shows delayed differentiation into mesodermal progenitors. MiR-1 also represses a number of nonmusical gene expressions, i.e. endoderm markers Afp and Hnf4a as well as neuron marker Ncam1 [9]. These data indicate that miR-1 plays a major role in promoting the progression of mesodermal progenitors by up regulating cardiac mesodermal gene expression while down regulating genes belong to other cell lineages. Further analysis identifies that the Notch ligand *Delta 1* (*Dll*1) is a direct target of miR-1, and the *Dll*1 knockdown mESCs show greater propensity toward cardiac cell fate and form beating cardiomyocyte earlier than wide type [9].

MiR-1's role in determination of cardiac progenitors has also been explored by *in vivo* study. MiR-1 deleted *Drosophila* shows

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overabundant progenitor pool and decreased cardioblasts. The Eve expressing progenitor cells undergo asymmetric division and give rise to two distinct lineages of daughter cells: one is pericardial cell, the other is muscle cell. However the Eve^{+} pericardial cell is missing in the miR-1 mutants [10]. This experiment suggests miR-1 expression is required for development of the pericardial cell lineage, and its absence leads to the missing of entire lineage. *In vivo* evidence also indicates *Dll*1 as a target of miR-1 [10].

Notch pathway mediates cell fate decision by asymmetric expression of Notch receptor and Notch ligand Delta between opposing cells [11], which eventually guide the distinct biochemical events within the cells [12]. The function of Notch pathway in cardiogenesis has also been studied extensively. Nemir and colleagues 'research on ESCs supports the idea that inhibition of Notch signaling is essential for mesodermal induction of ESC, and eventually myocardial cell fate [13]. An *in vivo* study also shows Notch signaling regulates cardiac progenitor cell fate by modulating balance between muscle and non-myogenic cell lineages [14].

Cardiogenesis is exceptionally sensitive to the dose of *Notch* and *Dll*. So the quantity of ligand and receptor present on the surface of cells is a tightly regulated parameter in the Notch pathway [12]. As a novel regulator of cell fate decision, miR-1 repression of *Dll 1* and "fine tune" the signal exchange in Notch pathway is likely one of the major mechanisms. In ESCs, miR-1 down regulation of *Dll1* might account for inhibition of Notch1 signaling in the process of myocardial progression. Nonetheless questions like how expression of miR-1 is induced and how ligand and receptor interact with each other in this process need to be further explored. In cardiac progenitors undergo asymmetric cell division, miR-1 seems to suppress *Dll1* and helps maintain active Notch receptor in signaling receiving cells. And the Notch receptor regulates specific gene expression thus dictates cell lineage. In order to specify the detailed mechanism of this process, it is important to examine miR-1's function in asymmetric division.

What worth mention is that miR-1 regulates cardiac cell fate by directing differentiation as opposed to proliferation in *Drosophila* [10]. So it will be interesting to determine whether it exerts similar function in mammals. To accommodate an advanced circulation system, mammals have adopted hearts with four chambers and the development which is a key event in the building plan of mammalian heart. Formation of the cardiac chambers displays simultaneous proliferation and differentiation. The proliferation and differentiation of chamber myocardium show highly-localized regulation which requires expansion of the complexity of regulatory network in *Drosophila*. Developing chambers manifest trans mural pattern of growth, with the highest proliferation rate in compact layer as well as progressive pattern of differentiation. This regional growth and differentiation of chamber myocardium ensues the "ballooning" model of chamber formation [3]. There are studies show miR-1 participates in the regulation of chamber expansion and differentiation. The miR-1 transgenic embryos show thin myocardium compact layer with decreased cycling myocardium. This is due to premature differentiation, early withdrawal from cell cycle, proliferation defect and failure of ventricular cardio myocyte expansion caused by excessive miR-1 [8]. Conversely, miR-1-2 deletion results in cardiac hypertrophy and ventricular septum defect (VSD). Gene expression analysis of mutants shows up regulation of cardiogenic transcriptional factors compared with wide type [5]. According to what mentioned above, miR-1 mediates cell cycle arrest and differentiation of cardiomyocyte during chamber development. The study also suggests cardiac expression of miR-1 is serum response factor (SRF)-dependent [8].

SRF has long been acknowledged as a critical regulator for chamber differentiation and functional maturation [15]. Cardiac conditional knockout (CKO) of SRF results in morphological and functional defects, presented as reduced thickness of a trial and ventricular compact layer, poor trabeculation and losing rhythmic heart beating, pericardial effusion which are due to disrupted sarcomere organization. The atrophic heart does not display significant change in proliferation rate, yet shows a impaired expression of cardiac specific genes [16,17]. The cardiac chamber differentiation process shows inverse relationship between proliferation and differentiation. SRF is not considered as a inhibitory transfactor for cell cycle, rather it exert gene silencing activity through miRNAs [3]. miR-1 mediates cell cycle withdrawal and directly targets Hand2 [8].

Hand factors are recognized as important regulators for ventricular cardiomyocyte proliferation, differentiation, morphogenesis and conduction [18]. Homozygous Hand2 null mouse displays a single left-sided ventricular chamber and aberrant connection of outflow tract that is lethal between E9,5-E10. Molecular analysis reveals impaired expression of cardiogenic transcription factors without influencing cardiomyocyte differentiation markers in mutants. Hand2 is crucial for chamber morphogenesis and development of cardiac neural crest-derived tissues [19], However overdose of Hand2 is also associated with heart abnormalities [20]. It is likely that miR-1 exert its negative regulation of myocyte proliferation partly via Hand2 during chamber differentiation [21]. However chamber formation is a local process, whether miR-1 expresses in a region-specific pattern, if so how it is regulated remains to be elucidated. However, it is safe to say that miR-1 induced by SRF helps refining transcription activity and modulates chamber development at least partially via targeting Hand2.

MiR-1 Regulates Electrical and Contractile Activity of the Heart

Efficient contraction of heart is the result of highly organized conductive system and effective electromechanical coupling. Electrical stimulation proceeds from apex toward the base in ventricles and repolarize in reverse order [22]. Spatial heterogeneous action-potential duration and conductance guarantee depolarization and repolarization propagate orderly [23]. To ensure the heterogeneity, heart has adopted a spatial specific transcriptional network that tightly regulates expression of ion channel and gap junction. When stimuli reach working cardiomyocyte, it triggers release of calcium which binds to troponin C followed by myosin-actin cross-bridge formation. In this process, myosin light chain kinase (MLCK) potentiates the force and rate of cross-bridge recruitment in cardiomyocyte and may serve as a major target in regulation of cardiac contraction [24].

Perturbation the dose of miR-1 results in electrophysiological and contractile defects. The miR-1 homozygous null mice show prolonged QRS complex and prolonged PR and QT intervalson Surface electrocardiograph, indicating defects in atrioventricular (AV) and ventricular conduction. Echocardiography also reveals severely impaired fractional shortening with poor systolic function [25]. MiR-1 over expression mice develops frequent atrioventricular block of varying degree as evidenced by prolonged PR interval [26]. The transgenic mice also show impaired contractile and diastolic function that might due to damaged sarcomere assembly [27].

The miR-1 deletion mice show elevated Irx4 and down regulated Irx5 which is a target of miR-1-2 [5]. Three members of the Iroquois homeobox (Irx) family, Irx3, Irx4 and Irx5 have been proved to play individual as well as cooperative and antagonistic roles in regulation

of electrophysiology. Irx3 establishes fast conduction in His-purkinje network [28]. Irx5 builds repolarization gradient of mouse ventricle [23]. Irx3; Irx5 double knockout (DKO) mice show AV and ventricular conduction defects, indicating a cooperative role of both in the modulation of proximal ventricular conduction. There is also an antagonistic mechanism between Irx5 and Irx3 in the regulation of ventricular depolarization [22]. Cooperation of Irxs happens between Irx4 and Irx5 in control of Kcnd2 promoter activation [29]. So precise dose of individual Irxs as well as relative ratio of Irxs is crucial for establishment of proper conduction. By directly targeting Irx5, miR-1 may modulate relative ratio between Irxs to regulate AV and ventricular conduction. How miR-1 influence expression of other Irxs still need to be further addressed.

miR-1 also regulates gap junction-forming and iron channel proteins directly [30,31]. miR-1 slows ventricular conduction through directly down regulation of Cx43 which expresses in working myocardium and facilitates coupling of the high speed conduction pathway to the surrounding myocytes [32]. MiR-1 also targets the cardiac L-type calcium channel gene CACNA1C (CAV1.2), the main calcium channel in heart [31]. SomiR-1 might control electrophysiology through targeting multiple levels. But how miR-1 orchestrates different levels of regulation needs to be further explored. In the miR-1 up-/down-regulated mice that manifest abnormal contractile function, two sets of genes are dysregulated. For miR-1 over expression mice, cardiac MLCK which functions to maintain cardiac contraction and sarcomeric assembly is significantly decreased. This is accompanied by decrease of calmuldin which is also critical for sarcomere assembly and function [27]. As for the miR-1 null mutants, a MLCK isoform, Telokin, which is supposed to specifically express in smooth muscle aberrantly upregulates in cardiomyocytes [25]. Both increased Telokin and decreased cardiac MLCK impair phosphorylation of MLC2 that results in defected assembly of the myosin thick filaments. Another study also indicates miR-1 plays important role in regulating contractility of cardiomyocyte by controlling calcium homeostasis [33]. MiR-1 mediates expression of different MLCK is forms and controls calcium homeostasis to maintain forceful contraction.

Concluding Remarks

Studies on miR-1's function in heart have uncovered novel mechanisms underlying broad spectrum of cardiac diseases. Although miR-1 has not been related to congenital heart deformities, there is ever increasing knowledge of miR-1' role in arrhythmia and cardiac dysfunction. Expression of miR-1 is lost in myotonic dystrophy patients, concomitant with up regulation of its targets Cx43 and CAV1.2 which may account for arrhythmia occurred in these patients [31]. Besides, its expression is significantly reduced in chronic a trial fibrillation patients [34], although the implication has not been fully understood. miR-1 may also play a role in ischemic arrhythmia in patients with coronary heart disease [30]. In samples from end-stage heart failure patients and hypertrophic cardiomyopathy mouse model, miR-1 is significantly down regulated [33,35]. There is also study shows miR-1 is involved in the development of diabetic cardiac dysfunction in mouse model [36].

MiR-1 is an example that a single miRNA plays critical roles in cardiogenesis as well as maintaining normal cardiac function. However, the target genes identified thus far are only a small fraction of the total genes directly respond to miR-1 in the heart. The application of genome-wide enhancer/repressor screen has aided in uncovering multiple factors which functions in common pathways targeted by miR-1 and other miRNAs [37]. With increasing knowledge about functions of miRNAs in heart, we are expecting to see a regulatory network consists of interrelated miRNAs that intertwine with well-described signaling and transcription factor networks to determine the development and pathologenesis of the heart [21].

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