

REVIEW

The emerging roles of long noncoding RNAs in common cardiovascular diseases

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Long noncoding RNAs (lncRNAs) are defined as noncoding RNAs that are longer than ~200 nucleotides and lack protein-encoding capacity. It has been shown that lncRNAs are involved in multiple human diseases by regulating gene expression at various levels. However, studies of lncRNAs in the cardiovascular system are still in their infancy. A growing body of evidence suggests that lncRNAs are also involved in common cardiovascular diseases, including cardiac development, atherosclerosis, myocardial infarction, heart failure, hypertension and aneurysms. In this review, we summarize the current understanding of lncRNAs in common cardiovascular diseases in an effort to better elucidate the molecular mechanism of cardiovascular diseases and provide a basis for exploring new therapeutic targets in those diseases.

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INTRODUCTION

It is well known that protein-coding genes account for only ~2% of the human genomic sequences and the overwhelming majority of the genome is transcribed into non-(protein)-coding RNA (ncRNAs).^{1,2} These ncRNAs can be subdivided into short and long ncRNAs.² MicroRNAs, an example of short ncRNAs, have been widely reported to be involved in almost all known physiological and pathological process.³ The long ncRNAs (lncRNAs) are defined as ncRNAs that are longer than ~200 nucleotides and lack protein-encoding capacity.^{2,4} Usually, the expression levels of lncRNAs appear to be lower than those of protein-coding genes.⁴ There is increasing evidence that lncRNAs have a role in numerous cellular processes, including stem cell differentiation and cell cycle regulation.^{5,6}

It is known that lncRNAs expression occurs in a disease-, tissue- and developmental stage-specific manner.^{7,8} It has been reported that lncRNAs can regulate gene expression at various levels, including epigenetic regulation, transcriptional and posttranscriptional regulation, in contrast to microRNAs (miRNAs or miRs) that regulate gene expression only at the posttranscriptional level.^{7,8} The lncRNA ANRIL exerts epigenetic regulation by binding a subunit of polycomb-repressive complex 2 complex, which trimethylates histone H3 on lysine 27.⁹ Alu lncRNAs can directly repress the activity of Pol II and regulate transcription.¹⁰ H19 lncRNA exerts posttranscriptional regulation by serving as the primary miRNA of miRNA-675.¹¹ A subclass of lncRNAs has been shown to have enhancer-like activity and act over long distances.¹² lncRNAs have been demonstrated to be measurable in urine and blood, which shows that they can serve as biomarkers of diagnosis and prognosis for diseases. An example is lncRNA-PCA3

levels in the urine as a marker for prostate cancer.^{13,14} More importantly, lncRNA-LIPCAR in plasma was found to be down-regulated early after myocardial infarction (MI) but upregulated in later stages, implicating it as a new biomarker for heart failure (HF).¹⁵ Cardiovascular diseases are one of the leading causes of death worldwide, and the morbidity is increasing yearly.¹⁶ Although research on lncRNAs in the cardiovascular system is just beginning, it is already clear that lncRNAs will become new therapeutic targets in cardiovascular diseases in the near future. The lncRNAs reported to be involved in cardiovascular diseases are listed in Figure 1.

LNCRNAS AND HEART DEVELOPMENT

Heart development is a biologic process of the concurrent differentiation of multiple cell types and involved in the precise time and spatial regulation of gene expression.¹⁷ lncRNAs have been reported to regulate gene expression at various levels.^{7,8} Therefore, it is not surprising that lncRNAs, including Braveheart (Bvht), Fendrr and Kcnq1ot1, are implicated in heart development, as has been demonstrated by several recent studies.^{18–21}

Kcnq1ot1

Kcnq1 is a gene encoding a potassium channel. Its expression is initially imprinted but becomes biallelic during the development of the heart, whereas the lncRNA *Kcnq1ot1* is transcribed from the intron 11 of the *Kcnq1* gene in the antisense direction.¹⁸ In September 2012, Korostowski *et al.*,¹⁸ at the Temple University, reported that *Kcnq1ot1* also switches to biallelic expression—especially in the heart—in the same time frame as that of *Kcnq1*. They further found that the

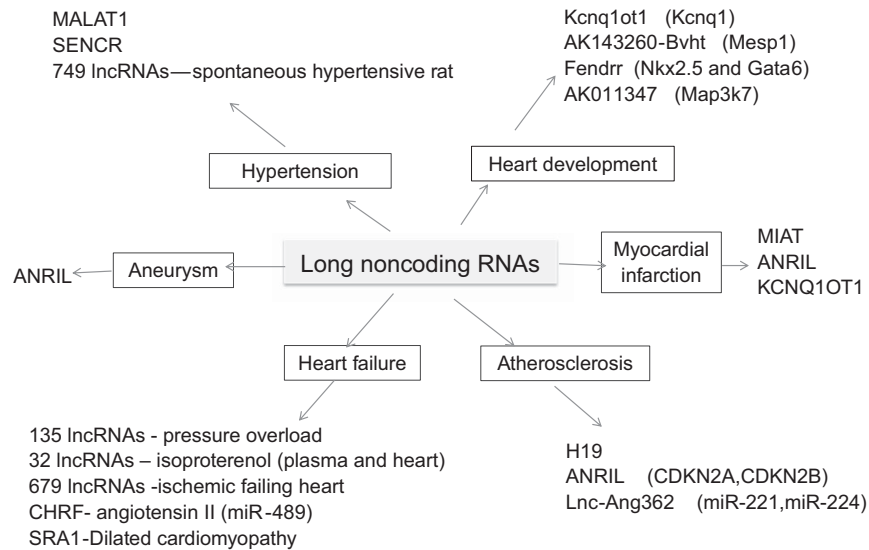


Figure 1 Long noncoding RNAs in cardiovascular diseases. A full color version of this figure is available at the *Hypertension Research* journal online.

imprinted *Kcnq1* level is not dependent on the transcription of *Kcnq1ot1* in early heart development but in the later stages of heart development. *Kcnq1ot1* has a role in regulating *Kcnq1* expression because *Kcnq1* levels significantly increased in K-term mice compared with wild-type mice at E16.5. Moreover, the increase in *Kcnq1* level is accompanied by an aberrant three-dimensional chromatin structure. Their studies show that *Kcnq1ot1* has a role in regulating *Kcnq1* levels in the embryonic heart by modulating chromatin flexibility and access to enhancers.¹⁸

Braveheart

In January 2013, Klattenhoff *et al.*,¹⁹ at the Massachusetts Institute of Technology, reported that a mouse-specific lncRNA, *AK143260* (called *Bvht*), located on mouse chromosome 18, is crucial for heart development. *Bvht* is expressed at early developmental stages in mouse embryonic stem cells and is also abundantly expressed in the adult heart.¹⁹ They found that depletion of *Bvht* in mouse embryonic stem cells results in the loss of beating cardiomyocytes and a failure to activate a network of cardiac transcription factors, including *Mesp1* (a marker of cardiovascular progenitor cells that ultimately determines all cell types in the heart) and *Nkx2.5*.¹⁹ They further found that *Bvht* acts upstream of *Mesp1*, by overexpression of *MesP1* rescued the depletion phenotype.¹⁹ They also observed that *Bvht* interacted with *SUZ12*, a component of polycomb-repressive complex 2 that catalyzes the trimethylation of histone H3 lysine 27 and mediates epigenetic regulation of cardiac commitment.¹⁹ Moreover, they found that isolated neonatal cardiomyocytes that were depleted of *Bvht* showed abnormal myofibrils and a- and b-myosin heavy chain expression was decreased, indicating a role of *Bvht* in maintaining cardiac fate in neonatal cardiomyocytes.¹⁹ Their study showed that *Bvht* has critical roles in cardiac development.

Fendrr

In January 2013, shortly after the *Bvht* report, Grote *et al.*,²⁰ at the Max Planck Institute for Molecular Genetics in Germany, reported that another mesoderm-enriched lncRNA, *Fendrr*, is essential for proper heart and body wall development in mice. *Fendrr* is a 2397-bp-long lncRNA that is transcribed divergently from the transcription factor gene *Foxf1*, which is specifically expressed in the lateral plate

mesoderm that gives rise to the heart and body wall muscles.²⁰ They found that deletion of *Fendrr* in mice resulted in embryonic lethality around E13.75, owing to myocardial dysfunction due to impaired development of the heart and body wall.²⁰ They also observed that deletion of *Fendrr* in mice increased the expression of *Nkx2.5* and *Gata6* (transcription factors for heart development) in the heart of E8.5 embryos.²⁰ Similar to *Bvht*, *Fendrr* epigenetically regulates some transcription factors key for cardiac development, including *GATA-6*, *NKX2-5*, *FOXF1*, *TBX3*, *IRX3* and *PITX2*. However, in contrast to *Bvht*, loss of *Fendrr* increased the trimethylation status of H3K4me3 at the promoters of *Nkx2.5* and *Gata6*, whereas the methylation status of H3K27me3 showed no significant change.²⁰ Their study showed that *Fendrr*, like *Bvht*, is essential for heart development by epigenetically regulating the expression of cardiac transcription factors.

AK011347

In December 2013, Zhu *et al.*,²¹ at the Nanjing Medical University in China, described the lncRNA profile of the fetal mouse heart at three key time points (E11.5, E14.5 and E18.5). They found that 1237 lncRNAs showed consistent expression changes more than twofold between the three time points. Their analysis indicated that *AK011347* might be involved in heart development through the target gene, *Map3k7*.²¹

LNCRNAs AND ATHEROSCLEROSIS

Atherosclerosis, the common pathologic basis of numerous cardiovascular and cerebrovascular diseases, is a complex chronic inflammatory process.²² MicroRNAs have been demonstrated to be widely involved in the initiation and progression of atherosclerosis and to serve as novel diagnostic and prognostic tools for atherosclerosis.²³ Several studies have recently begun to reveal the roles of lncRNAs in the development of atherosclerosis.

H19

Proliferation of vascular smooth muscle cells (VSMCs) is known to be important in the development of atherosclerosis. In June 2009, Li *et al.*,²⁴ at Sichun University in China, reported that homocysteine (50–1000 μM) induced the proliferation of VSMCs and increased the expression of the first reported lncRNA *H19*. They found that the

increase in *H19* was epigenetically regulated by hypomethylation of the sixth CTCF-binding site upstream of *H19* in human umbilical VSMCs.²⁴ Their study indicated that *H19* might have a role in the pathologic mechanism of atherosclerosis.

ANRIL

ANRIL is the abbreviation of the lncRNA (antisense noncoding RNA in the *INK4* locus) encoded by chromosome 9p21 locus, whose expression has been correlated with atherosclerosis severity.^{25–27} In February 2013, Congrains *et al.*,²⁶ at the Osaka University in Japan, reported that *ANRIL* knockdown with small interfering RNA in aortic VSMCs decreased the cell proliferation, and different *ANRIL* small interfering RNA targeting to exons 1 and 19 resulted in significant alterations in the expression of human atherosclerosis-related pathways genes.²⁷ In June 2013, Motterle *et al.*,²⁸ at the Queen Mary University of London, reported that the 9p21 risk genotype was associated with reduced expression of *CDKN2A*, *CDKN2B* and *ANRIL* in primary cultures of VSMCs and also related to VSMC proliferation. *ANRIL* expression was shown to be correlated with the expression of *CDKN2A* and *CDKN2B*.²⁸ In July 2013, Holdt *et al.*,²⁵ at the University Leipzig in Germany, reported that overexpression of *ANRIL* increased cell adhesion, promoted cell proliferation and attenuated cell apoptosis, which are all essential mechanisms in the development of atherosclerosis.²⁵ They further found that the promoter regions of target genes of *ANRIL* were rich in Alu motifs, which are essential for transregulation and proatherogenic functions.²⁵ Those studies demonstrated the role of *ANRIL* in the development of atherosclerosis.

lnc-Ang362

In July 2013, Leung *et al.*,²⁹ from Beckman Research Institute of City of Hope, reported that they identified 5 previously annotated lncRNAs and 24 novel lncRNAs (19 upregulated and 5 downregulated) in rat VSMCs that were treated by with angiotensin II (Ang II) ($0.1 \mu\text{mol l}^{-1}$) for 3 h. Moreover, the regulation of Ang II on the lncRNAs (*lnc-Ang219*, *lncRNA-Ang249*, *lncRNA-Ang362*, *lncRNA-Ang 162* and *lncRNA-Ang 112*) was time-dependent.²⁹ They also found that Ang II increased the expression of two proximal miRNAs of *lncRNA-Ang362*, miR-222 and miR-221, which have been implicated in VSMC proliferation.²⁹ They found that miR-221 and miR-222 are cotranscribed with *lnc-Ang362*, and small interfering RNA knockdown of *lnc-Ang362* in VSMC reduced the expression of the two miRNAs and VSMC proliferation.²⁹ Their studies revealed that *lncRNA-Ang362* has a role in VSMC proliferation by acting as a host transcript for the two miRNAs.

LNCRNAS AND MI

MI is a common lethal cardiovascular disease, especially in developed countries. Despite recent progress, the molecular mechanism of MI has still not been completely elucidated and needs further study. miRNAs have been demonstrated to be involved in the initiation and progression of MI and may serve in novel miR-based therapeutic approaches.³⁰ However, the roles of lncRNAs in the development of MI have not been widely studied. Several single-nucleotide (SNP) analyses have revealed an association between lncRNAs and MI.

MI-associated transcript

In October 2006, Ishii *et al.*,³¹ in Japan, reported that they had identified six SNPs in *MIAT* (MI-associated transcript), an lncRNA that confers susceptibility to MI. They found that the *MIAT* gene was located on chromosome 22q12.1 and consisted of five exons.³¹ Moreover, *in vitro* functional analyses revealed that one SNP in exon

5(A11741G) of the *MIAT* gene increased the transcriptional level of *MIAT* more than the normal A allele, whereas five other SNPs did not show transcriptional differences.³¹ Their results indicated that the altered expression of *MIAT* by the SNP might have a role in the pathogenesis of MI.

ANRIL

The human chromosome 9p21.3 region, encoding *ANRIL*, *CDKN2A* and *CDKN2B*, is one of the most validated regions associated with cardiovascular disease, and SNPs in Ch9p21.3 are strongly associated with MI.^{26,27,30} In February 2013, Johnson *et al.*³² reported in the Framingham heart study that they had sequenced the 9p21.3 region of patients with MI and subclinical coronary artery disease and controls, and found a strong association between several *ANRIL* SNP loci and MI. In February 2013, Ahmed *et al.*,³³ in Pakistan, reported that in a northern Pakistan population, one SNP locus of *ANRIL* (rs1333049: C>G) was significantly associated with MI, and the association with MI was confirmed in both males and females.

KCNQ1OT1

In September 2014, the level of the lncRNAs hypoxia-inducible factor 1A antisense RNA 2, member 1 opposite strand/antisense transcript 1 (*KCNQ1OT1*), and metastasis-associated lung adenocarcinoma transcript 1 were shown to be higher in the peripheral blood cells of patients with MI than in those of healthy volunteers.³⁴ Patients with ST-segment-elevation MI had lower levels of *ANRIL*, *KCNQ1OT1*, *MIAT* and metastasis-associated lung adenocarcinoma transcript 1, when compared with patients with non-ST-segment-elevation MI.³⁴

LNCRNAS AND HF

HF is the final stage of many cardiovascular diseases, including MI, cardiac hypertrophy (HY) and hypertension, and it is a common cause of death in developed countries. The molecular mechanism of HF is known to involve the reprogramming of gene expression.³⁵ Although it has been confirmed that miRNAs have a role in the development of HF,³⁶ the role of lncRNAs in HF is just beginning to be studied.

Sequencing identified 135 lncRNAs, including *H19*, in failing murine heart

In October 2011, Lee *et al.*,³⁷ at the University of California Los Angeles, reported the sequencing analysis of normal and failing murine hearts induced by pressure overload. They found that 15 lncRNAs were differently expressed between HY and sham-HY (HY stage, 1 week after transaortic constriction), whereas 135 lncRNAs were differently expressed between HF and sham-HF (HF stage, 8 weeks after transaortic constriction).³⁷ Moreover, the first reported lncRNA, *H19*, was reported to be significantly upregulated in the HF stage, compared with sham controls.³⁷ Their studies showed that those lncRNAs might be involved in the development of cardiac failure.

lncRNAs in the plasma and heart during murine HF

In October 2013, Li *et al.*,³⁸ at the Peking University of Basic Medical Sciences, reported that they had identified a distinct expression pattern of lncRNAs in the heart and plasma when HF was induced by subcutaneous injection of isoproterenol in a mouse model.³⁸ Their microarray analysis revealed that 518 lncRNAs were upregulated and 908 were downregulated in the heart of an HF model.³⁸ Quantitative PCR confirmed the increase of five lncRNAs (*Ak137898*, *AK049728*, *AK044955*, *ENSMUST00000142855* and *ENSMUST00000127230*) and decrease of five lncRNAs (*ENSMUST00000143888*, *UC.115*, *AK139454*, *NR028277* and *NR036631*) in failing hearts.³⁸ The

microarray also revealed aberrant lncRNAs during HF in the plasma (1619 up and 1582 down) and in the whole blood (1139 up and 1506 down).³⁸ QPCR confirmed lncRNAs changes in whole blood (up: *ENSMUST00000120957*, *EMSMUST00000117393*, *AK038798*; down: *AK036863*, *UC.184*, *ENSMUST00000167632*) and in the plasma (up: *AK020791*, *ENSMUST00000160947*, *ENSMUST00000119855*, *AK139989* and *AK153778*; down: *ENSMUST00000127429*, *ENSMUST0000022467*, *ENSMUST00000117372*, *NR033575*, *ENSMUST0000041159* and *AK143260-Bvht*).³⁸ They found that 32 lncRNAs were expressed in all three samples (heart, whole blood and plasma) during HF, indicating the possibility of using the levels of these lncRNAs as new biomarker of HF.³⁸

Deep sequencing identified lncRNAs in failing human heart

In January 2014, Yang *et al.*,³⁹ at the Washing University Medical School, reported their deep sequencing-based transcriptome analysis of ischemic (ICM) human failing heart. RNA sequencing found 18 480 lncRNAs, including 113 novel ones, in human hearts.³⁹ Moreover, 679 lncRNAs were differentially expressed in ICM human failing hearts and 570 lncRNAs were differentially expressed in non-ICM human failing hearts, some of which could be ameliorated by a left ventricular assisted device.³⁹ They further reported that the expression signature of lncRNAs, but not miRNA or mRNA, distinguished ICM cardiopathy and non-ICM cardiopathy.³⁹ Their studies were the first reports of aberrant lncRNAs in failing human hearts.

CHRF

Sustained cardiac HY is often accompanied by maladaptive cardiac remodeling, leading to decreased compliance and increased risk for HF. Maladaptive HY is considered a therapeutic target in patients with HF.⁴⁰

In February 2014, Wang *et al.*,⁴⁰ at the Institute of Zoology in China, reported that an lncRNA, *CHRF* (cardiac HY related factor), was involved the pathologic process of cardiac HY induced by Ang II by targeting miR-489. Specifically, they found that *CHRF* decreased the level of miR-489 by serving as an endogenous sponge of miR-489.⁴⁰

lncRNAs and dilated cardiomyopathy

Human dilated cardiomyopathy, characterized by cardiac chamber dilation and impaired systolic function, often leads to congestive HF. Genetic factors have a role in the etiology and pathogenesis of dilated cardiomyopathy. In March 2009, Friedrichs *et al.*,⁴¹ at the University Hospital Heidelberg in Germany, reported that they had identified a genomic region (5q31.2-3) harboring the risk alleles for dilated cardiomyopathy in three Caucasian populations. The steroid receptor RNA activator (*SRA*) gene generates both steroid receptor RNA activator protein and several noncoding *SRA* transcripts. Knockdown of *SRA1* in this region resulted in myocardial contractile dysfunction in the ventricular heart chamber of zebrafish, revealing an association between *SRA1* and dilated cardiomyopathy.⁴¹

lncRNAs in cardiac fibrosis

Cardiac fibrosis is the final event of myocardial interstitial remodeling, and the Ang II-induced proliferation of cardiac fibroblasts has a pivotal role in the development of cardiac fibrosis.⁴² We recently reported that treatment of cardiac fibroblasts with Ang II (100 nM) for 24 h induced substantial changes in the expression of lncRNAs and protein-coding genes associated with fibrosis.⁴³ Moreover, we found that Ang II dynamically downregulated the expression of lncRNA-*NR024118* and cyclin-dependent kinase inhibitor 1C in cardiac fibroblasts, indicating a potential role of *NR024118* in the proliferation of cardiac fibroblasts and cardiac fibrosis.⁴³ The Ang II type 1 receptor

has been widely shown to reverse the effects of Ang II, including the development of hypertension.^{44,45} Recently, we found that the decrease of *NR024118* induced by Ang II was AT1-receptor-dependent (not published). The expression of lncRNAs was closely regulated by Ang II, indicating their roles in cardiac fibroblasts and cardiac fibrosis.

LINC RNAs AND HYPERTENSION

Hypertension is one of the most common cardiovascular diseases, but its molecular mechanism has yet to be completely clarified. Oxygen free radicals have been considered to be closely associated with vascular endothelial function and pregnancy-induced hypertension.⁴⁶ Recently, several studies have revealed the roles of lncRNAs in the development of hypertension.⁴⁷⁻⁴⁹ *MALAT1* was found to regulate endothelial cell function and vessel growth,⁴⁷ and *SENCR* was identified as a new vascular cell-rich lncRNA associated with smooth muscle cell phenotype.⁴⁸ In November 2014, a paper reported that 749 differentially expressed lncRNAs were identified between Dahl salt-sensitive *vs.* spontaneously hypertensive rats.⁴⁹ However, the roles of lncRNAs in hypertension are only just beginning to be studied and require further, deeper studies.

LINC RNAs AND ANEURYSMS

Aneurysms are characterized by pathological widening of the vessel and thinning of the vessel wall, which often occur in large arteries and lead to death after rupture. Recently, microRNAs have been shown to be involved in aneurysm formation.^{50,51} However, the role of lncRNAs in aneurysms is just beginning to be studied. Several studies in 2008 and 2011 reported that genome-wide association studies identified *ANRIL* as a genetic-susceptibility locus associated with intracranial aneurysm and abdominal aortic aneurysm.^{52,53} In November 2012, Foroud *et al.*, in India, reported that their genome-wide association studies confirmed the role of *ANRIL* as a risk factor in intracranial aneurysms.⁵⁴ However, the mechanism of *ANRIL* in aneurysms is still unclear and needs to be clarified by functional experiments.

CONCLUSION

Research on lncRNAs in the cardiovascular system is still in its infancy. The reported studies show that lncRNAs are widely involved in cardiovascular diseases, although the mechanisms of action of only a few lncRNAs have been elucidated, and most have only been associated with cardiovascular diseases, without functional evidence. However, we believe that in the near future more lncRNAs will be implicated in the development of cardiovascular diseases, and their molecular mechanisms will be the basis for exploring new therapeutic targets.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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