Research Article
Open Access
CrossMark

Regulatory long noncoding RNAs in cardiovascular development and congenital heart defects

Abstract

Long noncoding RNAs (lncRNAs) have emerged as potent regulators of cardiac development and can drive transcriptome programming, affecting all aspects of gene regulation. The functional properties of lncRNAs are notoriously diverse and have been mechanistically challenging. Thus, focused an efforts for lncRNA annotation and functional interrogation in these contexts is highly required. Herein, we discuss current approaches for identifying putative regulatory lncRNAs for the mechanistic investigation of their function in cardiovascular development and congenital heart defects (CHDs).

Keywords: long noncoding RNA, epigenetics, transcriptome, RNA-seq, congenital heart defects, LncRNA

Volume 6 Issue 1 - 2023

Marlin Touma[1,2,3,4]
[1]Department of Pediatrics, University of California, USA
[2]Neonatal/Congenital Heart Laboratory, University of California, USA
[3]The Molecular Biology Institute, University of California, USA
[4]Eli and Edythe Stem Cell Institute, University of California, USA

Correspondence: Marlin Touma, MD, Department of Pediatrics, David Geffen School of Medicine, University of California Los Angeles, USA, Email mtoum@mednet.ucla.edu

Received: June 05, 2023 | **Published:** June 14, 2023

Abbreviations: CHDs, congenital heart defects; PRC2, polycomb repressive complex 2; CDKN2B, cyclin dependent kinase inhibitor 2b; SUZ12, polycomb repressive complex 2 subunit; MIAT, myocardial infarction associated transcript

Introduction

CHDs affect 1% of live births and are a major source of childhood morbidity and mortality.[1] Heart development and function are controlled by an intricate gene expression network that is precisely regulated by genetic and epigenetic mechanisms, including lncRNAs.[1–7] The recent discoveries of regulatory lncRNAs have led to increased interest in deciphering their functional roles in heart development and CHDs.[7–12] Growing resources of deep RNA-sequencing datasets have uncovered thousands of lncRNAs enriched in the cardiac transcriptome, dynamically transcribed during cardiac development, and differentially regulated in cardiovascular diseases.[6,7] Eventually, cutting-edge mechanistic experiments have challenged the functional mysteries of lncRNA, leading to highlighting novel roles of lncRNAs in cardiac development and disease. Bvht (Braveheart) was found to regulate the core cardiac transcription during cardio genesis, acting in trans by binding with SUZ12, a core component of the polycomb repressive complex 2(PRC2).[9] Fendrr (fetal-lethal non-coding developmental regulatory RNA) was indispensable for heart development.[10] More recently, Ppp1r1b-lncRNA was identified as a critical, functionally conserved, epigenetic regulator of myogenic differentiation by interacting with EZH2, the catalytic subunit of PRC2[11] several other lncRNAs have been implicated in cardiovascular disease, including MIAT (myocardial infarction associated transcript),[12] ANRIL (CDKN2B antisense RNA 1),[13] and Chaer (cardiac hypertrophy associated epigenetic regulator 1).[14]

The proven links between lncRNAs and heart development[6-16] indicate that lncRNAs comprise core transcriptional regulatory circuits with key cardiac transcription factors that are essential for cardio genesis. Furthermore, lncRNAs can potentially mediate pathological responses of the cardiovascular system to hemodynamic and environmental stress factors. However, knowledge about their contribution to the pathogenesis of CHDs remains limited. Therefore, dissecting the functional roles of this emerging layer of transcriptome regulation is a critical task that may lead to significant knowledge of CHDs pathogenesis and important translational applications for infants with CHDs.

Biochemical properties of the regulatory LncRNAs

LncRNAs are arbitrarily defined as RNA species of more than 200 nucleotides in length lacking a functional open reading frame (ORF). Like messenger RNAs (mRNA), most lncRNAs are transcribed by RNA polymerase II (RNAP II), 5' capped, poly a tailed, and subjected to alternative splicing.[17–19] Compared to protein-coding genes, lncRNAs are remarkably less conserved at the sequence level and predominantly expressed at lower levels. LncRNAs are commonly classified based on their genomic position and orientation compared in reference to the closest protein-coding gene.[17–19] Most lncRNAs are intergenic, located between two protein-coding genes without intersecting with the coding sequence. A subclass of intergenic lncRNA are the enhancer- associated lncRNAs, of which many are bidirectional, lacking a poly A tail, and transcribed at a low copy number. The second class is intragenic lncRNAs, which can be intronic, located within the intronic sequences of a protein- coding gene, but more commonly, intergenic lncRNAs overlap with protein-coding sequences, spanning one or more gene. Finally, bidirectional lncRNAs are transcribed in the close vicinity of a protein-coding gene on the opposite strand.[20,21] In each class, lncRNAs can be transcribed from the sense or the antisense strand. A subclass of intragenic overlapping antisense lncRNAs is the natural antisense transcripts (NATs), which complement and regulate their opposite coding genes expression.

Biological diversity of regulatory LncRNAs

The lncRNAs have increasingly expanded the functional complexity of transcriptome, affecting all aspects of gene regulation, including mRNA expression, epigenetic modification, and post□ transcriptional processing (Figure 1).[22] Compared to mRNAs, most lncRNAs are expressed at relatively lower levels and are commonly associated with chromatin-modifying complexes or splicing machinery. In some instances, lncRNAs are expressed at levels comparable to mRNAs and those are likely to function as structural scaffolds for nuclear domains. Indeed, the subcellular localization of a given lncRNA (cytoplasmic versus nucleus) may suggest distinct roles in regulating specialized cellular functions in these compartments.[23,24]

Importantly, certain subclasses of lncRNA may serve functions. For example, the natural antisense transcripts (NATs) are most likely to repress the expression of their complementary counterpart,[25] which in most cases lies directly in the opposite strand at the same genomic locus. The other classes, however, have diverse functions derived from their ability to fold into complex secondary and tertiary structures. LncRNAs can partially hybridize with DNA leading to the formation of scaffold structures for RNA processing, histone modification, protein binding, and to a minimal extent, protein-coding. LncRNAs may interact with proteins, modifying their structure, dictating their localizations, and executing their functions. Unlike microRNAs, which uniformly carry out repressive functions, lncRNAs may induce or suppress the expression of their target genes by employing a wide range of molecular mechanisms in cis or in trans.[22,23,26] LncRNAs can be transcribed across the promoter region of a given protein-coding gene and directly interfere with transcription factor binding, thereby affecting cellular differentiation and developmental decisions as exemplified by regulating homeotic HOX gene expression pattern and special localization.[27] However, direct interaction with promotor may not be necessary for lncRNA function, as certain lncRNAs, such as HOTAIR (HOX antisense intergenic RNA), can induce histone modifications to repress transcriptional initiation of overlapping protein-coding genes by recruiting the polycomb chromatin repressive complex PRC2 leading to transcriptional repression.[28] Other examples, including the prototypical lncRNA XIST (X inactive□specific transcript), can regulate gene silencing or activation indirectly by guiding chromatin- modifying enzymes and associated lncRNAs to their target genes.[29–30] Alternatively, lncRNA transcription can be transcribed from multiple sites in a stepwise manner upstream of a promoter, causing a chromatin- opening cascade, which proceeds progressively toward the mRNA transcription start site acting leading to transcription initiation.[30] Moreover, lncRNAs can influence RNA processing by interacting with RNA-binding proteins[31] at the post□transcriptional editing, including the splicing level.[20] LncRNAs can also serve as precursors to generate small interfering RNAs (siRNA) or act as a sponge for clearing siRNAs and titrating their activity.[32]

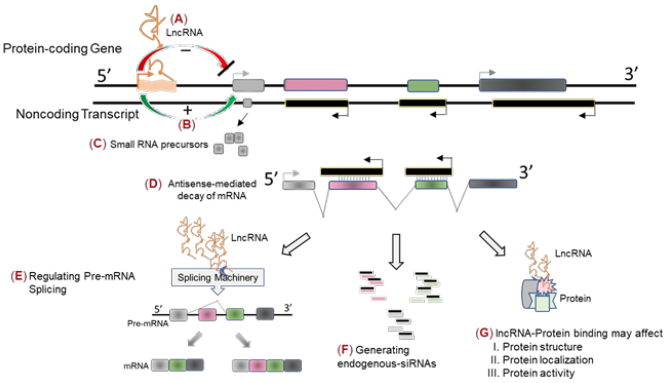

Figure 1 Functional diversity of long noncoding RNA (lncRNA).

Schematic illustration of the diverse functions of lncRNAs, including chromatin interaction and histone modification that may lead to transcriptional inhibition (A), or transcriptional activation (B). Regulating mRNA processing via antisense mediated decay (D) and inducing mRNA splicing machinery via recruiting RNA binding proteins and other cofactors (E). LncRNAs can also lead to the generation endogenous siRNA (F). LncRNA may bind certain proteins, altering their structure, cellular localization, and modifying theiractivities.

Functional assertation of regulatory LncRNA

Due to the lack of clear annotation and sequence conservation, functional characterization of lncRNA has been notoriously challenging. Navigating the most difficult issue in lncRNA biology, which is to identify candidate lncRNAs with putative function, is a very critical task to depict their biological function. Several criteria can be used for identifying putative regulatory lncRNA candidates and prioritizing them for functional studies (Table 1). As previously stated, the subcellular localization of a given lncRNA may allow a rough prediction of its potential functionality. Nuclear lncRNAs may influence transcriptional outputs through epigenetic modifications, interactions with transcription factors, and affecting nuclear export of mRNA. In contrast, cytoplasmic lncRNAs may function by influencing mRNA stability, splicing, and translation initiation, acting as competing endogenous RNAs, or influencing post-translational modification.[33] Tissue and cell specificity are other key features in lncRNA expression. Most regulatory lncRNAs display tissue and cell specificity that has surpassed that of protein-coding transcripts.[34–38] In the heart, lncRNA specificity has been shown most clearly during cardiomyocyte progenitor differentiation and early cardiogenesis,[39] suggesting that lncRNA transcription is more intimately linked with primordial tissue and plastic cell phenotypes. Furthermore, evidence of a correlated expression with developmental or physiological indices or cellular state may support functional lncRNAs. Indeed, during heart development, the regulatory lncRNAs tend to be tightly regulated even within a small interval, potentially indicating a driving role of lncRNA in transcriptome programming at a higher order in a highly precise manner in response to developmental cues. Remarkably, the dynamic nature of lncRNA expression in parallel to protein-coding genes may suggest functional relevance (guilt-by-association). Moreover, the association with specific chromatin states, such as identifying chromatin marks and interaction with histone modification enzymes at gene promoters, may indicate epigenetic regulation by lncRNA in response to developmental environmental stimuli. Most importantly, the existence of a human ortholog or conserved function in more than one species represents top indicators of lncRNA function.

Table 1 Prediction Criteria for functional regulatory lncRNAs

Subcellular localization
Exhibiting tissue specificity
Exhibiting cellular specificity
Exhibiting developmental regulation
Correlated expression with protein-coding genes in response to developmental Cues or physiological stimuli
Association with chromatin modulation marks
Interaction with Histone modification enzymes at gene prompters
Identifying a human orthologues with conserved function in two or more species

These criteria provide foundations to identify putative regulatory lncRNAs and pave the way to investigate the underlying mechanisms. At the mechanistic and technical levels, in addition to using RNA interference and modified antisense oligonucleotide approaches, new approaches for targeting lncRNAs are needed to overcome several challenges. For example, a fraction of the newly discovered cardiac lncRNAs act in cis as epigenetic regulators. Particularly the enhancer-associated lncRNAs that function primarily at their endogenous site of production, modulating the nascent transcript. Therefore, identifying chromatin marks and histone modifications at gene promoters and enhancers and dissecting the downstream molecular mechanisms requires employing sophisticated molecular genetics tools, such as chromatin isolation by RNA purification (ChIRP)[11,40] and capture hybridization analysis of RNA targets (CHART).[41] Furthermore, advanced genome-editing strategies can serve to modify cardiovascular lncRNA expression in vivo. New techniques

Citation: Touma M. Regulatory long noncoding RNAs in cardiovascular development and congenital heart defects. *Int J Mol Biol Open Access.* 2023;6(1):14–17. DOI: 10.15406/ijmboa.2023.06.00147

to generate targeted mutations in the genome, such as Cas9/CRISPR systems will immensely benefit lncRNA research. Moreover, hiPSCs (human induced pluripotent stem cells) can provide powerful platforms for future studies designed to establish the mechanisms of human cardiac lncRNA and their potential translational applications as putative disease modifiers, diagnostic biomarkers, and therapeutic targets in CHDs.

Conclusion

Human cardiac lncRNAs are key regulators of heart development and potential contributors to CHDs. However, the mechanistic functions of the vast majority of predicted regulatory lncRNA candidates remain to be fully revealed. Rigorous investigation of their regulatory function and potential applications as putative disease modifiers, diagnostic biomarkers, and therapeutic targets may open new paradigms and novel approaches to improve outcomes of infants with CHDs. Complementary and multidisciplinary research forum, from deep sequencing platforms and bioinformatics tools to comprehensive functional interrogation methods in novel model systems of heart development and CHDs, is necessary to circumvent these challenges in lncRNA biology in general, and particularly to elucidate the roles of lncRNAs in the context of complex developmental perturbations such as CHDs.

Acknowledgments

None.

Funding statement

This work was supported by grants from NIH/NHLBI 1R01 HL153853-01 (M.T.) and the 2022 UCLA Academic Senate Faculty Research Fund (M.T.), and the Children's Discovery Institute and Seed Grant (M.T.).

Author contributions

M.T. Conceived and designed the manuscript and completed manuscript writing and editing.

Conflicts of interest

The author declared there are no conflicts of interest towards this article.

References

1. Touma M, Reemtsen B, Halnon N, et al. A path to implement precision child health cardiovascular medicine. *Front Cardiovasc Med*. 2017;4:36.
2. Muntean I, Toganel R, Benedek T. Genetics of congenital heart disease: past and present. *Biochem Genet.* 2017;55(2):105–123.
3. Srivastava D, Olson EN. A genetic blueprint for cardiac development. *Nature.* 2000;407(6801):221–226.
4. Olson EN. Gene regulatory networks in the evolution and development of the heart. *Science*. 2006;313(5795):1922–1927.
5. Garry DJ, Olson EN. A common progenitor at the heart of development. *Cell.* 2006;127(6):1101–1104.
6. Touma M, Kang X, Zhao Y, et al. Decoding the long noncoding RNA during cardiac maturation:a roadmap for functional discovery. *Circ CardiovascGenet*. 2016;9(5):395–407.
7. Matkovich SJ, Edwards JR, Grossenheider TC, et al. Epigenetic coordination of embryonic heart transcription by dynamically regulated long noncoding RNAs. *Proc Natl Acad Sci USA*. 2014;111(33):12264–12269.
8. Wang Z, Wang Y. Dawn of the Epi–LncRNAs: new path from Myheart. *Circ Res.* 2015. 116(2):235–236.
9. Klattenhoff CA, Scheuermann JC, Surface LE, et al. Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. *Cell.* 2013;152(3):570–583.
10. Grote P, Wittler L, Hendrix D, et al. The tissue–specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. *Dev Cell.* 2013;24(2):206–214.
11. Kang X, Zhao Y, Van Arsdell G, et al. Ppp1r1b–lncRNA inhibits PRC2 at myogenic regulatory genes to promote cardiac and skeletal muscle development in mouse and human. *RNA*.2020;26(4):481–491.
12. Ishii N, Ozaki K, Sato H,et al. Identification of a novel non– coding RNA, MIAT, thatconfers risk of myocardial infarction. *J Hum Genet.* 2006;51(12):1087–1099.
13. Fang J, Pan Z, Wang D, et al. MultipleNoncoding ANRIL transcripts are associated with risk of coronary artery disease: a promising circulating biomarker. *J Cardiovasc Transl Res*. 2021;14(2):229–237.
14. Wang Z, Zhang XJ, Ji YX, et al. A long non–coding RNA defines an epigenetic checkpoint in cardiac hypertrophy. *Nat Med.* 2016;22(10):1131–1139.
15. Han X, Zhang J, Liu Y, et al. The lncRNA Hand2os1/Uph locus orchestrates heart development through regulation of precise expression of Hand. *Development.* 2019;146(13):dev176198.
16. Anderson KM, Anderson DM, McAnally JR, et al. Transcription of the non– coding RNA upper hand controls Hand2 expression and heart development. *Nature.* 2016;539(7629):433–436.
17. Lee JH, Chen Gao, Peng G, et al. Analysis of transcriptome complexity through RNAsequencing in normal and failing murine hearts. *Circulation research.* 2011;109(12):1332–1334.
18. Wang J, Zhang J, Zheng H, et al. Mouse transcriptome: neutral evolution of 'noncoding'complementary DNAs. *Nature.* 2004;431(7010):1 p.
19. Derrien T, Vaysse A, Andre C, et al. Annotation of the domestic dog genome sequence: finding the missing genes. *Mamm Genome.* 2012;23(1–2):124–131.
20. Kellis M, Wold B, Snyder MP, et al. Defining functional DNA elements in the human genome. *Proc Natl Acad Sci USA.* 2014;111(17):6131–6138.
21. Cabili MN, Trapnell C, Goff L, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev.* 2011;25(18):1915–1927.
22. Mercer TR, Dinger ME, Mattick JS. Long non–coding RNAs: insights into functions. *Nat Rev Genet.* 2005;10(3):155–159.
23. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell.* 2011;43(6):904–914.
24. Brockdorff N, Ashworth A, Kay GF, et al. The product of the mouse Xist gene isa 15 kb inactive X–specific transcript containing no conserved ORF and located in the nucleus. *Cell.* 1992;71(3):515–526.
25. Ritter O, Haase H, Schulte HD, et al. Remodeling of the hypertrophied human myocardium by cardiac bHLH transcription factors. *J Cell Biochem.* 1999;74(4):551–561
26. Mercer TR, Dinger ME, Sunkin SM, et al. Specific expression of long noncoding RNAs in the mouse brain. *Proc Natl Acad Sci USA.* 2008;105(2):716–721.
27. Rinn JL, Kertesz M, Wang JK, et al. Functional demarcation of active and silentchromatin domains in human HOX loci by noncoding RNAs. *Cell.* 2007;129(7):1311–1323.
28. Zhao J, Sun BK, Erwin JA, et al. Polycomb proteins targeted by a short repeat RNA to themouse X chromosome. *Science.* 2008;322(5902):750–756.

29. Ogawa Y, Sun BK, Lee JT. Intersection of the RNA interference and X–inactivation pathways. *Science.*

1. 2008;320(5881):1336–1341.

30. Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. *NatStruct Mol Biol.* 2013;20(3):300–307.

31. Wang X, Arai S, Song X, et al. Induced ncRNAs allosterically modify RNA–binding proteins in cis to inhibit transcription. *Nature. 2008;*454(7200):126–130.

32. Feng J, Bi C, Clark BS, et al. The Evf–2 noncoding RNA is transcribed from the Dlx–5/6 ultraconserved region and functions as a Dlx–2 transcriptional coactivator. *Genes Dev.* 2006;20(11):1470–1484.

33. Ravasi T, Suzuki H, Pang KC, et al. Experimental validation of the regulated expression of large numbers of non–coding RNAs from the mouse genome. *Genome Res.* 2006;16(1):11–19.

34. Gan J, Sonntag HJ, Tang MK, et al. Integrative analysis of the developing postnatal mouseheart transcriptome. *PLoS One.* 2015;10(7):e0133288.

35. Kurian L, Aguirre A, Sancho–Martinez I, et al. Identification of novel long noncoding RNAs underlying vertebrate cardiovascular development. *Circulation.* 2015;131(14):1278–1290.

36. Paralkar VR, Mishra T, Luan J, et al. Lineage and species–specific long noncoding RNAs during erythro– megakaryocytic development. *Blood.* 2014;123(12):1927–1937.

37. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, et al. Simple combinations of lineage–determining transcription factors prime cis–regulatory elements required for macrophage and B cell identities. *Mol Cell.* 2010;38(4):576–589.

38. Yan L, Tang M, Guo H, et al. Single–cell RNA seq profiling of human preimplantation embryos and embryonic stem cells. *Nature Structural and Molecular Biology.* 2013;20(9):29.

39. Han P, Li W, Lin CH, Yang J, Shang C, *et al*. A long noncoding RNA protects the heart from pathologicalhypertrophy. *Nature.* 2014;514:102–106.

40. Chu C, Quinn J, Chang HY. Chromatin isolation by RNA purification (ChIRP). *J Vis Exp.* 2012;61:3912.

41. Chu C, Qu K, Zhong FL, et al. Genomic maps of long noncoding RNA occupancyreveal principles of RNA–chromatin interactions. *Mol Cell.* 2011;44:667–678.

Citation: Touma M. Regulatory long noncoding RNAs in cardiovascular development and congenital heart defects. *Int J Mol Biol Open Access.* 2023;6(1):14–17. DOI: 10.15406/ijmboa.2023.06.00147