Long Non-Coding RNAs in Cardiac Remodeling

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Abstract
Cardiac remodeling occurs after stress to the heart, manifested as pathological processes, including hypertrophy and apoptosis of cardiomyocytes, dysfunction of vascular endothelial cells and vascular smooth muscle cells as well as differentiation and proliferation of fibroblasts, ultimately resulting in progression of cardiovascular diseases. Emerging evidence has revealed that long non-coding RNAs (lncRNAs) acted as powerful and dynamic modifiers of cardiac remodeling. LncRNAs including Chaer, Chast, Mhrt, CHRF, ROR, H19, and MIAT have been implicated in cardiac hypertrophy while NRF, H19, APF, CARL, UCA, Mhrt and several other lncRNAs (n379599, n379519, n384640, n380433 and n410105) in cardiomyocyte loss and extracellular matrix remodeling. In addition, MALAT1 and TGFB2-OT1 have been reported to contribute to vascular endothelial cells dysfunction while lincRNA-p21 and Inc-Ang362 to vascular smooth muscle cells proliferation. Thus, manipulation of lncRNA expression levels through either the inhibition of disease-up-regulated lncRNAs or increasing disease-down-regulated lncRNAs represents novel therapeutic strategies for cardiac remodeling.

Introduction
Cardiovascular diseases, especially coronary atherosclerotic disease and heart failure (HF), affect more than 20% of the population aged 40 and over. Though the clinical management is improving, mortality rates of HF remain about 50% within 5 years of diagnosis, making HF a leading cause of morbidity and mortality in industrialized countries [1, 2]. Cardiac remodeling, which refers to changes that result in the rearrangement of normal structures of the heart and vessel [3], is a chronic maladaptive process characterized

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by progressive myocardial hypertrophy, apoptosis, ventricular dilatation, fibrosis, vascular dysfunction, finally resulting in HF [4, 5]. Preventing or reversing cardiac remodeling is a key strategy for the treatment of HF [6] and further efforts are highly needed to explore the mechanisms underlying cardiac remodeling.

Non-coding RNAs (ncRNAs) cover more than 98% of the human genome [7]. Among which, small ncRNAs, such as microRNAs (miRNAs, miRs), have been intensely investigated for many years while studies on long ncRNAs (lncRNAs) just started in recent years. LncRNAs are defined as transcripts that are longer than 200 nucleotides and do not code for proteins in most cases, but basepair with DNA or RNA in a sequence-specific manner, thus regulating gene expression. LncRNAs can be classified into 4 main categories based on the gene-regulating mechanism: i) signal lncRNA, which can regulate gene expression in a time and space manner; ii) decoy lncRNA, which can titrate transcription factors and other proteins away from chromatin, or titrate miRNAs out from their target; iii) guide lncRNA, which can recruit chromatin modifying enzymes to target genes, either in cis (near the site of lncRNA production) or in trans to distant target genes; and iv) scaffold lncRNA, which can facilitate the assembling of multiple proteins to form ribonucleoprotein (RNP) complexes, which may act on chromatin, affecting histone modifications [7-12].

Emerging data have shown that lncRNAs play a role in both the development and pathology of cardiovascular system, providing novel insights and therapeutic targets for cardiovascular diseases. Here we reviewed the progress of lncRNAs' roles in cardiac remodeling and HF, especially the interaction between lncRNAs and other RNAs, mainly miRNAs and mRNAs (Table 1 and Fig. 1).

**LncRNAs in cardiac hypertrophy**

Cardiac hypertrophy is an initially adaptive response to stress or volume overload stimuli, reducing the increased wall tension and helping to maintain cardiac output [3]. The adaptive process is beneficial and can improve muscular economy at the very start, however, persistent exposure of the heart to increased load would lead to the impairment of cardiac microcirculation, resulting in tissue hypoxia and a subsequent loss of cardiomyocytes [13, 14], ultimately resulting in HF. With the advent of high-throughput RNA sequencing, a number of studies have been carried out to investigate the role of lncRNAs in cardiac hypertrophy.

**ChaeR**

Two high-throughput RNA sequencing studies revealed approximately 15 differentially regulated lncRNAs in mouse cardiac hypertrophy model [15, 16]. Among them, Chaer (Cardiac Hypertrophy-Associated Epigenetic Regulator) can directly interact with the catalytic subunit of polycomb repressor complex 2 (PRC2), thereby inhibiting histone H3 lysine 27 methylation at the promoter regions of genes involved in cardiac hypertrophy and thus contributing to the development of cardiac hypertrophy [17]. Along with other PRC2-interacting lncRNAs, such as Fendrrand and Bvht, which have been shown to be important in epigenetic programming during heart development, Chaer is also an epigenetic regulatory lncRNAs. Since Chaer-PRC2 interaction is only transiently enhanced at the onset of hypertrophy, while the downstream effect of this interaction seems to be long lasting in mouse transverse aortic constriction (TAC) model, inactivation of Chaer in the stressed heart may serve as a novel therapy for pathological remodeling in hypertrophic cardiomyopathy.

**Mhrt**

LncRNA Mhrt (Myosin Heavy Chain Associated RNA Transcripts) is the first example of lncRNA serving as chromatin remodelers and it can inhibit cardiac hypertrophy in pathological remodeling. Mhrt directly interacts with a histone acetylation factor named Brg1 and antagonizes the function of Brg1, which is activated as a chromatin-remodeling factor...
by stress to trigger aberrant gene expression and cardiomyopathies [18, 19]. The reciprocal Mhrt-Brg1 inhibition constitutes a negative feedback circuit in maintaining cardiac function. It is worth mentioning that Mhrt directly interacts with the helicase core of Brg1, implying Mhrt–helicase interaction may be a new mechanism by which lncRNA controls chromatin structure.

Chast

Chast (Cardiac Hypertrophy-Associated Transcript) is another pro-hypertrophic lncRNA which shows a cis-regulatory action on the gene located on the opposite strand, named Plekhm1. Chast was found to be specifically up-regulated in hearts from both mouse TAC model and aortic stenosis patients. Further studies mechanistically revealed that Chast negatively regulated Plekhm1, thus impeding cardiomyocyte autophagy and driving hypertrophy, while Chast expression was induced partly by the pro-hypertrophic transcription factor NFAT [20]. Further studies also showed that Chast had a strong effect on pathways associated with cardiac muscle morphogenesis, cardiomyopathies, and Wnt signaling by bioinformatic analysis while activation of Wnt signaling has been described as a cause of cardiac hypertrophy.

CHRF

Unlike the gene- or chromatin- regulation effect of other lncRNAs, CHRF (Cardiac Hypertrophy-Related Factor) was found to function as a sponge of miRNA. CHRF was found to be up-regulated in both hypertrophic mouse heart and human HF samples as well. In vitro studies mechanically indicated that CHRF played an anti-hypertrophic role by down-regulating miR-489 expression level while miR-489 had a pro-hypertrophic effect by increasing its target gene Myd88, which could down-regulate the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway [21].

Table 1. An overview of lncRNAs in cardiac remodeling

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TAC, Transverse aortic constriction; AngII, Angiotensin-2; ECs, Endothelial cells; VSMCs, Vascular smooth muscle cells; HUVECs, Human Umbilical Vein Endothelial Cells
LncRNA ROR has been reported to contribute to cardiac hypertrophic responses by acting as a sponge of miR-133, whose expression was increased while ROR was knocked down. In line with this, over-expression of miR-133 also prevented the elevation of ROR. The ROR/miR-133 negative feedback circle protected cardiomyocytes from phenylephrine-induced cardiomyocyte hypertrophy [22].

LncRNA H19 was firstly verified to be up-regulated in pathological cardiac hypertrophy and HF by the RNA-sequencing approach [15]. A gain-of-function and loss-of-function study performed in vitro further indicated that H19 protected cardiomyocytes from phenylephrine-induced hypertrophy through miR-675. Furthermore, CaMKIIα, a direct target of miR-675, partially mediated the effect of H19 on cardiomyocyte hypertrophy [23].

In an AngII-induced cardiac hypertrophy mouse model, lncRNA MIAT (Myocardial Infarction–Associated Transcript) was significantly increased and was necessary for cardiac hypertrophy. MIAT over-expression in H9C2 cells significantly reduced the miR-150 expression and MIAT acted as a sponge of miR-150 during the development of hypertrophy [24].

In addition to cardiac hypertrophy, post-ischemic remodeling, mainly manifested as the loss of cardiomyocytes and extracellular matrix remodeling, is also a common kind of cardiac remodeling. Ischemia injury releases reactive oxygen species and causes inflammation in the heart, leading to dysfunction of the energy metabolism of cardiomyocytes and finally resulting in autophagy, apoptosis or necrosis of the cardiomyocytes [25-27]. Loss of cardiomyocytes further contributes to systolic dysfunction and extracellular matrix remodeling. Several lncRNAs have been reported to participate in this process.

LncRNAs in post-ischemic remodelling, mainly cardiomyocytes loss and extracellular matrix remodeling

In addition to cardiac hypertrophy, post-ischemic remodeling, mainly manifested as the loss of cardiomyocytes and extracellular matrix remodeling, is also a common kind of cardiac remodeling. Ischemia injury releases reactive oxygen species and causes inflammation in the heart, leading to dysfunction of the energy metabolism of cardiomyocytes and finally resulting in autophagy, apoptosis or necrosis of the cardiomyocytes [25-27]. Loss of cardiomyocytes further contributes to systolic dysfunction and extracellular matrix remodeling. Several lncRNAs have been reported to participate in this process.

NRF and H19 in myocardial necrosis

LncRNA NRF (Necrosis-Related Factor) was able to induce cardiomyocyte necrosis and exacerbate ischemia/reperfusion injury in H2O2-treated cardiomyocytes and myocardial infarction mice by sponging miR-873 and its target gene RIPK1 (Receptor-Interacting serine/threonine-Protein Kinase 1)/RIPK3 (Receptor-Interacting serine/threonine-Pro-
tein Kinase 3) [28]. Besides NRF, H19 also inhibited H$_2$O$_2$-induced cardiomyocyte necrosis in vitro by serving as a sponge for miR-103/107 and it can further regulate FADD, a target gene of miR-103/107 [29].

**APF in myocardial autophagy**

LncRNA APF (Autophagy Promoting Factor) acted as an enhancer of the miR-188-3p/ATG7 axis both in vitro and in vivo, regulating autophagy. APF knockdown by APF siRNA exhibited a significant reduction in myocardial infarction sizes and the amelioration of myocardial function in the ischemia/reperfusion mouse hearts by targeting miR-188-3p and ATG7 [30].

**CARL, UCA and Mhrt in myocardial apoptosis**

LncRNA CARL (Cardiac Apoptosis-Related LncRNA) inhibited anoxia-induced mitochondrial fission and apoptosis in cardiomyocytes by impairing miR-539-dependent downregulation of PHB2 [31]. Besides that, lncRNA UCA1 (Urothelial Carcinoma-Associated 1) contributed to cardiomyocyte apoptosis by suppressing p27 expression in vitro [32]. Moreover, the Mhrt/Nrf2 pathway was also reported to participate in the regulation of doxorubicin-induced cardiomyocyte apoptosis [33].

**LncRNAs (n379599, n379519, n384640, n380433 and n410105) regulate ECM remodeling**

A deep sequencing and genome-wide transcriptome analysis of RNAs from cardiac samples of 15 patients with ischemic cardiomyopathy (ICM) and 15 controls identified 35 lncRNAs that displayed a strong positive correlation with extracellular matrix (ECM) protein-coding genes and further gain- and loss-of-function studies in cardiac fibroblasts identified that 5 of the 35 lncRNAs (n379599, n379519, n384640, n380433 and n410105) participated in the TGF-β pathway to modulate the expression of ECM genes and myofibroblast differentiation [34].

**LncRNAs in dysfunction of vascular endothelial cells and smooth muscle cells**

Vascular remodeling, mainly characterized by vascular endothelial cells (VECs) dysfunction and vascular smooth muscle cells (VSMCs) proliferation, is closely linked to numerous pathological processes such as arteriosclerosis, thrombus formation and plaque erosion, leading to acute myocardial infarction or chronic ischemic heart disease. LncRNAs have also been reported to participate in the regulation of vascular remodeling.

**MALAT1 and TGFB2-OT1 in VECs dysfunction**

LncRNA MALAT1 (Metastasis-Associated Lung Adenocarcinoma Transcript 1) has been found to be highly expressed in VECs and the expression of MALAT1 was significantly increased in proliferating VECs in hypoxic conditions. Additionally, MALAT1 improved blood flow in recovery and capillary density after hindlimb ischemia, implying the angiogenesis effect of MALAT1 in the ischemic heart [35].

LncRNA TGFB2-OT1 (TGFB2 Overlapping Transcript 1) was also shown to be involved in VEC dysfunction. TGFB2-OT1 in HUVECs could regulate the levels of miR-3960, miR-4459 and miR-4488 and then regulate the expression of the miRNA targets CERS1, NAT8L and LARP1, of which NAT8L and CERS1 may participate in autophagy by regulating mitochondrial function. Moreover, over-expression of TGFB2-OT1 could induce IL-6 and IL-8 production in VECs, which indicates that TGFB2-OT1 can trigger the inflammation linkage reaction [36].

**LincRNA-p21 and Lnc-Ang362 in VSMCs proliferation**

LincRNA-p21 has been reported to be dramatically down-regulated in an animal model of atherosclerosis, and it could repress VSMC cell proliferation and induce apoptosis in vitro...
using loss- and gain-of-function approaches. Furthermore, lincRNA-p21 was proven to be a promising transcriptional target of p53, which could feed back to enhance p53 transcriptional activity. Finally, lincRNA-p21 expression was significantly decreased in coronary artery tissues of coronary artery disease patients. These data elucidated an important role and a proper mechanism of lincRNA-p21 in angiogenesis [37]. Besides that, another study found that Inc-Ang362 was responsible for AngII-induced VSMC proliferation by targeting miR-221 and miR-222, implying that Inc-Ang362 is a therapeutic target of AngII-associated cardiovascular disease [38].

**LncRNAs in genetic cardiomyopathy**

Hypertrophic cardiomyopathy (HCM) represents a most common genetic cardiomyopathy and is also a leading cause of cardiac sudden death in young people [39]. Although a total of 1400 mutations in more than 10 genes have been identified as genetic variants responsible for HCM pathology, the predominant mechanisms underlying the pathogenesis of HCM is still unknown. Since lncRNAs are involved in a variety of pathologically processes of HCM, such as varying degrees of myocardial hypertrophy, cardiomyocyte disarrangement as well as interstitial fibrosis [40, 41]. The IncRNA expression profile of HCM patients has been screened by microarray and related pathologic pathways were further analyzed by Gene ontology (GO) enrichment and KEGG analysis. They found that IncRNAs were involved in the pathogenesis of HCM, mainly through pathways underlying ribosome and oxidative phosphorylation [42]. Furthermore, another study identified 2 specific mitochondrial IncRNAs named uc004cov.4 and uc022bqu.1 as potential useful clinical biomarkers for hypertrophic obstructive cardiomyopathy (HOCM) [43].

**Conclusions**

LncRNAs could regulate multiple pathological processes of cardiac remodeling, including myocardial hypertrophy, autophagy, necrosis, apoptosis, fibrosis as well as vascular cell apoptosis and proliferation. Therefore, to manipulate IncRNA expression levels through either the inhibition of disease-up-regulated lncRNAs or increasing disease-down-regulated lncRNAs represents novel therapeutic strategies for cardiac remodeling. To date, various studies have been successfully performed in modulating lncRNAs in animal models by loss- and gain-of-function approaches; however, due to the innate immunity response and unpredictable toxicity of lncRNAs, no clinical trial has been performed. Nevertheless, anti-miR-122 has come to a phase II clinical trial in the treatment of hepatitis C virus infection [44, 45], indicating the possibility of lncRNAs. In the future, with the development of sequencing and interfering technology, more lncRNAs associated with cardiac remodeling will be revealed, and more meaningful translational studies are highly needed.

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**Disclosure Statement**

The authors declare there are no conflicts of interest.
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