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Long non-coding RNAs as new regulators of cardiac electrophysiology and arrhythmias: Molecular mechanisms, therapeutic implications and challenges

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ABSTRACT

Long non-coding RNAs (lncRNAs or lncRs) as a new class of regulatory transcripts have been intensively studied for their roles in cardiovascular biology in the past decade. We now know that lncRNAs are significantly implicated in diverse cardiovascular conditions and associated risk factors, including myocardial infarction, coronary heart disease, atherosclerosis/coronary artery disease, vascular disease, cardiac hypertrophy, heart failure, etc. Though in its early stage, research on control of cardiac electrophysiology by lncRNAs has generated some interesting observations and mechanistic insight of significant relevance to translational medicine. This review article focuses on lncRNA regulation of cardiac electrophysiology and arrhythmias with brief discussion on some fundamental aspects of relevant background information for better understanding of the subject. It provides critical analysis of published studies in the literature together with unpublished observations from our own laboratories. In addition to discuss the phenotypes associated with deregulation of lncRNAs, we also try to dissect out the cellular and molecular mechanisms for lncRNAs as regulators of arrhythmogenesis. This review then further touches on the therapeutic implications of lncRNAs and potential strategies for the development of lncRNA-based drugs. Finally, future directions to lncRNA research on cardiac electrophysiology and arrhythmias are anticipated.

Contents

Abbreviations: AF, atrial fibrillation; AP, action potential; APD, action potential duration; ATO, arsenic trioxide; CA, cardiac arrhythmias; CCRR, lncRNA named cardiac conduction-regulating RNA; ceRNA, competing endogenous RNA; CH, cardiac hypertrophy; CHF, congestive heart failure; CV, conduction velocity; Cx43, connexin43; FD, functional domain; HF, heart failure; KCNA2, the gene encoding Kv1.2 K^+ channel protein; KCNA2as, lncRNA named KCNA2 antisense RNA; KCNQ1, the gene encoding slow delayed rectifier K^+ channel α-subunit; KvLQT1, the pore-forming α-subunit of slow delayed rectifier K^+ channel; lncRNA or lncR, long non-coding RNA; KVLQT1ot1, lncRNA named KCNQ1 overlapping transcript 1; LQT, long QT syndrome; miR or miRNA, microRNA; MI, myocardial infarction; MIAT, lncRNA named myocardial infarction associated transcript; siRNA, small interfering RNA; TSPO, translocator protein; ZFAS1, lncRNA named Zinc finger antisense 1.

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1. Introduction

1.1. LncRNAs as a new layer of regulatory network of gene expression

One of the concept-transforming discoveries in the field of life science over the past decades is the Human Genome Project revealing that protein-coding genes account for only 2% of the human genome, despite the fact that >90% of the genome is actively transcribed. While the 2% of the human genome represents estimated 20,500 protein-coding genes, the transcripts from >90% of the genome are believed to have no or minimal coding potential but are functionally active as RNA molecules. These functional RNAs are arbitrarily divided into two groups with a threshold of 200 nucleotides (nts): small and long non-coding RNAs (lncRNAs or lncRs). MicroRNAs (miRNAs or miRs) represent the best studied class of small ncRNAs that elicit primarily posttranscriptional regulation of gene expression. On the other hand, lncRNAs represent one of the most prominent but least understood transcriptomes, despite that they have rapidly emerged as a new layer of regulatory network of gene expression. Amaral et al. (Amaral, Clark, Gascoigne, Dinger, & Mattick, 2011; Guttman et al., 2009) proposed a slightly different definition for lncRNAs: the ncRNAs that may function as either primary or spliced transcripts, independent of small RNAs, excluding the structural RNAs.

The functional ncRNA was initially identified in bacteria and then in eukaryotic cells in 1980s. H19 and Xist were the first characterized lncRNAs in the pre-genomic era. Yet, not until the early 2000s with the Human Genome Project being publicized, have lncRNAs formally come into the bench-top of research laboratories as an independent category of regulatory RNAs.

In general, lncRNAs are expressed at low levels with 10-fold lower median expression abundance than their protein-coding counterparts. Individual lncRNAs have specific subcellular distribution: in the nucleus or in the cytoplasm. In addition, many lncRNAs exhibit striking tissue-specific expression patterns and have only weak cross-species sequence conservation.

LncRNAs typically have poor conservation across species with only rather restricted regions of conserved bases surrounded by large seemingly unconstrained sequences. Yet, it is important to know that lack of conservation do not invoke a lack of function. In fact, lncRNAs have been found to participate in a wide spectrum of biological events and pathophysiological processes. They can regulate neighboring genes in cis-formation. They can also function in trans-acting formation to regulate the expression of genes, which are not located closely with them. More specifically, the subcellular localization of lncRNAs is critical for their functions. In the nucleus, lncRNAs can interact with DNA to form RNA-DNA complexes to reprogram gene expression, act as molecular scaffold, activate or suppress transcription (Kuo et al., 2019; Wang et al., 2018). Other lncRNAs are enriched in the cytoplasm where they can impact protein localization or modulate mRNA stability and translation (Amaral et al., 2011; Guttman et al., 2009). Predominantly cytoplasmic lncRNAs may function through multiple mechanisms, including influencing the stability of an mRNA, affecting translation initiation, acting as competing endogenous RNAs, or influencing post-translational modification. Nuclear lncRNAs may influence transcriptional outputs through multiple mechanisms, including epigenetic modifications, interactions with transcription factors, and affecting mRNA processing or export.

For half a century before 2000s, we have considered and believed proteins as the primary protagonists of cellular functions is the pillar of the central dogma of molecular biology. Over the past decade, however, such a concept has been changed and revolutionized by ncRNA research. LncRNAs as functional molecules have received widespread attention as potentially new and crucial players of biological regulation. Given the fact that there are 172,216 and 131,697 lncRNA transcripts for humans and mice, respectively, in the NONCODE database, we are merely standing at the starting line for our understanding of this category of functional/regulatory RNAs.

1.2. LncRNAs as a new regulator of cardiac pathophysiology

Since the discovery of lncRNAs, functional characterization has rapidly introduced these molecules as a new category of diagnostic and therapeutic opportunities for cardiovascular risk mitigation owing to their differential expression and active participation in the pathophysiological conditions of cardiovascular disease. We now know that lncRNAs are implicated in diverse cardiovascular conditions and associated risk factors, including myocardial infarction (MI), coronary heart disease (CHD), atherosclerosis/coronary artery disease (CAD), vascular disease, cardiac hypertrophy (CH), heart failure (HF), etc.

1.2.1. MI, CHD and CAD

The first study associating lncRNA with cardiovascular disease was published as early as in 2006 by Ishii et al. (Ishii et al., 2006). These authors identified a novel ncRNA, MIAT representing MI associated transcript that confers risk of myocardial infarction on chromosome 22q12.1. Specifically, upregulation of lncR-MIAT by the single nucleotide polymorphism (SNP) predicts left ventricular dysfunction in acute MI. (For clarity and uniformity, in this article individual lncRNA is labeled as "lncR" followed by the specific name of that lncRNA with a hyphen in between to discriminate it from protein-coding genes. We also propose to a uniform nomenclature for designating lncRNAs by adding prefix "lncR-" to the name of individual lncRNA in future publications on lncRNAs.) The second study was published in 2007 by McPherson et al. (McPherson et al., 2007). The results from this study associate common variants at a locus on chromosome 9p21 near the *DKN2A* and *CDKN2B* genes with CHD and MI in six independent samples (>23,000 participants) from four Caucasian populations using genome-wide association scanning. Two single nucleotide polymorphisms (SNPs) at the loci rs10757274 and rs2383206 that contain no annotated genes define an allele that is associated with ~20% increase of risk in the 50% of individuals who are heterozygous for the allele and an ~40% increase of CHD in the 25% of Caucasians who are homozygous for the allele. In the same year, Helgadottir et al. (Helgadottir et al., 2007) identified a common variant on chromosome 9p21 that affects the risk of MI with ~21% of individuals in the population are homozygous for this variant, and their estimated risk of suffering MI is 1.64 times as great as that of non-carriers.

One year later, the first evidence for the role of lncRNAs in vascular disease was published by Broadbent et al. (Broadbent et al., 2008) who described that a large antisense non-coding RNA gene (ANRIL) is expressed in tissues and cell types that are affected by atherosclerosis and is a prime candidate gene for the chromosome 9p21 CAD locus. More specifically, SNPs on lncR-ANRIL contribute to the susceptibility of CAD.

These seminal studies stimulated tremendous interest in investigating the relationship between the chromosome 9p21 and CHD. From 2009 to 2012, a number of important studies were published on this topic. For instance, Smith et al. (Smith et al., 2009) characterized common genetic determinants for ischemic stroke on chromosome 9p21 in a large sample ($n = 4565$). Their findings indicate that ischemic stroke shares pathophysiological determinants with CHD and other arterial diseases and highlights the need for large sample sizes in stroke genetics. In the same year, Gori et al. (Gori et al., 2010) reported that common SNPs on chromosome 9p21 locus rs2891168 and rs10811661 are independently associated with MI and type 2 diabetes, respectively, in an Italian population. Scheffold et al. (Scheffold et al., 2011) identified six sequence variants on chromosome 9p21.3 that are associated with a positive family history of MI. Congrains et al. (Congrains et al., 2012) unraveled that the risk alleles for atherosclerosis-related phenotypes are consistently associated with a lower expression of lncR-ANRIL.

Whilst the published studies have been primarily focused on genetic links of lncRNAs in patients, fundamental research on the mechanistic insight of lncRNAs in the setting of MI has been sparse. A recent work from our group identified lncR-ZFAS1 as a SERCA2a inhibitor to cause

intracellular Ca^{2+} overload and contractile dysfunction in a mouse model of MI (Zhang et al., 2018). This lncRNA was early found markedly decreased in its level in bloodstream and increased in the myocardium of patients with acute MI (AMI), and was proposed as a new biomarker for AMI. In a study conducted by Wang et al. (Wang et al., 2015), autophagy-promoting factor (APF) was discovered to exacerbate MI injury by interacting with miR-188-3p. MiR-188-3p suppresses the expression of ATG7, a key regulator of autophagy that is known to be crucial in regulating cell death and survival in MI. Another study from our group identified lncR-MIAT as a pro-fibrotic lncRNA governing cardiac fibrosis in post-infarct myocardium through sponging miR-24 by the ceRNA mechanism (Qu et al., 2017). LncR-MIAT is upregulated in a mouse model of MI, and knocking it down improves cardiac function, along with inhibition of collagen production and cardiac fibroblast proliferation. A study reported by Yan et al. (Yan et al., 2015) demonstrated that lncR-MIAT could also act as a ceRNA of miR-150 to regulate VEGF expression contributing to pathological angiogenesis. In addition, lncR-MIAT can also act on miR-22-3p, leading to cardiomyocyte apoptosis involved in the pathogenesis of diabetic cardiomyopathy (DCM) (Zhou et al., 2017).

1.2.2. CH and HF

Not until six years after the establishment of the roles of lncRNAs in CHD in 2014, was the first paper describing a role for lncRNAs in cardiac hypertrophy (CH) published by Han et al. (Han et al., 2014). These authors found that a cluster of antisense lncRNA transcripts from myosin heavy chain 7 (Myh7) loci, named myosin heavy chain associated RNA transcripts (MHRT), are cardiac-specific and abundant lncRNAs in adult hearts. LncR-MHRT transcription is inhibited by pathological, and such stress-induced lncR-MHRT repression is essential for cardiomyopathy to develop: restoring lncR-MHRT to the pre-stress level protects the heart from hypertrophy and failure. In the same year, another group led by Li showed that an lncRNA named cardiac hypertrophy related factor (CHRF) regulates CH by targeting miR-489 (Wang et al., 2014). The mechanism of action was attributed to a binding of miR-489, which derepresses the miR-489 target Myd88 to regulate cardiomyocyte hypertrophy. More recently, the lncRNAs cardiac hypertrophy-associated transcript (Chast) and cardiac-hypertrophy-associated epigenetic regulator (Chaer) were identified by two independent research laboratories (Viereck et al., 2016; Wang et al., 2016). Both lncRNAs are found overexpressed upon pressure overload-induced HF in mice with lncR-Chast overexpression inducing cardiomyocyte hypertrophy in vitro and in vivo (Viereck et al., 2016), whereas silencing of lncR-Chast attenuating cardiac remodeling (Wang et al., 2016). Another two independent groups found that lncR-MIAT is a pro-hypertrophic lncRNA in a mouse model of CH induced by angiotensin II (Li, Wang, Sun, & Zhu, 2018; Zhu, Yuan, Rao, & Wang, 2016). Terminal differentiation-induced ncRNA (TINCR) was also found to play an important role in CH with forced expression of this lncRNA attenuating CH in mice (Shao et al., 2017).

1.2.3. Cardiac arrhythmias (CA)

A study published in 2009 by Newton-Cheh et al. (Newton-Cheh et al., 2009) must be particularly addressed here because it represents the first to correlate lncRNA with sudden and/or arrhythmic cardiac death (SCD) closely relevant to the content of this review article. These authors examined their hypothesis that alleles of the common variants on chromosome 9p21, which have been previously associated with multiple manifestations of vascular disease, might also be associated with SCD in the general population. Their findings indeed identified such a common variant that is significantly associated with sudden and/or arrhythmic death in individuals of European ancestry in a combined nested case-control analysis from six prospective cohorts. The risk allele is common with approximately 50% of the population carrying one copy and having an estimated 29% increased risk of sudden/arrhythmic death after controlling for other CHD risk factors as compared to non-carriers. For the approximate 25% of the population who are homozygous for the risk allele, the odds ratio of sudden/arrhythmic death is elevated by an estimated 60% as compared to those homozygous for the minor allele.

Yet, not until nearly 10 years later in 2018, was the first basic study published on the control of ventricular arrhythmias by lncRNAs (to be introduced detailly in a later subsection) and the pertinent molecular mechanisms (Zhang, Jiao, et al., 2018). However, the clinical implications of lncRNA regulation of cardiac electrophysiology remain presently unclear and translational medical studies on this matter are still lacking.

1.3. Basis of cardiac electrophysiology and arrhythmias

Cardiac electrophysiology or cardiac electrical activities can be reflected at four different levels: electrocardiogram in the heart, action potential (AP) in cardiac cells (cellular level), ion currents across the cell membrane (subcellular level), and ion channels and transporters in the membrane structures and their encoding genes in the nucleus (molecular level) (Yang, Lu, & Wang, 2008). A typical cardiac AP contains a characteristic long plateau phase distinct from APs in non-cardiac cells. A cardiac AP can be divided into 4 phases designated by the numbers 0 through 4, beginning with initial rapid depolarization (phase 0) and ending with the return to the resting state (phase 4). A cardiac AP is orchestrated by multiple categories of ion channels and transporters, the transmembrane proteins embedded across the cytoplasmic membrane of cardiomyocytes, and cellular Ca^{2+} handling proteins. Four major categories of ion channels exist in cardiac cells, including Na^+ channels, Ca^{2+} channels, K^+ channels, and Cl^- channels (Yang et al., 2008).

Under resting state, cardiac cells are normally sitting at a negative transmembrane potential (intracellular ~−80 mV). Upon activation, cells are depolarized by rapid entry of Na^+ through Na^+ channels to generate a large inward-flowing (depolarizing) Na^+ current (I_{Na}). Ca^{2+} entry into the cell through the L-type Ca^{2+} current (I_{CaL}) also contributes to phase 0 depolarization. This membrane depolarization is immediately followed by a brief rapid repolarization phase (phase 1) due to K^+ efflux through a rapidly activating and inactivating transient outward K^+ current (I_{to}). In atrial myocytes, another K^+ current named ultra-rapid delayed rectifier (I_{Kur}) is activated in accompany with I_{to} (Wang, Fermini, & Nattel, 1993b). Then cardiac cells enter the characteristic plateau phase (phase 2) during which there is a delicate balance between inward currents I_{CaL} and I_{Na} and outward K^+ currents. During this phase there is progressive time-dependent sequential activations of delayed rectifier K^+ currents, first the rapid delayed rectifier K^+ current (I_{Kr}) and then the slow delayed rectifier K^+ current (I_{Ks}) (Li, Feng, Wang, Fermini, & Nattel, 1995; Wang, Fermini, & Nattel, 1993a). I_{Kr} acts to terminate the AP with an appropriate delay by producing rapid phase 3 repolarization, and I_{Ks} serves as a repolarization reserve to prevent excessive slowing of repolarization. Finally, inward rectifier K^+ current (I_{K1}) aids to complete the terminal phase of repolarization (Wang, Yue, White, Pelletier, & Nattel, 1998).

Cardiac electrophysiology is manifested by excitation conduction, membrane repolarization, automaticity, intracellular Ca^{2+} handling, spatial heterogeneity of these above properties, and myocardial architecture (gaps and scars). CA can occur when there is abnormality in any of these six properties.

Cardiac conduction refers to the propagation of excitation within a cell (intracellular conduction) and between cells (intercellular conduction), and cardiac conduction velocity is determined by the rate of membrane depolarization responsible for excitation generation and the intercellular conductance for excitation propagation. I_{Na} is a critical determinant of membrane depolarization velocity thereby the intracellular conduction. However, for a whole heart to function properly, action potential must propagate smoothly throughout the heart from sinus node to atria then to ventricles through AV node. In this regard,

connexin43 (Cx43) gap junction channel is of key importance in ventricular muscles; it is critical for intercellular propagation of excitation (Yang et al., 2007). Slowing or blockade of the conduction pathway can result in CA.

Cardiac repolarization follows depolarization with the membrane potential begins to return towards the resting state. The rate of membrane repolarization determines the length of action potential duration (APD) and effective refractory period (ERP) thereby the timeframe of channel availability for the generation of a next AP in a cardiac cell. The repolarization is governed by the delicate yet dynamic balance between the inward and the outward currents. The inward-flowing currents such as Na^+ and L-type Ca^{2+} currents tend to depolarize the membrane and delay repolarization to prolong APD, whereas the outward-going K^+ currents, mainly including I_{K1}, I_{to}, I_{Kr} and I_{Ks}, tend to repolarize membrane to shorten APD. Excessive delay of repolarization due to inhibited outward currents or enhanced inward currents can cause abnormal APD prolongation leading to long QT syndrome (LQTS) (polymorphic ventricular arrhythmias) upon the occurrence of early afterdepolarizations (EADs). On the other hand, aberrantly accelerated repolarization can cause abnormal APD shortening favoring reentrant arrhythmias and short QT syndrome (Yang et al., 2008).

Cardiac intracellular calcium handling aims to maintain proper intracellular Ca^{2+} homeostasis, and this process is coordinated by interplay of a number of Ca^{2+} handling proteins. During an excitation Ca^{2+} enters cardiomyocytes mainly via L-type Ca^{2+} current (I_{CaL}). This triggers the release of additional Ca^{2+} from sarcoplasmic reticulum (SR) Ca^{2+} stores through closely coupled SR Ca^{2+} release channel RyR2 by the Ca^{2+}-induced Ca^{2+} release mechanism. SR Ca^{2+} content depends on cellular Ca^{2+} entry mainly via I_{CaL}, Ca^{2+} removal from the cell (particularly via the sarcolemmal Ca^{2+} pump and forward-mode Na^+-Ca^{2+} exchange), and Ca^{2+} pumping into the SR by the Ca^{2+}-ATPase Ca^{2+} pump (encoded by SERCA2a) (Zhang, Jiao, et al., 2018). I_{CaL} accounts for excitation-contraction coupling and contribute to the plateau duration of AP. Intracellular Ca^{2+} homeostasis is crucial to cardiac rhythmic activities; abnormal Ca^{2+} handling contributes to arrhythmogenesis directly by triggering abnormal depolarizations and indirectly by modulating action potential duration. Ca^{2+} overload is a causal factor for arrhythmogenesis (Yang et al., 2008).

2. LncRNAs as modulators of CA under various diseased states

2.1. LncRNAs regulate cardiac conduction and ventricular arrhythmias in congestive heart failure (CHF)

2.1.1. LncR-CCRR

Individual cardiac muscle fibers are connected by the highly organized intercalated discs where gap junctions are concentrated allowing for well-orchestrated spatial propagation of cardiac excitations or APs through tight cardiomyocyte-cardiomyocyte coupling. Intercellular conduction of excitation through gap junctions, or more specifically gap junction channel proteins, forms electrical coupling that is essential for the normal impulse propagation throughout the heart. Impaired electrical coupling promotes arrhythmogenicity. In ventricular myocardium, gap junctions contain hundreds to thousands of connexins, particularly connexin43 (Cx43), the pore forming subunits of gap junction channels that create a conduit connecting the cytoplasm between cardiomyocytes. A hallmark of the electrical disorders with regard to impulse conduction in HF is the electrical uncoupling due to diminished presence of Cx43 in gap junctions, resulting in slowing of conduction velocity and dispersion of impulse propagation leading to an increased risk of re-entrant excitation, predisposing to cardiac arrhythmia and even sudden cardiac death. One of the multiple regulators of Cx43 presence in gap junction membrane is Cx43-interacting protein CIP85 that is known to induce the endocytic trafficking of Cx43 for degradation by the lysosome-mediated mechanism (Cochrane, Su, & Lau, 2013). Loss of function of CIP85 can lengthen the half-life of Cx43 or increase the presence of Cx43 in gap junctions thereby improving cardiac conduction.

Our group identified an antiarrhythmic lncRNA, cardiac conduction-regulatory RNA (CCRR) that acts to improve the presence of Cx43 in gap junction thereby maintaining normal cardiac conduction (Zhang et al., 2018). This lncRNA (AK045950) has not been previously studied and it was initially screened as a downregulated lncRNA by microarray profiling of lncRNAs expression in a mouse model of pressure-overload CHF created by transverse aortic constriction (TAC). Bioinformatics analyses suggest it with a high profile linking to HF. With quantitative RT-PCR analysis, we established that lncR-CCRR is abnormally downregulated in mice with CHF induced by TAC and in patients with CHF. Using optical mapping techniques, we further demonstrated that downregulation of lncR-CCRR slows cardiac conduction and enhances arrhythmogenicity. Moreover, silencing lncR-CCRR by siRNA in healthy mice causes conduction slowing and the associated arrhythmogenesis as those seen in CHF, indicating that downregulation of lncR-CCRR alone is sufficient to induce cardiac electrophysiological disturbances leading to the occurrence of re-entrant arrhythmias. In sharp contrast, lncR-CCRR overexpression mitigates the conduction anomalies and ventricular arrhythmias in CHF mice or in healthy mice pretreated with lncR-CCRR siRNA.

At the subcellular level, CHF or lncR-CCRR silence causes destruction of intercalated discs and gap junctions to slow longitudinal cardiac conduction, and lncR-CCRR overexpression improves these detrimental alterations, indicating the involvement of Cx43, the major gap junction ion channel protein responsible for the intercellular conduction of cardiac excitation.

At the molecular level, Cx43 is downregulated in CHF or when lncR-CCRR is silenced. On the other hand, lncR-CCRR overexpression binds CIP85 to hinder the function of this protein and to subsequently increase the level of Cx43 in the cytoplasmic membrane. In other words, lncR-CCRR:CIP85 interaction interrupts CIP85:Cx43 interaction, resulting in upregulation of Cx43 expression and consequent improvement of cardiac conduction.

We further identified a sequence region of lncR-CCRR (290 nts) that is conserved across species and experimentally verified it the functional domain of lncR-CCRR in the context of cardiac electrophysiology. Our in-depth analysis supports this domain being responsible for lncR-CCRR:CIP85 interaction and the consequent disruption of CIP85:Cx43 interaction. Notably, this functional domain of lncR-CCRR recapitulates the anti-arrhythmic property of the full-length lncR-CCRR: improving cardiac conduction and suppressing the associated ventricular arrhythmias (Fig. 1).

Our study therefore suggests lncR-CCRR to be an anti-arrhythmic lncRNA that acts by maintaining normal cardiac conduction and downregulation of lncR-CCRR cause conduction slowing leading to enhanced propensity of arrhythmias, which may account at least partly for the electrical disorders in CHF. The fact that overexpression of full-length lncR-CCRR or the functional domain of lncR-CCRR abrogates the electrical disturbances in the setting of CHF suggests lncR-CCRR replacement a potential therapeutic approach for pathological arrhythmias (Zhang, Sun, et al., 2018).

2.1.2. LncR-KCNA2as

Voltage-gated K^+ channel subfamily A member 2 also known as Kv1.2 is a protein that is encoded by the *KCNA2* gene in humans, and homotetrameric *KCNA2* forms a delayed-rectifier K^+ channel protein that carries a K^+ current named I_{Ks} playing a role in cardiac repolarization in rodents (Barry, Trimmer, Merlie, & Nerbonne, 1995). It needs to be clarified that the I_{Ks} carried by *KCNA2*/Kv1.2 is entirely different from the commonly recognized typical canonical I_{Ks} carried by the channels co-assembled by *KCNQ1*/KCLQT1 and *KCNE1*/MiRP1 K^+ channel subunits which accounts for sub-populations of genetic and acquired long QT syndrome (Sanguinetti et al., 1996; Yang et al., 2008). In order to

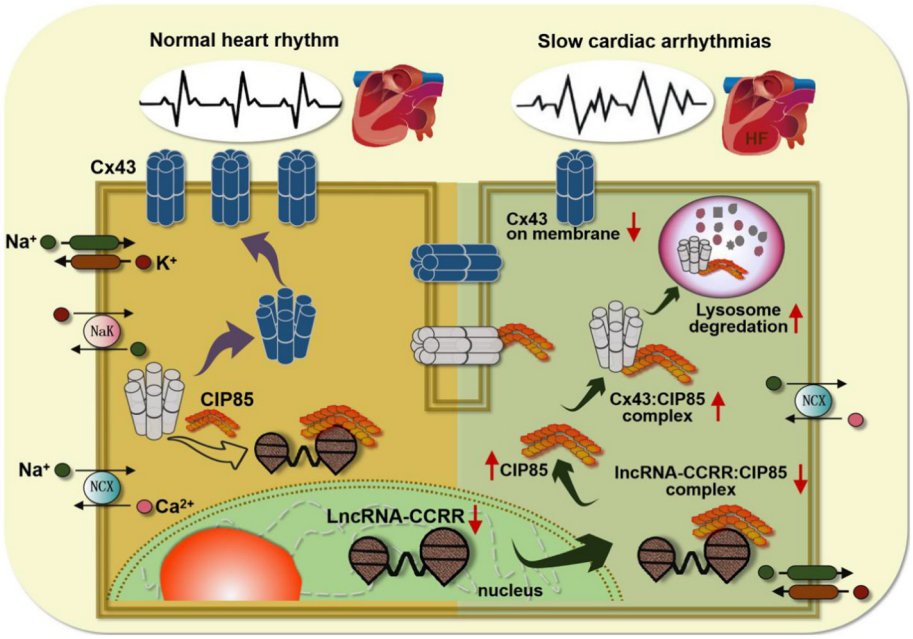

Fig. 1. Illustrative cartoon showing the mechanism by which lncR-CCRR (cardiac conduction-regulatory RNA) produces anti-arrhythmic action. LncR-CCRR binds CIP85 to prevent connexin43 (Cx43):CIP85 binding and internalization for lysosome metabolism. In this way, lncR-CCRR prolongs the half-life of Cx43 thereby maintaining the gap junction membrane density of Cx43, resulting in improved cardiac intercellular conduction.

discriminate the *KCNA2*/Kv1.2-generated current from the canonical I_{Ks} and avoid the potential confusion, here we rename the former I_{Ksr}.

The study by Long et al. (Long et al., 2017) showed that the expression of an lncRNA KCNA2 antisense RNA (KCNA2as) is significantly upregulated in a rat model of congestive HF (CHF) induced by TAC and in a cellular model of cardiomyocyte hypertrophy induced by phenylephrine stimulation as well. LncR-KCNA2as upregulation is accompanied by concordant decreases in *KCNA2* mRNA and Kv1.2 protein levels in the left ventricular myocardium of rats with CHF. Consistently, forced overexpression of KCNA2as mediated by viral vector AAV9 causes abnormal downregulation of *KCNA2* and Kv1.2. At the ionic and cellular levels, artificial silence of *KCNA2* or overexpression of lncR-KCNA2as decreases I_{Ksr} and prolongs APD in cardiomyocytes, consistent with the prolongation of QTc interval observed in CHF rats. Moreover, these manipulations increase the propensity of norepinephrine-induced arrhythmias. Conversely, *KCNA2* overexpression attenuates the CHF-induced I_{Ksr} reduction, APD prolongation, and ventricular arrhythmias. Knockdown of lncR-KCNA2as produces qualitatively the same effects as *KCNA2* overexpression.

The authors of this study did not get insight into the mechanistic link between lncR-KCNA2as and *KCNA2* expression. It thus remains unclear whether lncR-KCNA2as can act as an antisense RNA (asRNA) to *KCNA2* to induce degradation of the latter.

While the results of this study suggest that lncR-KCNA2as upregulation contributes to reduction of I_{Ksr}, prolongation of APD, providing the substrate for induction of EADs, which would eventually result in triggered activities leading to long QT syndrome (LQTS) type of ventricular arrhythmias in rats with CHF, it should be noted here that I_{Ksr} is virtually absent in human heart according to patch-clamp recordings in isolated human cardiac cells (Li, Feng, Yue, Carrier, & Nattel, 1996). Consistently, Kaab et al. (Kaab et al., 1998) reported that *KCNA2* is expressed at low abundance in human ventricle. Similarly, Bertaso et al. (Bertaso, Sharpe, Hendry, & James, 2002) showed that level of *KCNA2*/Kv1.2 is also very low in samples of human atrial appendage. Hence, it is anticipated that lncR-KCNA2as unlikely contributes significantly to humans due to the minimal role of Kv1.2/I_{Ksr} in membrane repolarization thereby cardiac electrophysiology in human heart. Precaution must then be taken when interpreting the clinical application of the finding about lncR-KCNA2as regulation of I_{Ksr}, although the authors of this study claimed that lncR-KCNA2as may be a new target for the prevention and treatment of ventricular arrhythmias in patients with CHF. In addition, how exactly lncR-KCNA2as affects expression of *KCNA2* has not been elucidated either.

2.2. LncRNAs regulate cardiac conduction and the associated arrhythmias in myocardial ischemia (MI)

The international authority Framingham study announced that CHD mainly caused by acute MI (AMI) is the direct and main cause of 75% to 80% of sudden cardiac death (SCD) (Motloch et al., 2017). The most serious complication of AMI is ventricular arrhythmias, including premature ventricular beats (PVB), ventricular tachycardia (VT), and ventricular fibrillation (VF). The mechanisms for these electrical disturbances are complicated and different phases of AMI have different ionic correlates thereby different arrhythmogenic substrates (Carmeliet, 1999). In the early phase (phase 1a), reentry due to cardiac conduction slowing (decreases in I_{Na} and Cx43) is the predominant type of ventricular arrhythmia, and in late phase EADs due to delayed cardiac repolarization (decreased K^+ currents) and enhanced I_{CaL} are the main cause for ischemic arrhythmias (Carmeliet, 1999). Overall, the exact mechanisms underlying ischemic arrhythmias remain incompletely understood. Recent studies suggest the crucial involvement of lncRNAs in regulating ion channel gene expression to shape the cardiac electrophysiology.

2.2.1. LncR-CCRR

In the above subsection, we have described the role of lncR-CCRR in regulating cardiac conduction and the associated arrhythmias in the setting of CHF. Our subsequent investigation unraveled that the anti-arrhythmic property of lncR-CCRR is also manifested in the setting of AMI. Like in CHF, the heart undergoes similar electrophysiological alterations with enhanced arrhythmogenicity in AMI. A typical change common to AMI and CHF is the disruption and loss of gap junction channel Cx43 leading to cardiac conduction slowing and interruption that can develop into re-entrant arrhythmias. Our unpublished findings clearly demonstrated the crucial role of lncR-CCRR in controlling the cardiac electrical activities in AMI via the same mode of action as in CHF.

2.2.2. LncR-ZFAS1

Intracellular Ca^{2+} homeostasis is crucial to cardiac rhythmic activities and emerging evidence suggests Ca^{2+} overload as a causal actor for arrhythmogenesis. Intracellular Ca^{2+} handling is therefore a critical process for maintaining normal cardiac electrophysiology, which is tightly controlled by several Ca^{2+} handling proteins in a highly coordinated manner. Sarcoplasmic reticulum (SR) Ca^{2+}ATPase 2a (SERCA2a) is one of these regulatory proteins and it mediates Ca^{2+} reuptake into SR in cardiac muscles. SERCA2a activity is susceptible to various cellular environmental cues and is under the tight regulation by an array of factors (Talukder et al., 2007; Vangheluwe, Raeymaekers, Dode, & Wuytack, 2005). SERCA2a function can be impaired as a result of expression deregulation (mostly downregulation), and such a change can cause deficiency of Ca^{2+} reuptake back to SR leading to intracellular Ca^{2+} overload. The consequences of intracellular Ca^{2+} overload are enhancement of arrhythmogenicity due to increased propensity of EAD or DAD (delayed afterdepolarization), impairment of cardiac contractile function and promotion of cardiomyocyte apoptosis. Indeed, SERCA2a dysfunction has been associated with a wide spectrum of cardiovascular diseases such as MI, CH, and HF, as well as CA in all these pathological conditions. Our recent study identified lncR-ZFAS1, an antisense lncRNA to the 5′ end of the protein-coding gene Znfx1, as a critical regulator of SERCA2a function.

In our earlier study, we established lncR-ZFAS1 as an independent predictor of AMI (Zhang et al., 2016). One intriguing observation is that lncR-ZFAS1 level is markedly decreased in the bloodstream, but elevated in the myocardium. This prompted us to propose that in addition to being a biomarker of MI, lncR-ZFAS1 might also contribute to the development of AMI. Our subsequent detailed experimental investigation indeed generated strong evidence in support of our hypothesis.

LncR-ZFAS1 expression is robustly increased in cytoplasm and SR in a mouse model of MI created by coronary artery occlusion and a cellular model of hypoxia. At the subcellular level, lncR-ZFAS1 deleteriously alters the Ca^{2+} transient leading to intracellular Ca^{2+} overload in cardiomyocytes. At the molecular level, lncR-ZFAS1 directly binds SERCA2a protein to hinder its activity and to repress its expression as well. Notably, a sequence domain of lncR-ZFAS1 gene that is conserved across species mimics the effects of the full-length lncR-ZFAS1. Mutation of this domain or application of an antisense fragment to this conserved region efficiently cancels out the deleterious actions of lncR-ZFAS1. Clearly, lncR-ZFAS1 is an endogenous SERCA2a inhibitor acting by binding to SERCA2a protein to limit its intracellular level and inhibit its activity.

Although this study did not particularly focused on arrhythmogenesis, impairment of SERCA2a by lncR-ZFAS1 suggests it a pro-arrhythmic lncRNA. Such a deduction is rational because SERCA2a dysfunction has been associated with enhanced arrhythmogenesis and SERCA2a overexpression suppresses ventricular arrhythmias in various pathological conditions of the heart (Prunier et al., 2008). For instance, SERCA2a gene therapy in a wide variety of preclinical models, including acute ischemia/reperfusion, chronic pressure overload and chronic MI, has resulted in a reduction in ventricular arrhythmias by stabilizing SR Ca^{2+} load, reducing ryanodine receptor phosphorylation and decreasing SR Ca^{2+} leak, and reducing cellular triggered activity in vitro and spontaneous and catecholamine-induced ventricular arrhythmias in vivo in failing rat hearts (Cutler, Wan, Laurita, Hajjar, & Rosenbaum, 2009; Lyon et al., 2011; Prunier et al., 2008; Sikkel, Hayward, MacLeod, Harding, & Lyon, 2014). SERCA2a overexpression suppresses cellular alternans to interrupt an important pathway to cardiac fibrillation in the intact guinea pig heart (Cutler et al., 2009).

2.2.3. LncR-MIAT

In the mitochondrion of cardiac cells there is sitting peripheral benzodiazepine receptor called translocator protein (TSPO) that is a five transmembrane domain protein with a primary role in maintaining mitochondrial membrane potential ($\Delta\Psi m$) equilibrium (Rupprecht et al., 2010; Veenman & Gavish, 2006). Emerging lines of evidence have suggested that TSPO plays a significant role in CA, CVD, MI, CH, atherosclerosis, etc. (Akar, Aon, Tomaselli, & O'Rourke, 2005; Aon, Cortassa, Akar, & O'Rourke, 2006; Brown & O'Rourke, 2010; Li et al., 2010). Specifically, TSPO ligands produce protective effects in cardiovascular systems through maintaining $\Delta\Psi m$, proper cardiac electrical activity, intracellular calcium homeostasis, and mitochondrial energy provision. In addition to its mitochondrial localization, TSPO can also be found in the nuclear fraction and plasma membrane (Rupprecht et al., 2010; Veenman & Gavish, 2006). Relevant to this review article is the common and frequent association of TSPO with ventricular fibrillation (VF) and atrial fibrillation (AF) (Aon et al., 2009; Li et al., 2011; Schmidt, Kisselbach, Schweizer, Katus, & Thomas, 2011; Wann et al., 2011). Studies have documented that TSPO ligands can reduce or even eliminate VF in MI/reperfusion injury animal models. Moreover, inhibition of TSPO by its antagonist significantly reduces the incidence of AF induced by ischemia, stretch, and cholinergic agitation (Li et al., 2010, 2011).

We have mentioned in an earlier section that MIAT (myocardial infarction associated transcript) was found to confer risk of MI upon its upregulation due to SNP and to predict left ventricular dysfunction in AMI (Ishii et al., 2006). In an effort to gain in-depth understanding of the pathophysiological role of lncR-MIAT and to delineate the mechanistic link of lncR-MIAT to its detrimental effect on heart, we investigated the effect of this lncRNA on TSPO in a mouse model of AMI. Robust elevation (>5 fold) of lncR-MIAT transcript level is consistently noticed in the infarcted myocardium of MI mice relative to sham-operated control counterparts. Upregulation in AMI hearts or forced expression of lncR-MIAT in healthy animals causes significant impairment of cardiac contractile function, as well as of electrical activities. We further observed that lncR-MIAT downregulates TSPO mRNA and protein levels. Moreover, lncR-MIAT can also bind directly to TSPO and to enhance opening of the mitochondrial permeability transition pore (mPTP) leading to depolarization of $\Delta\Psi m$ or loss of mitochondrial membrane potential and increase in arrhythmogenic potential of MI hearts. Silencing lncR-MIAT mitigates the deleterious alterations of MI hearts. Furthermore, we identified a homologous region of lncR-MIAT sequence, which is common to all four human sapiens lncR-MIAT variants (Accession: NR_003491.3, NR_033319.2, NR_033320.2, and NR_033321.2) and to *Mus musculus* lncR-MIAT variant (Accession: NR_033657.1) as well. We subsequently established this region as a functional domain of lncR-MIAT, which retains the full ability of full-length lncR-MIAT to bind TSPO and to enhance opening of mPTP thereby the pro-arrhythmic property of lncR-MIAT.

2.2.4. LncR-NONRATT021972

Diabetic cardiac autonomic neuropathy (DCAN) is a serious and common complication in diabetes mellitus (DM). One of the problems of DCAN is the decrease in heart rate variability (HRV) mediated by superior cervical ganglia (SCG). HRV is a physiological phenomenon of variation in the time interval between heartbeats. Although cardiac automaticity is intrinsic to pacemaker tissues, heart rate and cardiac rhythm are largely under the control of the autonomic nervous system. Reduced HRV has been shown to be a predictor of mortality after MI, and victims of SCD generally have lower HRV than healthy individuals (Abildstrom et al., 2003; Bigger Jr. et al., 1992; Kleiger, Miller, Bigger Jr., & Moss, 1987). A range of other outcomes/conditions may also be associated with lower HRV, including CHF, diabetic neuropathy, depression, post-cardiac transplant, susceptibility to SIDS and poor survival in premature babies. A study reported significant upregulation of lncR-NONRATT021972 in SCG of DM rats (Xu et al., 2016). Knockdown of lncR-NONRATT021972 by siRNA rescued decreased HRV in DM rats. This beneficial effect is accompanied by decreased expression upregulation of TNF-α, blocked serine phosphorylation of insulin receptor substrate (IRS) 1, and enhanced expression downregulation of IRS1 in SCG. These findings suggest that lncR-NONRATT021972 plays an important role in maintaining normal cardiac electrophysiology. However, it

is not elucidated in this study how lncR-NONRATT021972 affects the expression of TNF-α and IRS1 and the activity of IRS1.

2.3. LncRNAs regulate cardiac repolarization and the associated arrhythmias in MI

2.3.1. LncR-MALAT1

The lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been reported to be highly expressed in MI/reperfusion injury that is associated with frequent occurrence of ventricular arrhythmias (Zhao et al., 2017). To understand if lncR-MALAT1 contributes to regulation of arrhythmogenic potential and decipher the downstream mechanisms, Zhu et al. (Zhu et al., 2018) conducted a study with a rat model of MI. In their report, cardiac lncR-MALAT1 level was found significantly elevated in AMI rats relative to the sham-control animals. Artificial knockdown of lncR-MALAT1 by sh-MALAT1 attenuates the AMI-induced decreases in peak current density of transient outward K^+ current I_{to} in AMI rats, along with rescuing of expression downregulation of *KCND2* and *KCND3* mRNAs and their corresponding proteins Kv4.2 and Kv4.3. These findings suggest lncR-MALAT1 might elicit pro-arrhythmic effects in the setting of AMI. To understand the mode of action of lncR-MALAT1, the authors established lncR-MALAT1 as a ceRNA for miR-200c in cardiomyocytes as it does in endometrioid endometrial carcinoma, and then subsequently established HMGB1 as a target gene of miR-200c in cardiac cells as in breast and lung cancer cells previously identified by others. These authors then further characterized the functional role of HMGB1 and confirmed the published finding that HMGB1 inhibits the expression levels of Kv4.2 and Kv4.3, the α-subunits of K^+ channels for I_{to} and reduces I_{to} current density. Based upon these findings together, the authors proposed that abnormal upregulation of lncR-MALAT1 diminishes the functional availability of miR-200c, resulting in derepression or expression upregulation of HMGB1 that in turn suppresses the expression of Kv4.2 and Kv4.3 thereby inhibiting I_{to}, possibly leading to enhanced arrhythmogenic potential with LQTS. It is therefore anticipated that lncR-MALAT1 knockdown improves cardiac electrophysiology in the setting of MI.

Unfortunately, whether lncR-MALAT1 really alters, and if yes, then how it modulates (pro- or anti-arrhythmic) the propensity of ischemic arrhythmias was not evaluated in this study. Thus, the role of lncR-MALAT1 in arrhythmogenecity remains unclear as the function of I_{to} in arrhythmias is unclear. I_{to} has been known to produce paradoxical effects in cardiac repolarization: decrease or blockade of I_{to} could either lengthen or shorten APD depending on species, myocardial region or cell subtype. In cells with spike-and-dome morphology such as epicardial cells and atrial cells in human and canine hearts, decrease of I_{to} causes shortening of APD due to an elevation of the plateau level towards the reversal potentials of inward currents (I_{Na} and I_{CaL}) thereby diminishment of these APD-prolonging currents. On the other hand, in cardiac cells without spike-and-dome morphology such as endocardial cells and Purkinje fibers, inhibition of I_{to} usually causes lengthening of APD. Nonetheless, there is a possibility that decrease in I_{to} can cause regional heterogeneity of repolarization favoring arrhythmogenesis, and it is likely that lncR-MALAT1 could act by such a mechanism.

2.4. LncRNAs regulate atrial fibrillation (AF)

Atrial fibrillation (AF), rapid and irregular activations of atrial myocytes, is the most frequently encountered clinical arrhythmia that is often accompanied by increased risk for stroke and HF. AF is highly prone to various stresses, and once triggered, it tends to self-perpetuate in the clinical setting. The adverse properties of AF are conferred by atrial electrical remodeling (AER) and/or atrial structural remodeling processes favoring the recurrence and maintenance of AF (Wang, Lu, & Yang, 2011). AER, which can also occur in the absence of progressive underlying heart disease or any anatomical and structural lesions in atrium, is characterized by shortening of effective refractory period primarily because of the shortening of atrial action potential duration (APD). Diminishment of L-type Ca^{2+} current (I_{CaL}) or augmentation of inward rectifier K^+ current (I_{K1}), or the combination of the two, is the key electrophysiological alterations in the adverse AER that shortens atrial APD favoring the initiation and persistence of AF (Wang et al., 2011).

In addition, intracellular Ca^{2+} homeostasis is crucial to cardiac rhythmic activities and emerging evidence suggests Ca^{2+} overload as a causal actor for arrhythmogenesis promoting focal firing, substrate evolution, and AER during AF. Ectopic activity contributes to AF by acting as a trigger to initiate reentry in a vulnerable substrate and is governed by factors controlling afterdepolarizations and triggered activity: early afterdepolarizations with prolonged APD and delayed afterdepolarizations due to spontaneous sarcoplasmic reticulum (SR) Ca^{2+} releases (Wang et al., 2011).

Control of AF by lncRNAs is a topic attracting intensive attention in the past years. Several studies have analyzed the differential expression of lncRNAs in AF patients (Chen et al., 2016; Su, Li, Zhao, Yue, & Yang, 2018; Xu et al., 2016; Yu et al., 2017) and in animal models. However, only a handful of lncRNAs have been experimentally investigated for their role in controlling AF, though such studies have been steadily increasing.

2.4.1. LncR-MIAT

We identified a set of differentially expressed lncRNAs (≥2-fold changes) between atrial tissues from AF patients and subjects with sinus rhythm (SR). Our bioinformatics analyses predicted the potential links of the differentially expressed lncRNAs to AF. In particular, we observed a significant downregulation of lncR-MIAT in AF relative to SR subjects and in a mouse model of AF as well. Artificial knockdown of lncR-MIAT by lentivirus-mediated siRNA silencing in otherwise healthy mice increased AF vulnerability by increasing the rate of AF induction and duration of AF persistence. At the cellular level, lncR-MIAT downregulation shortened action potential duration in single atrial myocytes. At the molecular level, lncR-MIAT downregulation decreased expression of CACNA1C, the gene encoding the pore-forming α-subunit of L-type Ca^{2+} channel protein Cav1.2 and the corresponding ion current I_{CaL}, and concomitantly increased expression of inward rectifier K^+ channel gene KCNJ2/Kir2.1 and its derived ion current I_{K1}, creating the two most critical electrical substrates for AF. We further elucidated that the molecular changes are consequent to the loss of lncR-MIAT actions as a ceRNA on miR-135a and as an antisense RNA (asRNA) on KCNJ2, and experimentally established CACNA1C as a target gene for miR-135a. In other words, lncR-MIAT acts via dual mechanisms to limit the two most critical processes of AER: sequestering miR-135a to release expression repression of CACNA1C and binding KCNJ2 mRNA to depress its level (unpublished observations), leading to shortening of APD and ERP favoring re-entry thereby occurrence of AF.

We identified two functional domains in the lncR-MIAT sequence with one conferring the ceRNA function of lncR-MIAT on miR-135a and the other conferring the asRNA function on KCNJ2. The ceRNA domain mimicked the regulatory effect of full-length lncR-MIAT on the expression of CACNA1C. Similarly, the asRNA domain of 21 nts reproduced the inhibitory effect of full-length lncR-MIAT on the transcript level of KCNJ2. Notably, these short stretches could recapitulate the phenotypical alterations of atrial remodeling and the associated vulnerability of AF.

Our findings indicate that lncR-MIAT is an anti-AF lncRNA acting by dual mechanisms as a ceRNA and an asRNA, and loss of lncR-MIAT favors AF. Therefore, lncR-MIAT replacement might be a new therapeutic option for AF.

It is interesting to note that the expression alterations of lncR-MIAT are tissue- and/or disease-dependent. In the setting of MI, ventricular lncR-MIAT is robustly upregulated as already described above, whereas in AF, atrial lncR-MIAT is markedly downregulated. And further notable

is that either excess/abnormal upregulation or downregulation of lncR-MIAT is pro-arrhythmic. This indicates that expression of lncRNAs is delicately regulated and intracellular "homeostasis" of lncRNAs is of paramount importance for maintaining normal cellular functions. Such a tight and delicate regulation of lncRNA expression is one of the mechanisms for tightly balanced regulation of expression and function of protein-coding genes. Another point worthy of mentioning here is that there are presumably distinct regulatory mechanisms for the expression of lncR-MIAT in ventricle and atrium. Better understanding of such a tissue-specific and differential expression of lncRNA may help us decipher the pathophysiological importance of lncRNA regulation. In the case of lncR-MIAT in MI, ventricular upregulation contributes to the risk of ischemic myocardial injuries including arrhythmias and atrial downregulation may well be a risk factor for enhanced propensity of AF in MI.

2.4.2. LncR-KCNQ1ot1

The study reported by Shen et al. (Shen et al., 2018) demonstrated that KCNQ1ot1 (KCNQ1 overlapping transcript 1; more detailed information regarding lncR-KCNQ1ot1 is to be discussed in a following subsection.) is upregulated in a mouse model of AF induced by angiotensin II, which is accompanied by the same pattern of increase in CACNA1C transcripts. Electrophysiological measurements showed that lncR-KCNQ1ot1 shortens effective refractory period (ERP), prolongs atrial APD, delays interatrial conduction time (IACT), and increases incidence of AF and AF duration. These effects of lncR-KCNQ1ot1 could be attributed to the ceRNA action of this lncRNA on miR-384 that targets CACNA1C. Knockdown of lncR-KCNQ1ot1 produces anti-AF effects by releasing miR-384 to enhance repression of CACNA1C thereby suppressing AF. The authors proposed that a new axis lncR-KCNQ1ot1–miR-384–CACNA1C that controls AF (Fig. 2).

Yet, despite that the findings of this study are interesting, the data needs cautious interpretation as ERP and APD go in an opposite direction in response to AngII administration, which is somewhat odd because normally ERP is in parallel to APD. A recent study aiming to investigate the patterns of AER and atrial structural remodeling in AngII-mediated AF demonstrated that atrial ERP and APD are both lengthened in mice treated with AngII (Jansen et al., 2018). Moreover, Shen et al. (Shen et al., 2018) demonstrated that lncR-KCNQ1ot1 could also act in two opposite directions (lengthen ERP and shorten APD) with one single mechanism (upregulating CACNA1C), which is also somewhat unusual and puzzled. Also it should be clarified that during AER, APD is shortened, but not prolonged (Yue et al., 1997). Furthermore, in their study it was not shown how AngII alters CACNA1C expression at the protein level though changes of its transcript level were demonstrated. More difficult to understand and confusing is the result that silencing CACNA1C increases the level of miR-384.

2.4.3. LncR-TCONS_00075467

A research group led by Hou (Li et al., 2017) utilized RNA sequencing technique in conjunction with quantitative real-time polymerase chain reaction (qRT-PCR) to analyze the lncRNA expression profiles in right atrial tissues from a rabbit model of AF induced by right atrium tachypacing and from non-AF control animals. A total of 99,843 putative new lncRNAs were identified, in which 1220 differentially expressed transcripts exhibited >2-fold changes. These authors conducted detailed experimental investigations on lncR-TCONS_00075467 after bioinformatics analysis to predict the functions and interactions of the aberrantly expressed genes. Their results showed that lncR-TCONS_00075467 is significantly downregulated in AF rabbits. This expression repression increases the susceptibility of AF. Consistently, atrial ERP is shortened, along with simultaneous shortening of APD in rabbits seven days after infection by lentivirus carrying a shRNA to silence lncR-TCONS_00075467. At the ionic level, I_{CaL} density is reduced by lncR-TCONS_00075467 shRNA in isolated atrial myocytes. The authors moved further to establish that lncR-TCONS_00075467 acts as a ceRNA for miR-328 that subsequently represses expression of *CACNA1C*/Cav1.2. Knockdown of lncR-TCONS_00075467 increases the functional availability of miR-328, leading to diminishment of *CACNA1C*/Cav1.2 and I_{CaL} favoring AER and re-entry thereby the vulnerability of AF. Given the fact that our earlier study has established L-type Ca^{2+} channel β-subunit (*CACNB1*)/Cavβ1 as a target gene for miR-328

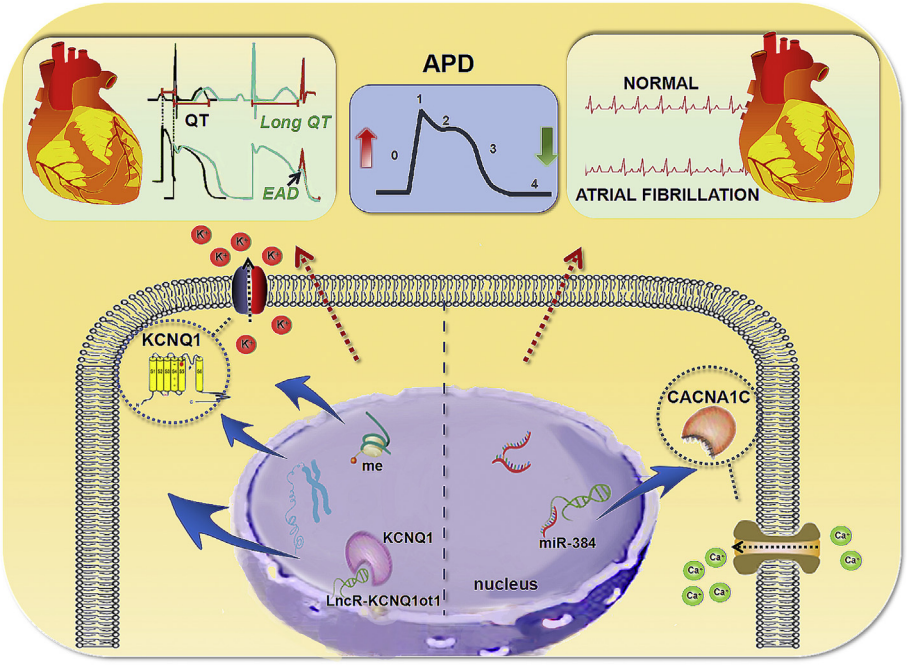

Fig. 2. Flow-chart depicting the roles and molecular mechanisms of lncR-KCNQ1ot1 in controlling cardiac electrophysiology and the associated arrhythmias. asRNA: antisense RNA that acts on their target RNAs based on sequence complementarity mechanism; CACNA1C: the gene encoding the pore-forming α-subunit of cardiac L-type Ca^{2+} channel; Cav1.2: the pore-forming α-subunit of cardiac L-type Ca^{2+} channel; LQTS: long QT syndrome; AF: atrial fibrillation; APD: action potential duration; ERP: effective refractory period; EAD: early afterdepolarization; ceRNA: competing endogenous RNA that acts to sponge miRNAs and reduce their functional availability.

(a pro-AF miRNA established by our group), it is rational to believe that lncR-TCONS_00075467 can also act indirectly on *CACNB1*/Cavβ1 through miR-328 (Lu et al., 2010).

Earlier in 2015, the same group identified two lncRNAs TCONS_00032546 and TCONS_00026102 that are possibly associated with neural development, migration and neurodegenerative disorders, by using transcriptomic analysis of lncRNAs in AF and non-AF canine cardiac fat pads (Wang et al., 2015). These authors found that lentivirus-mediated silencing of lncR-TCONS_00032546 significantly shortens atrial ERP, whereas silencing of lncR-TCONS_00026102 prolongs it, presumably by promoting and inhibiting the neurogenesis, respectively. However, whether these two lncRNAs affect AF and if yes, how they regulate AF were uninvestigated in this study.

In addition, a genome-wide association study (GWAS) identified a couple of novel genetic loci associated with early-onset AF on chromosomes 1q32.1/PPFIA4 and 4q34.1/HAND2 that encode lncRNAs, though detailed experimental investigation was not performed (Lee et al., 2017).

2.5. LncRNAs regulate susceptibility of long QT syndrome

Long QT syndrome (LQTS) is a cardiac electrical disorder characterized by delayed membrane repolarization at the subcellular level manifested by prolongation of corrected QT intervals (QTc interval) and T wave morphology changes in the ECG (Sanguinetti et al., 1996; Yang et al., 2008). LQTS increases the risk of fatal ventricular arrhythmias.

2.5.1. LncR-KCNQ1ot1

KCNQ1 is a gene encoding a pore-forming α-subunit of slow delayed rectifier K^+ channel protein that generates slow delayed rectifier K^+ channel current I_{Ks} responsible for the late phase of cardiac repolarization (Sanguinetti et al., 1996). Loss-of-function mutations in KCNQ1 are found in LQTS patients (Sanguinetti et al., 1996; Yang et al., 2008). Moreover, preferential maternal transmission of KCNQ1 variants is closely linked to channel dysfunction thereby LQTS, because of an excess of mutations of maternal origin among LQTS patients (Itoh et al., 2016), which is in agreement with a different disease-effect depending on the paternal origin of the mutation.

Intriguingly, inside the KCNQ1 sequence in human chromosome 11p15.5, there is an lncRNA named the KCNQ1 opposite strand/antisense transcript 1 (KCNQ1ot1). Specifically, lncR-KCNQ1ot1 gene spans from introns 11–10 of the KCNQ1 gene and its promoter region falls within the intron 11 of KCNQ1, which is transcribed from the opposite strand to the KCNQ1 transcript. LncR-KCNQ1ot1 is imprinted being exclusively expressed from the paternal allele (i.e. the lncR-KCNQ1ot1 gene is exclusively transcribed from the unmethylated paternal allele). Notably, lncR-KCNQ1ot1 has been identified as a regulator of expression of several genes, including KCNQ1, within the chromosome 11p15, through a mechanism that involves the formation of repressive chromatin structures. It is thus plausible that lncR-KCNQ1ot1 might participate in controlling the risk of developing LQTS.

In an earlier study by Korostowski et al. (Korostowski, Sedlak, & Engel, 2012) who first identified lncR-KCNQ1ot1 as an lncRNA regulator of gene expression, lncR-KCNQ1ot1 was found to play a role in modulating KCNQ1 levels with its absence leading to overexpression of KCNQ1 via affecting chromatin conformation in later developmental stages of the heart.

Later, Zhang et al. (Zhang et al., 2014) used an RNA-guided chromatin conformation capture method to demonstrate that the lncR-KCNQ1ot1 drives an intrachromosomal loop between the KCNQ1 promoter and a neighbor sequence, which is required for the maintenance of imprinting. Targeted suppression of lncR-KCNQ1ot1 causes loss of KCNQ1 imprinting by preventing the creation of the intrachromosomal loop. In this way, lncR-KCNQ1ot1 regulates the expression of KCNQ1 harboring mutations or LQTS risk alleles.

A more recent study suggests that differential methylation of lncR-KCNQ1ot1 promoter polymorphism is associated with symptomatic LQTS (Coto et al., 2017). These investigators compared the allele and genotype frequencies of a common lncR-KCNQ1ot1 promoter polymorphism (rs11023840) between the 131 patients and the 240 control subjects and found a significant higher frequency of AA genotype in the patients compared with healthy controls. They then investigated the methylation status by genotyping for DNA digested with the methylation-sensitive *Hpa*II restriction enzyme, and found a higher frequency of the A-allele among the patients compared with the controls. The methylation silencing of this lncRNA linked to the A-allele could promote the expression of KCNQ1 harboring the risk variant. In other words, the lncR-KCNQ1ot1 rs11023840 AA genotype is a risk factor for developing symptomatic QTc prolongation. This mechanism supports a role for the differential methylation/imprinting of lncR-KCNQ1ot1 in the risk for symptomatic LQTS. However, it should be noted the precise mechanisms that link lncR-KCNQ1ot1 variants to a prolonged QTc interval remain to be elucidated.

The role of lncR-KCNQ1ot1 expression in LQTS has also been supported by the coexistence of severe long-QT and Beckwith-Wiedemann syndrome (Gurrieri et al., 2013).

In addition to the genetic and epigenetic mechanisms for the control of LQTS by lncR-KCNQ1ot1, our recent study also revealed the possibility of this lncRNA to participate in the acquired or drug-induced LQTS (Jiang et al., 2018). More detailed information on this property of lncR-KCNQ1ot1 is to be presented in a subsequent section.

3. LncRNAs modulate cardiac arrhythmias by interacting with therapeutic drugs

3.1. Therapeutic drugs regulate the expression of lncRNAs to modulate drug-induced long QT syndrome

3.1.1. Arsenic trioxide (ATO) and lncR-KCNQ1ot1

Arsenic trioxide (ATO) is a known anti-acute promyelocytic leukemia (APL) reagent, whose clinical applications are limited by its serious cardiac toxicity and fatal adverse effects, such as sudden cardiac death resulting from LQTS. The mechanism of cardiac arrhythmia due to ATO exposure is primarily slowing of cardiac repolarization leading to prolongation of APD thereby QTc interval. A recent study from our group unraveled the involvement of lncRNA in ATO-induced LQTS in vivo and in vitro (Jiang et al., 2018).

We observed that after ATO treatment, the expression levels of lncR-KCNQ1ot1 and KCNQ1 are both markedly downregulated in myocardium and cardiomyocytes; consistently, QTc is significantly lengthened. Artificial lncR-KCNQ1ot1 knockdown produces similar electrical anomalies as ATO does: prolongation of APD in isolated ventricular cells and of LQTS in mice, along with expression downregulation of KCNQ1. On the other hand, silencing KCNQ1 exerts no effect on lncR-KCNQ1ot1 expression. It was proposed that ATO inhibits the expression of lncR-KCNQ1ot1 that in turn downregulates the expression of KCNQ1 to cause slowing of cardiac repolarization thereby APD and QTc prolongation (Fig. 2).

This is the first demonstration that therapeutic drug can alter lncRNA expression that subsequently alters expression of related protein-coding genes and the associated cellular functions (Jiang et al., 2018). In the case of lncR-KCNQ1ot1, it is speculated that lncR-KCNQ1ot1 replacement might be used as a new approach for the management of LQTS induced by ATO in APL patients receiving it as a therapeutic agent. Yet, it remains unknown how ATO alters expression of lncR-KCNQ1ot1.

It is interesting to note that in our study, lncR-KCNQ1ot1 positively regulates the expression of KCNQ1 with silencing of this lncRNA downregulating KCNQ1 expression, which is in contradiction to previously published studies (Coto et al., 2017; Korostowski et al., 2012). One possible explanation to this discrepancy is that the studies by other

laboratories concerned about the effects of imprinting alterations of lncR-KCNQ1ot1 on KCNQ1 expression, whereas our study focused on the effects of expression alterations of lncR-KCNQ1ot1 on KCNQ1 expression. While this paradox suggests mechanism-dependent differences in regulation of KCNQ1 expression by lncR-KCNQ1ot1, future studies are definitely required to clarify this issue.

3.1.2. Melatonin and lncR-MEG3

Another study from our group generated the data leading to the same conclusion that therapeutic agents can alter lncRNAs expression to regulate downstream functional signaling molecules and regulate cellular functions (Zhang et al., 2018). In this study, we investigated the anti-pyroptotic property of melatonin in atherosclerotic endothelium and explored the underlying molecular mechanisms. In high-fat diet (HFD)-treated ApoE$^{-/-}$ mice as an atherosclerotic animal model, intragastric administration of melatonin markedly reduces the atherosclerotic plaque in aorta. Meanwhile, melatonin enhances expression of lncR-MEG3 and attenuates the expression of pyroptosis-related genes, including NLRP3, ASC, cleaved caspase-1, etc., in aortic endothelium of melatonin-treated animals. We further unraveled that lncR-MEG3 promotes pyroptosis via acting as an endogenous sponge to suppress the function of miR-223 thereby increasing the expression of NLRP3, the target gene of miR-223. Our results suggest that melatonin prevents endothelial cell pyroptosis via the MEG3/miR-223/NLRP3 axis in atherosclerosis. Intriguingly, in our earlier study, we have established *KCND2* as a target gene for miR-223 and in a rat model of AMI, upregulation of miR-223 in myocardium causes remarkable downregulation of *KCND2* and its encoded protein Kv4.2, resulting in pronounced reduction of I_{to} density and APD prolongation (Liu et al., 2016). More strikingly, direct intramuscular injection of miR-223 antisense inhibitor into the ischemic myocardium decreases the propensity of ischemic arrhythmias. In a separate study by another group (Wang et al., 2012), miR-223 was found to be upregulated by >2 fold in atria of AF patients versus healthy control subjects. Taken together these three published studies, it is not irrational to believe that regulation of lncR-MEG3 by melatonin could alter cardiac electrical activities, though the precise outcomes and mechanisms require more rigorous studies to elucidate.

Another study described that losartan, an angiotensin II receptor subtype 1 (AT1) blocker clinically used as an anti-hypertensive, increases expression of lncR-NR024118 in the presence of AngII in adult rat cardiac fibroblasts (Jiang, Zhang, & Ning, 2015). Given the critical role of AngII in arrhythmogenesis, it is not unreasonable to speculate that AT1 blocker could affect arrhythmias through altering lncR-NR024118 expression, though this study did not touch on this issue.

3.2. LncRNAs directly regulate the effects of therapeutic drugs

There have not been any published studies on lncRNA regulation of actions of therapeutic drugs in the context of arrhythmogenicity, despite that emerging evidence has strongly indicated the roles of lncRNAs in shaping cancer drug responses. Nevertheless, we have good reasons to believe that with increasing attention on lncRNA research, this issue would be clarified in the very near future.

4. Cellular and molecular mechanisms for lncRNAs as regulators of arrhythmias

Functional diversity is a characteristic of lncRNAs and such diversity is conferred by the capacity of individual lncRNAs to regulate expression of multiple protein-coding genes via multiple modes of actions. The diverse actions of lncRNAs can be ascribed to the diverse molecular mechanisms: even a single lncRNA can act by multiple mechanisms. At the cellular and subcellular levels, lncRNAs have been reported to regulate cardiac excitation conduction (specifically inter-cell conduction), cardiac repolarization (APD), excitability (ERP), and intracellular Ca^{2+} homeostasis. At the molecular level, lncRNAs in the heart can act through the following mechanisms.

(1) Interacting with chromatin remodeling factors to reprogram gene expression.
(2) Guiding chromatin-modifying complexes to their required genomic destination and acting as scaffolds or docking stations for complex recruitment.
(3) Activating transcription of certain genes by guiding transcription factors to their promoters.
(4) Suppressing transcription by sequestering transcription factors.
(5) Interacting with mRNA to regulate their translation and splicing.
(6) Influencing the epigenetic programs of the transcriptome.
(7) Acting as ceRNAs by binding to complementary miRNAs via base pairing to sequester them serving as miRNA sponges.
(8) Directly interacting with target proteins to mediate their trafficking and signaling and regulate the function of bound proteins.
(9) Acting as antisense RNAs (asRNAs) to target mRNAs and alter cellular functions.
(10) Modifying the cardiotoxicity of therapeutic drugs to alter heart function and pathology.

For the specific aim of this review article, we will be highlighting the mechanisms (7)–(10) below, as these mechanisms of lncRNA actions have been documented in published studies regarding the regulation of cardiac electrophysiology and arrhythmias.

4.1. Direct regulation of cardiac ion channel genes/proteins by lncRNAs

4.1.1. Functional modulation of cardiac ion channel proteins or other related proteins

A number of lncRNAs have been documented to directly bind proteins critical in shaping cardiac electrophysiology. In all these cases, lncRNA:protein interaction renders loss-of-function of the targeted proteins. To date, it is known that lncRNAs can interact with ion channel-regulating proteins, intracellular Ca^{2+} handling proteins, and mitochondrial membrane potential-maintaining proteins. For instance, lncR-CCRR acts on CIP85 to disrupt CIP85:Cx43 interaction and prevent the endocytic trafficking of Cx43 for degradation. In this way, lncR-CCRR improves cardiac conduction producing anti-arrhythmic actions. lncR-ZFAS1 can bind SERCA2a to limit the functional availability of this critical Ca^{2+} handling protein, leading to intracellular Ca^{2+} overload creating a substrate for the generation of arrhythmias. As already mentioned above, lncR-MIAT can bind directly to TSPO to enhance opening of the mitochondrial permeability transition pore, followed by loss of mitochondrial membrane potential and increase in arrhythmogenic potential.

There have not been any studies showing the direct interactions between lncRNAs and cytoplasmic ion channel proteins.

4.1.2. Expression regulation of cardiac ion channel-encoding genes

While the lncRNAs described above act directly on ion channel proteins to limit their functions, lncRNAs can also serve as regulators and modulate the expression of ion channel-encoding genes. For instance, we have discussed that lncR-KCNA2as was found to alter the expression of *KCNA2* that codes for Kv1.2 K^+ channel protein. Another example is lncR-KCNQ1ot1 regulation of *KCNQ1* expression, and this observation has been reported by two laboratories. It appears that lncR-KCNQ1ot1 downregulates *KCNQ1* via affecting chromatin conformation. Still another study from our group demonstrated that lncR-MIAT can directly alter the expression of *KCNJ2* mRNA and the corresponding Kir2.1 protein levels.

4.2. LncRNAs as asRNAs to target genes of cardiac ion channels

Nucleotide antisense fragments are known to be able to bind mRNAs to hinder protein translation process and induce breakdown of targeted mRNAs. In the cells, there are natural antisense RNA molecules (asRNAs) that function as regulators of gene expression by binding to mRNAs. In theory, there are at least two different modes for lncRNAs to become asRNAs: (1) lncRNAs as exact antisense transcripts to their mRNA counterparts and (2) lncRNAs containing antisense or complementary sequence region(s) to target mRNAs.

Emerging studies have generated evidence for lncRNAs as asRNAs to regulate expression of genes. In the context of cardiac electrophysiology and arrhythmias, lncRNAs have been shown to regulate ion channel genes by asRNA mechanism. As described in Section 2.1, lncR-KCNA2as counteracts the expression of *KCNA2* through its antisense property, despite that the authors of this study did not go insight to delineate the exact way for such a regulation. Similarly, lncR-KCNQ1ot1 can also act as an asRNA to regulate *KCNQ1* level.

While lncR-KCNA2as and lncR-KCNQ1ot1 are antisense transcripts to their respective genes, lncR-MIAT was identified as a partial antisense molecule to *KCNJ2*. We found that lncR-MIAT contains a short stretch of sequence complementary to *KCNJ2* mRNA sequence, and examined its effects on *KCNJ2* expression. Our results demonstrated that this lncR-MIAT fragment antisense to *KCNJ2* significantly reduces the transcript level of the latter and causes remarkable decreases in Kir2.1 protein and I_{K1} current density. Our finding predicts a decent number of lncRNAs with the possibility of being asRNAs in the gene expression regulatory network.

4.3. LncRNAs as ceRNAs for miRNAs (miRNAs as intermediators of lncRNA functions)

In general, all lncRNAs have the potential to serve as ceRNAs to indirectly alter the expression levels of protein-coding genes. In this way, miRNAs intermediate the effects of lncRNAs. Thus, the model of actions can be simplified as: lncRNA/ceRNA → miRNA → target genes of miRNA → cellular function. Such a signaling pathway exists commonly in organisms and in the case of lncRNA regulation of cardiac electrophysiology and arrhythmias. We have discussed above several cases of such a mechanism of action. For example, lncR-MALAT1 → miR-200c → HMGB1 → *KCND2*/Kv4.2 and *KCND3*/Kv4.3 → I_{to}, lncR-KCNQ1ot1 → miR-384 → *CACNA1C*/Cav1.2 → I_{CaL}, and lncR-TCONS_00075467 → miR-328 → *CACNA1C*/Cav1.2 & *CACNB1*/Cavβ1 → I_{CaL}.

It needs to be noted that in theory, lncRNAs that carry the binding sites of miRNAs could all act as ceRNAs; in reality, however, this is not the case. We have tested no less than 50 lncRNAs and found the majority of these lncRNAs actually do not serve as sponge to the anticipated target miRNAs. In sharp contrast, the level of lncRNAs more often goes in the same direction of the candidate target miRNAs; or the reciprocal relationship does not exist between lncRNAs and miRNAs that have one or more binding sites in many cases. This emphasizes the importance of experimental verifications; merely using theoretical complementarity predictions can often point one to an entirely wrong direction.

In addition, when doing verifications, one needs to keep it in mind that luciferase reporter gene assay may not always exhibit the expected outcomes. That is, throwing in miRNA mimic may or may not affect luciferase activities elicited by the vector carrying an lncRNA fragment encompassing the miRNA binding domain. The causes for this remain yet to be delineated.

4.4. LncRNAs as modulators of potency and efficacy of therapeutic drugs

To date, only two such lncRNAs have been defined: ATO alters expression of lncR-KCNQ1ot1 (Jiang et al., 2018) and melatonin alters lncR-MEG3 (Zhang, Liu, et al., 2018). In the case of ATO, altered expression of lncR-KCNQ1ot1 regulates the expression of *KCNQ1*/KvLQT1 to change the likelihood of LQTS (this has been described in detail in Section 3.1). As for melatonin, it changes lncR-MEG3 expression that could likely alter expression *KCND2*/Kv4.2.

5. Therapeutic implications of lncRNAs and potential strategies for drug development

Given the facts that the expression of lncRNAs is relatively tissue/cell specific, and the cellular function and pathological roles of these RNAs are diverse, there is no doubt that lncRNAs have significant implication in clinical applications and great potential for new drug development for the treatment of cardiovascular disease including cardiac electrical disorders. In general, there are two different directions for the development of lncR-based therapeutic drugs. One is loss-of-function approach by repressing the expression or knocking down the cellular level of lncRNAs, and the other is gain-of-function strategy by introduction of exogenous lncRNAs into cells or induction of overexpression of lncRNAs in target cells.

Yet, from a translational perspective, major challenges are present not only because of our current poor understanding of the function of lncRNAs but also due to the multiple technical constraints unique to lncRNAs. First, moderate level of lncRNA sequence conservation across species indicates that the knowledge acquired from animal models may not be applied to man. Second, many lncRNAs function in the *cis*-acting regulation, and, although some have transcript-specific *trans*-actions, it is difficult, if not impossible, to uncouple RNA-specific effects from *cis*-acting genome elements. Third, diverse and multiple mechanisms of a given lncRNA make it hard to dissect out an exact and precise process to interfere. Forth, lncRNAs are mostly large molecules that are difficult to penetrate into cells to take their actions. And finally, despite a broad interest in lncRNAs, only a handful of transcripts have been well characterized for their cellular functions and pathophysiological roles.

5.1. How can lncRNAs be used as therapeutic targets for cardiac disease?

Silencing of lncRNAs is definitely one of the options for manipulating the levels of these transcripts for the therapeutic purpose for the lncRNAs that are abnormally upregulated during the pathogenesis and contribute to disease progression. This approach has been achieved by chemically modified siRNAs in non-human species for basic and preclinical studies. In particular, for nuclear resident lncRNAs, chemistries that trigger RNase H-dependent degradation in the nucleus where the mature transcripts are produced, are reportedly more efficient and are proposed to be the better choice for therapeutic applications of siRNA technique. However, therapeutic application of synthetic siRNAs and their chemical derivatives are limited by their low efficacy, potency, and stability, and by the risk of off-target effects in other organs as well.

CRISPR-CAS (clustered regularly interspaced short palindromic repeats–CRISPR-associated protein)–based modification of lncRNA expression has been shown to possess advantages over RNAi. These advantages include increased degree of loss-of-function, improved specificity, and the enhanced ability to modulate gene expression in *cis*. However, disadvantages co-exist side-by-side. By using CRISPR-based loss-of-function approaches in mouse cell lines, a study showed that approximately 50% of lncRNAs influence the expression of neighboring protein-coding genes. And even worse is that these effects are non-specific but being largely independent of the lncRNA sequence.

In addition to RNAi approach, antisense oligodeoxynucleotides (ODNs) are another option for loss-of-function of lncRNAs. This issue is to be discussed in the subsequent subsection.

5.2. How to achieve lncRNA replacement therapy: identification and characterization of the functional domain of lncRNAs?

In many situations, abnormal downregulation of lncRNAs is a determinant of detrimental alterations of cellular functions promoting

pathogenesis and disease progression. In such a case, supplement of lncRNAs should be a rational approach to correct the anomalies and afford beneficial effects to organisms. However, lncRNAs are in general very long in their sequences and are not easy to be efficiently introduced into cells. While plasmid and viral vectors could be a solution for some lncRNAs, their application is often limited by their insufficient capacity of carrying large constructs. This difficulty hampers the development of lncRNA replacement therapy. One way to surrogate the over-length issue is to identify and apply the functional domain (FD) of lncRNAs that retains the cellular function of full-length lncRNAs for a particular disease entity. In theory, the lncRNAs using the sequence complementary mechanism for their actions can all fit into such a theme. For example, lncRNA-ceRNA and lncRNA-asRNA are the best subcategories of lncRNAs for such a purpose. One can simply artificially synthesize nucleotide fragments encompassing the miRNA or mRNA binding sites and apply them to cells. These fragments should be able to mimic or reproduce the ability of their respective full-length lncRNAs to bind target miRNAs or target mRNAs and take the corresponding cellular actions. Unfortunately, in reality such a seemingly simple approach is not attractive and straightforward at all. For lncRNA-ceRNA, one could more simply directly manipulating target miRNAs without the needs to interfere lncRNAs. Another intrinsic caveat of this approach is that miRNA has multiple target genes and change one miRNA could affect expression of many protein-coding genes. Such a non-gene-specific action is unavoidable and non-desirable.

On the other hand, our group has developed for the first time a strategy to identify FDs of lncRNAs and verify their cellular and pathophysiological effects. This strategy focuses on the lncRNAs that directly interact with proteins. Such an approach has an obvious advantage over ceRNA in that lncRNA:protein interaction is more protein-specific and manipulating the FD of an lncRNA should theoretically only alter the function of target protein without off-target effects on other non-binding proteins. We have applied such an approach to animal studies and successfully validated the delivery efficiency and functional effects of FDs of three different lncRNAs. We are providing the step-by-step protocols for identification and validation of lncRNA FD below (Fig. 3).

1) Computational prediction of RNA-protein interaction and experimental verification of direct lncRNA:protein interaction;
2) Searching the candidate FD by sequence alignment for high degree conservation of lncRNA region across species. It should be noted the presence of sequence conservation may not always predict functions of lncRNAs, and this is why verification of direct lncRNA:protein interaction should proceed conservation analysis;
3) Theoretical prediction of the ability of the candidate FD to bind the target protein as its full-length lncRNA, using the computational docking software Hex 8.0 or other tools. In this way, we are able to identify the nucleotides encompassed by the candidate FD as the core motif for specific binding to the specific amino acids of the target protein;
4) Synthesis and chemical modification of conserved region (the candidate FD);
5) Construction of viral vector (for in vivo applications) or plasmid vector (for in vitro cellular investigations);
6) Experimental examinations of the effects of the candidate FD, as compared with those of full-length lncRNAs and negative control constructs;
7) Nucleotide substitution mutation within the core motif of the candidate FD to disrupt the binding site for target protein, followed by computational docking analysis to indicate the loss of the original binding sites in the mutant FD. This is next followed by experimental verification of loss of function of the mutant construct;
8) Verification of loss of function of the candidate FD using antisense technique (oligo deoxynucleotides fragment antisense to FD thereby its full-length counterpart) or siRNA. In theory, an antisense to FD acts by dual mechanisms: inducing degradation of, and masking the binding site in the candidate FD. In addition, FD antisense could also bind to its full-length lncRNA and act as an FD-masking fragment to mitigate the function of this lncRNA.
9) Finally, all negative control constructs of the candidate FD and antisense fragments need to be verified for their anticipated effects;

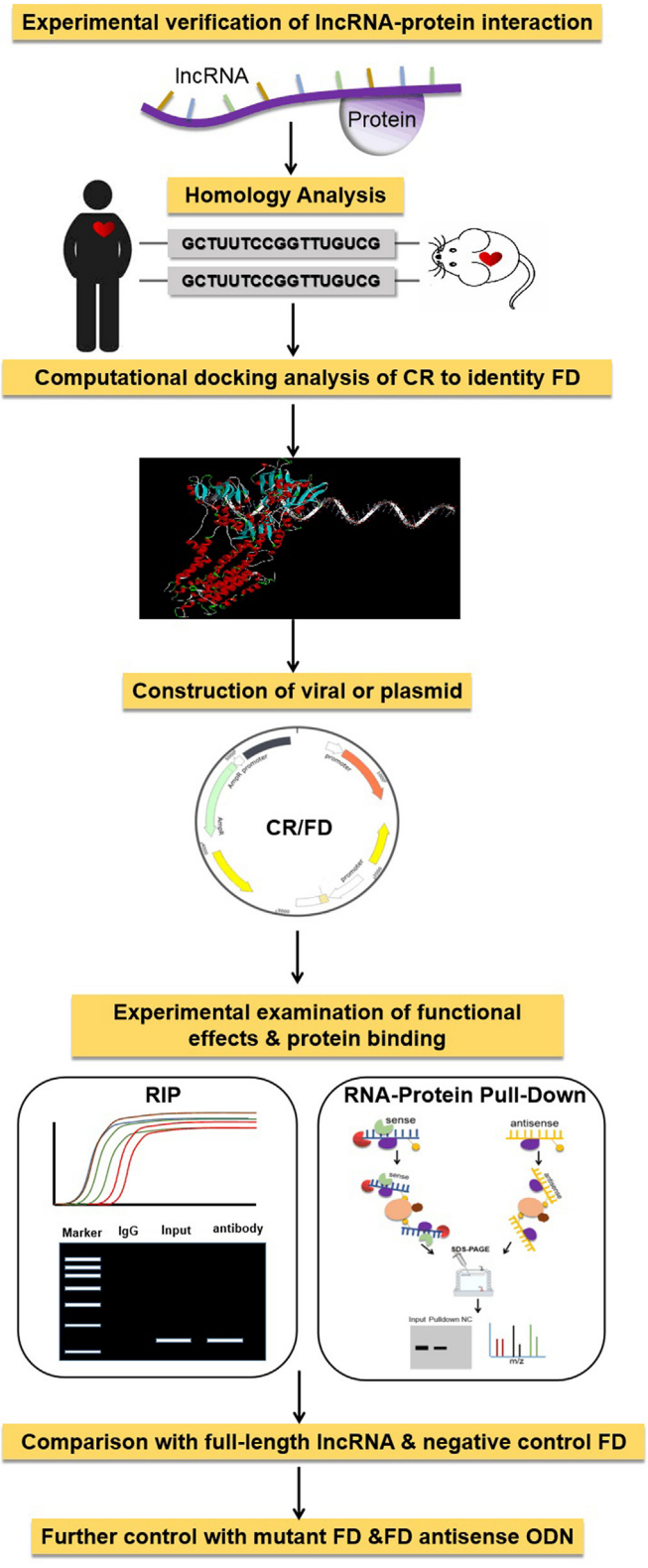

Fig. 3. Flow chart displaying the step-be-step protocol for identifying and validation of functional domain (FD) of lncRNA based on conserved region (CR). ODN: oligodeoxynucleotides.

10) Delivery of FD into animals to confirm its expected actions in appropriate pathological models. In theory, an appropriate FD construct can be applied to humans for a particular disease state.

It should be noted that the FD strategy has only been tested in animal models, and there is still a long way to go to make it applicable to clinical practice. Before that to happen, rigorous and vigorous future studies are absolutely required. Nonetheless, for the time being, this approach can be utilized for fundamental studies aiming to decipher the mechanistic link between lncRNAs and their cellular function and the associated pathophysiological roles.

6. Conclusions and future perspectives

Thus far, there have been several studies in the literature on the regulation of cardiac electrophysiology and arrhythmias under several diseased states of the heart, and increasing efforts in this regard are expected in the near futures. While these studies have opened a new research field with findings that can definitely advance our understanding of lncRNAs as a new layer of the regulatory network for cardiac electrophysiology and bare significant clinical implications, we must admit that we have made only the first step towards in-depth understanding of these RNA molecules. Especially, studies on human hearts have been restricted in lncRNA expression profiles and functional investigations using human specimens have been lacking. It is also noticed that to date, the published studies focusing on lncRNAs and cardiac arrhythmias are rather sparse with only a few reports in this regard including lncR-CCRR in HF, lncR-KCNA2as in HF, lncR-MALAT1 in MI, lncR-TCONS_00075467 in AF, lncR-KCNQ1ot1 in LQTS, and lncR-NONRATT021972 in diabetic cardiac autonomic neuropathy.

Future studies may be directed to identify more lncRNAs that can interact directly with ion channel/transporter proteins or ion channel/transporter-encoding genes and alter cardiac electrical activities and arrhythmogenicity. Close attention may also be paid to miRNAs that intermediate the role of lncRNAs in regulating ion channels/transporters; this is because a variety of miRNAs have been found to participate in the regulation of cardiac electrophysiology under varying pathological conditions of the heart and also because it is technically more straightforward for identifying miRNA inter-mediators. For example, both lncR-H19 and lncR-MD1 can function as ceRNAs as well as precursors for miRNAs (Rashid, Shah, & Shan, 2016) that are related to regulation of cardiac electrophysiology. LncR-H19 acts as a molecular sponge for let-7 family of miRNAs (Cesana et al., 2011). Intriguingly, we have reported earlier that let-7e replacement yields potent anti-arrhythmic efficacy via targeting β_1-adrenergic receptor and the anti-arrhythmic efficacy of let-7e is similar to propranolol, a non-selective β-AR blocker and metoprolol, a selective β_1-AR blocker (Li et al., 2014). It is therefore likely that lncR-H19 could well modulate cardiac electrophysiology through its ceRNA action on let-7. On the other hand, lncR-MD1 acts as a natural decoy for two muscle-specific miRNAs, miR-133 and miR-135 (Kallen et al., 2013). As discussed above, our unpublished findings indicate that miR-135 is a target for lncR-MIAT to act as a ceRNA and is linked to the susceptibility of AF. It is not unreasonable to deduce that lncR-MD1 might possess similar property to lncR-MIAT by acting as a ceRNA of miR-135. Moreover, as a decoy RNA for miR-133, lncR-MD1 could also impose regulatory effects on cardiac electrophysiology since miR-133a and miR-133b has been demonstrated to regulate and repress KvLQT1 protein expression to control repolarization reserve of the heart thereby potentially affecting vulnerability of LQTS and AF. Furthermore, lncR-MD1 primary transcript harbors the pri-miR-133b sequence (Rashid et al., 2016), likely conferring itself the ability to control cardiac electrophysiology and the associated CA. While due to the ethical issue and tissue availability, studies on human specimens will still be limited, and under such a situation using human cardiac cell lines is an alternative solution for mechanistic investigations. Also noteworthy is that recent sequencing-based, transcriptome-wide studies have unraveled that lncRNAs are frequently modified and these chemical modifications determine the fate and activity of these functional RNAs. Three major epitranscriptomic marks include, pseudouridine (Ψ), N^6-methyladenosine (m^6A) and 5-methylcytosine (m^5C) (Jacob, Zander, & Gutschner, 2017; Warda et al., 2017). While the chemical modifications of lncRNAs are deemed to altering their functionalities including their roles in shaping cardiac electrophysiology, to date there have not been any studies linking such epitranscriptomic alterations to the regulation of cardiac electrorheology. For instance, we have mentioned above that lncR-MALAT1 is a decoy RNA or ceRNA for miR-200c, which governs the expression of Kv4.2 and Kv4.3 thereby arrhythmogenic potential. Notably it has recently been documented that this lncR is m^6A modified (Coker, Wei, & Brockdorff, 2019), and it would be interesting to clarify whether the effects of lncR-MALAT1 on Kv4.2 and Kv4.3 is related to its m^6A modification. Clearly, as an emerging new field of research, chemical modifications of RNAs open up a novel arena for studying lncRNA function.

In addition, translational studies on lncRNAs and cardiovascular disease will also be seeing their boom in the future, partially owing to our ever-improving understanding of lncRNA functions and to the now-available approach for identifying the functional domain of lncRNAs for practical applications as well.

Declarations of competing interest

The authors declare that there are no conflicts of interest.

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