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Noncoding RNAs in cardiovascular diseases

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Abstract

Purpose of review: Human genome is pervasively transcribed, producing coding and noncoding RNAs. Recent studies have revealed the roles of a class of noncoding RNAs, the long noncoding RNAs (lncRNAs), in heart failure and other cardiovascular diseases. This review provides a brief summary of recent findings on lncRNA function.

Recent findings: Recent studies have documented the roles of lncRNAs in cardiac regeneration, conduction, hypertrophy/dysfunction, and endothelial function. LncRNAs perform these functions through acting as competing RNA (by binding and sequestering miRNAs) or acting as guides to protein targeting. A few lncRNAs also encode small peptides (for example; *Dworf* RNA) and in the context of heart regulate cardiac calcium homeostasis.

Summary: Noncoding RNA provides a versatile mechanism of gene regulation and thereby present as novel targets for intervention in various cardiovascular disease. Future studies aimed at defining the context-dependent lncRNA mechanisms will be required to advance our understanding and relish the goal of RNA therapeutics.

Keywords

Heart failure; LncRNA; miRNA and Cardiac regeneration

Introduction

Despite significant improvements, heart failure remains a leading health epidemic [1]. Therefore, identification and characterization of promising new avenues to explore for risk mitigation in HF are essential. The human genome is extensively transcribed, resulting in the production of numerous noncoding RNAs (ncRNAs) [2–4]. The ncRNAs are generally classified based on size as small (<200 nucleotides, such as miRNA, piRNA, snoRNAs, etc.) and long noncoding RNAs (lncRNAs), which are generally defined as transcripts larger than 200 bases [4–9]. LncRNAs comprise a diverse class of RNAs, namely long intergenic RNA (lincRNA), enhancer RNAs (eRNAs), and sense or antisense transcripts (AS) [9, 10]. They are often expressed in a high cell- and tissue-specific manner and have been proposed to carry out varied functions, including transcriptional regulation in cis or trans, nuclear

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Conflicts of interest
There are no conflicts of interest.

domain formation/ organization, regulation of protein function and stability, and at times encoding for small proteins [5, 6, 8, 11–17]. Likewise, a subset of lncRNAs are produced from enhancer or promoter regions and likely regulate transcriptional activity from these regulatory elements. Abnormalities of lncRNAs function and expression have been directly linked with cardiovascular biology and disease. The roles of lncRNAs in cardiovascular disorders are beginning to emerge; of particular interest is the dysregulation of lncRNA regulatory circuits in cell fate, cardiac hypertrophy, vascular disease, atherosclerosis, and metabolic syndrome [5, 6, 8, 11–17]. In light of these discoveries, in this review, we provide information on some of the recent findings and highlight the role of lncRNAs in cardiac regeneration, cardiac arrhythmia, cell death, and endothelial biology. We also summarize new findings on a class of non-conventional lncRNAs that code for the small peptide (micro peptide) and regulate cardiac muscle function.

LncRNAs in Cardiac regeneration

The neonatal heart has regenerative capacity driven largely thorough cardiac myocyte proliferation, the adult heart, on the other hand, has limited regeneration capacity [18, 19]. The mechanisms of this post-mitotic cell cycle exit, associated with cessation in cardiac proliferation and regeneration of myocardium, are not clearly defined. Several new studies have implicated lncRNAs in cardiac myocyte proliferation and thereby ushered in the new field of cardiac regeneration. A brief summary of these findings is presented here.

a) CAREL: A lncRNA named <u>ca</u>rdiac <u>r</u>egeneration-related long noncoding ribonucleic acid (CAREL or NONMMUT070401) was found to be induced in neonatal mice upon cessation of cardiac myocyte proliferation [20]**. Gain of function studies using CAREL-Transgenic mice showed its ability to suppress cardiac proliferation as evidenced by increased pH3$^+$ and Aurora B$^+$ myocyte. Furthermore, in the context of myocardial infarction (MI), silencing of CAREL improved heart function associated with increased cell proliferation and regeneration in neonatal and adult mice. Mechanistically, CAREL was proposed to act as a competing RNA for miR-296 and overexpression of miR-296, mimics CAREL loss of function phenotype. Targeting of *Trp53inp1* and *Itm2a* by miR-296 was proposed to be a plausible mechanism of action. Thus, the findings implicate CAREL in the suppression of cardiomyocyte cell proliferation and induction of cell cycle exit through inhibition of miR-296.

b) CRRL (cardiomyocyte regeneration-related lncRNA): Like CAREL another long noncoding RNA namely CRRL (<u>c</u>ardiomyocyte <u>r</u>egeneration-<u>r</u>elated <u>l</u>ncRNA) was shown to be a negative regulator of cardiomyocyte proliferation and cardiac repair [21] *. CRRL was identified to be induced in adult as compared to fetal cardiac tissues. Suppression of CRRL in a rat model of MI improved cardiac function and promoted cardiomyocyte proliferation and repair. CRRL exerts these effects through binding to miR-199a-3p and thereby suppressing its activity and increasing levels of its target *Hopx*. The latter is implicated in the regulation of cardiac gene expression and cardiac development [22].

c) AZIN2-sv/NONHSAT002258: AZIN2-sv, a non-coding splice variant of the *Azin2* gene, was identified as a potential cardiac lncRNA that was highly expressed in adult heart, and conserved in rodents [23] *. *In vitro* and *in vivo* inhibition of *Azin2-sv* in cardiomyocytes enhanced proliferation rate, whereas its overexpression significantly reduced proliferation. The mechanism of action of *Azin2-sv* in cardiomyocytes was mediated through the miRNA-214/PTEN/Akt axis. Specifically, *Azin2-sv* acted as a miR-214 sponge thereby depressing PTEN expression. Furthermore, stabilizing of PTEN was further reinforced by the direct binding of *Azin2-sv* to PTEN and enhancing proliferation.

Similar to the above-mentioned lncRNAs several others have been shown to mediate cardiomyocyte proliferation mainly by perturbing miRNAs and direct binding to proteins [12, 24–26] *.

Even though the initial studies described here have provided the impetus for defining the role of lncRNA in cardiac regeneration, future studies are required to rigorously define (by integrating multiple biochemical, molecular approaches) the role of lncRNAs on proliferation and maturation of cardiac myocyte. Likewise, careful studies designed to determine the effect of postnatal cardiac myocyte proliferation on cardiac hypertrophy and failure are required to ascertain the impact of lncRNAs on myocardial regeneration.

LncRNAs in cardiac conduction system:

Several ncRNAs (mostly miRNA) have been shown to regulate cardiac rhythm. Recently cardiac conduction regulatory RNA (CCRR) was identified as an antiarrhythmic lncRNA [27]**. CCRR is downregulated in failing human and mouse hearts. CCCR downregulation was associated with slow cardiac conduction and enhanced arrhythmogenicity in mice. CCRR overexpression rescues these detrimental effects. The biological role of CCRR is manifested through its interaction with a protein CIP85 whereby the CCRR-CIP85 complex occludes CIP85 mediated degradation of gap junction protein CX43. Knockdown or downregulation of CCRR causes perturbation of cell-cell junction integrity (intercalated discs and gap junctions) thorough degradation of CX43 by CIP85 interaction. Downregulation of CX43 mediates electrical uncoupling and thereby increased the propensity to cardiac arrhythmias. Even though these studies do not exclude mechanism other than lncRNAs in the maintenance and alteration of Intercalated disc (ID) and Gap junction, CCRR still provides a potential therapeutic avenue for targeting pathological arrhythmias.

LncRNAs in cardiac hypertrophy and dysfunction:

Maladaptive cardiac remodeling due to sustained cardiac hypertrophy leads to decreased compliance and increased risk for heart failure. Several signaling pathways are known to contribute to the pathogenesis of pathological hypertrophy, and heart failure, a subset of those are driven by Ca^{2+} dysregulation [28, 29]. Here lncRNAs that are regulating these pathways and their role in cardiac function is described.

a) LncRNA ZFAS1: Long noncoding RNA ZFAS1 (ZNFX1 antisense1) is produced from a snoRNA host gene. In MI mouse models, *Zfas1* was shown to be induced mainly in the cytoplasm and sarcoplasmic reticulum[30, 31]**. Overexpression of *Zfas1* in mice decreased while its knockdown rescued contractile dysfunction in the context of MI. *Zfas1* binds SERCA2A protein and impairs its activity, leading to altered Ca^{2+} transient and intracellular Ca^{2+} overload in cardiomyocytes. Finally, only a small conserved sequence domain of *Zfas1* gene is required and sufficient for its function. Given the targeting of SERCA2A in HF, ZFAS1 provides another opportunity for modulating SERCA2A activity and cardiac function in HF

b) Airn: Several long noncoding RNAs cluster with imprinted genes, of these antisense Igf2r RNA noncoding antisense (*Airn*), has been extensively studied. The *Airn* regulates expression of *Igf2r* and *Slc22a2* and *Slc22a3* genes either through transcriptional interference or through epigenetic silencing [32]. However, several isoforms of *Airn* are known to exist. Cardiac-specific *Airn* Isoform (Airn*-001*) is shown to regulate cell death [33]. Further detailed mechanistic studies showed direct binding of *Airn-01* to IGF2BP2 protein. This RNP complex is required for IGF2BP2 translation and also for the translation of several proteins that are targeted by IGF2BP2.Thus, *Airn* regulates cardiac translational output through IGF2BP2 and thereby cell survival.

LncRNAs regulating endothelial function:

Recent studies have implicated a crucial role of lncRNAs in in vascular biology. Several lncRNAs with a role in vascular remodeling, endothelial cell proliferation, angiogenesis, and cholesterol metabolism have been described [6, 8, 34]. Here we highlight two recent studies characterizing the role of lncRNAs in macrophage and BMC in the pathogenesis of artheoscloris.

a) MeXis: The liver X receptor (LXR) is a member of the nuclear receptor family of transcription factors that regulate cholesterol homeostasis and is implicated in the pathogenesis of atherosclerosis [35]. Activation of LXRs promotes reverse cholesterol transport in part through plasma membrane transporter ABCA1. LncRNA MeXis (Macrophage-expressed LXR-induced sequence) was shown to be induced upon LXR treatment [36]**. This induction of MeXis was correlated with the activation of its neighboring gene *Abca1*. MeXis regulates *Abca1* expression through remodeling the local chromatin accessibility and direct recruitment of transcriptional coactivator DDX17. MeXis loss of function (LOF) in mice leads to decreased *Abca1* expression, and impaired cholesterol efflux leading to atherosclerosis. MeXis human homolog TCONS00016111 was also found to regulate ABCA1 expression. Therefore, lncRNA MeXis-ABCA1 provide a novel regulatory target involved in the pathogenesis of atherosclerosis.

b) MALAT1: MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1) is a nuclear-enriched long coding RNA that lack PolyA tail and was initially implicated in cancer metastasis and cell migration. Recent studies have highlighted its role in the heart where it is involved in cardiac fibrosis, stress remodeling, and angiogenesis. However, its exact role in the endothelial function and the pathogenesis of atherosclerosis was not known. Using *Apoe*$^{-/-}$ and *Malat1*$^{-/-}$ mice, the role of *Malat1* in plaque size and infiltration of $CD45^{+}$ cells was revealed. Bone marrow transplantation experiments using *Malat1*$^{-/-}$ bone marrow cells in Apoe$^{-/-}$ mice further confirmed its role in atherosclerotic lesion formation and proatherosclerotic phenotype [37]*. *Malat1* was found to act as a sponge for miR-503. MALAT1 expression also correlated with enhanced human plaques and worse prognosis.

LncRNA coding for micropeptide: Recent genome-wide studies provide credence to the hypothesis that lncRNAs can also serve as a reservoir for coding functional micro peptides [38–40].The micro peptide discovery has been hindered largely because of the difficulty in identifying functional small open reading frames (ORFs). Regardless of this limitation, several micro peptides that affect the cardiovascular system has been identified. Of note are the conserved peptide myoregulin and DWORF (DWarf Open Reading Frame) both affecting SERCA (sarcoendoplasmic reticulum calcium transport ATPase) activity [41, 42]. However, the molecular underpinning of micro peptide function and their relevance to heart failure was not demonstrated.

Recently using DWORF-KO mice investigator have now determined that the effect of DWORF is mediated through its high-affinity binding to SERCA2A [43]**. This high-affinity binding is sufficient to compete with a SERCA2A inhibitor phospholamban (PLN) and restore SERCA2A activity and $Ca2^+$ transient in PLN-Tg mice. Furthermore, the relevance of DWORF to human HF was shown using a dilated cardiomyopathy mouse model of muscle-specific LIM domain protein knockout (MLP-KO). These mice show severe cardiac dilation, dysfunction and reduced SERCA2A activity. Overexpression of DWORF through a transgenic approach led to the rescue of cardiac function that was associated with increased SERCA2A activity and enhanced calcium transition. Thus, DWORF as a potent activator of SERCA2A within the heart is an attractive candidate for a heart failure therapy.

Conclusion

Here, the latest reports describing the role of lncRNAs in cardiovascular biology and disease are reviewed. Although several more lncRNAs are shown associated with cardiac development and disease, their functionality and therapeutic utility remain to be determined. More mechanistic studies that employ genome-wide tools with specific subcellular localization and classic RNA-protein interaction studies will be required to define the actual mode of lncRNA function. Likewise, more work is needed to determine whether lncRNAs could be used as biomarkers for cardiovascular diseases. The restricted spatiotemporal expression of lncRNAs provides a novel tool to treat cardiac cell type-specific lncRNAs without fortuitous effect on other cells and organs. Finally, novel tools for lncRNA cargo delivery have to be developed for effective treatment. In conclusion, these initial studies and future mechanistic details will enhance the likelihood of therapeutic targeting of specific lncRNAs.

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KEY POINTS

- Recent discoveries have highlighted the roles of lncRNAs in the regulation of cardiac myocyte proliferation, cardiac conduction, and cholesterol efflux
- LncRNAs regulate cardiac phenotypes by targeting miRNA activity (through direct binding to them) and or by protein through the formation of RNA-Protein complex (RNP).
- Future mechanistic studies aimed at defining lncRNA cell type-specific functions will be critical for their therapeutic targeting in the context of Heart failure