Long Noncoding RNAs in the Heart
The Regulatory Roadmap of Cardiovascular Development and Disease

Katey J. Rayner, PhD; Peter P. Liu, MD

Cardiac development is anchored on an intricate program of gene regulation and coordination, associated with critical timing and cell–cell interactions. Rather than a single master regulatory process, as originally envisioned to reside in a transcriptional complex or protein signaling cascade, cardiac development is likely regulated by a network of coordinated gene expressions, critically timed, and calibrated. Noncoding RNAs (ncRNA) are now seen as key new players in this regulatory network, and the road map is only beginning to be constructed.

Long noncoding RNAs (lncRNA) are RNA transcripts longer than 200 nucleotides expressed by the genome, but do not themselves code proteins. They are transcribed across the genome, including the intergenic regions as potentially overlapping sense and antisense transcripts that can flank protein-coding genes. Coordinated activities of lcnRNAs likely play a major role in the regulatory networks of organ development, normal organ function, and disease pathogenesis.

Until recently, ncRNAs were considered generic regulators of cell function, controlling the basic pathways of mRNA splicing and protein translation. However, in the past 10 years large scale community projects such as ENCODE (Encyclopedia of DNA elements) or FANTOM (Functional Annotation of the Mammalian Genome) have taught us that ncRNAs outnumber protein-coding genes. LncRNAs have the unique capability of modifying gene function in diverse ways, for example: (1) they can bind mRNA transcripts to either stabilize or promote translation (eg, enhancer function), or cause steric hindrance to block translation (eg, acting as decoys); (2) they can act as a sponge for miRNAs, lncRNAs activate protein expression by sequestering gene-repressing miRNAs, or (3) lncRNAs can associate with chromatin-remodeling complexes to repress transcription. Some examples of different types of lncRNA in cardiovascular development and disease are listed in Table.

To help unravelling some of these complex networks in cardiac development, Drs He et al from Dr Joseph Wu laboratory performed a bioinformatic comparison of lncRNA expression patterns based on RNASeq in fetal and adult human hearts, published in this issue of Circulation Cardiovascular Genetics. RNASeq can directly define the primary structures of the analyzed lcnRNAs. Dr He et al undertook the unique approach of integrating the expression of these IncRNAs with their nearby coding genes in adult versus fetal tissue (so called guilt by association analysis), to gain insight into how lncRNAs may be functionally altering cardiac developmental gene programs.

Additional pathway analysis identified a signature of protein-coding mRNAs and their coregulated lncRNAs that are functionally involved in cardiac output, development, and growth, and others that associate with hypertrophy and cardiac dysfunction. Notably, the pattern of IncRNA–mRNA coregulation was distinct in adult compared with fetal tissue: adult lncRNAs primarily clustered with disease-associated mRNAs (eg, ANKRD1, associated with cardiomyopathy), whereas fetal lncRNAs clustered with mRNAs involved in cellular programming and development (WDR1, myocardial growth). Although the authors did not explore the functional outcome of the IncRNA–mRNA coexpression in this study, their findings suggest that lncRNAs are intricately involved in regulating cardiac development and set the stage for further elucidation of these pathways.

Beyond direct regulation of the protein-coding genome, He et al revealed that cardiac lncRNAs may act in concert with epigenetic histone modifications during development. They find that the adult heart is enriched in lncRNAs in proximity to promoter regions with chromatin modifier histone H3K methytransferrases, similar to what had been reported previously. There were also interactions with other chromatin modifiers such as polycomb repressive complex when compared with the fetal heart. Moreover, the coexpressed IncRNA–mRNA pairs often share promoter regions, suggesting that coordinated activation of IncRNAs and their protein-coding partners could provide a feedback relationship where IncRNAs represses genes no longer needed during phases of...

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Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org DOI: 10.1161/CIRCGENETICS.116.001413
development (Figure). Furthermore, many of the IncRNAs expressed in adult and fetal cardiac tissue contain binding sites for transcription factors, such as Activating Transcription Factor (ATF) and RAR Related Orphan Receptor (RORα), which may be differentially activated during development or disease. Although these mechanisms need to be validated experimentally, this study elegantly highlights how IncRNAs can act as a nexus of control of cardiac development and could thus be further probed for their contribution to cardiac dysfunction.

Table. Select Examples of Different Types of Long Noncoding RNA (lncRNA) in Cardiovascular Development and Disease

<table>
<thead>
<tr>
<th>IncRNA Type</th>
<th>Examples</th>
<th>Potential Function</th>
</tr>
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<tbody>
<tr>
<td>Enhancer lncRNAs</td>
<td>HOTTIP</td>
<td>Binds genes such as WDR5 and transcription initiator H3K4me3 to activate genes</td>
</tr>
<tr>
<td>lncRNA</td>
<td>Braveheart</td>
<td>Acts upstream of MesP1 gene for cardiac lineage specification</td>
</tr>
<tr>
<td>lncRNA</td>
<td>MIAT</td>
<td>RNA Splicing, also a potent susceptibility locus for myocardial infarction</td>
</tr>
<tr>
<td>Natural antisense RNA</td>
<td>β-MHC antisense</td>
<td>Regulates isoform switching between α- and β-MHC</td>
</tr>
<tr>
<td>Natural antisense RNA</td>
<td>ANRIL</td>
<td>Scaffold for polycomb repressive complexes that regulates CDKN2A/B, the most potent genetic locus for coronary atherosclerosis</td>
</tr>
<tr>
<td>uaRNA</td>
<td>DMPK-3’UTR</td>
<td>Induction of Nkx2.5 leading to myotonic muscular dystrophy</td>
</tr>
</tbody>
</table>

ANRIL indicates antisense noncoding RNA in the INK4 locus (or CDKN2B-AS); DMPK, dystrophia myotonica protein kinase; HOTTIP, HOXA transcript at the distal tip; lincRNA, large intervening or intergenic noncoding RNA; MHC, myosin heavy chain; MIAT, myocardial infarct associated; uaRNA, 3’UTR–associated RNA transcript; and UTR, untranslated region.

Unlike protein-coding genes and miRNAs, IncRNAs are often poorly conserved between species and are highly tissue specific. This study represents the first in-depth analysis of IncRNAs in cardiac development in humans. Although this approach naturally represented a snapshot of IncRNA profiles in time, and prevented a thorough analysis of IncRNAs expression at each stage of cardiac development or identification of contributing cell types to the same degree as what can be accomplished in mice, these data provide a unique resource for the future study of IncRNAs that may functionally contribute...
to human disease. To further support this, the authors used data integrated from single nucleotide polymorphism (SNP) databases to interrogate whether transcription factor–binding sites for adult and fetal lncRNAs could be influenced by variation in the genetic sequence between individuals. They suggest that SNPs present in transcription factor–binding sites of lncRNAs may result in their altered expression during development. Although they did not test their hypotheses experimentally, their data demonstrate the additional layer of potential complex regulation when assigning function to ncRNAs as causative factors in cardiovascular disease.

Many important limitations exist within this study, but do represent important opportunities for further exploration. First, the authors focus on lncRNAs that are coexpressed with protein-coding mRNAs, but do not actually verify whether these transcripts lead to a functional protein. There exists the possibility that although coexpressed, an lncRNA may in fact target its protein-coding partner to prevent its translation. The specific targets of these lncRNAs were not investigated and were beyond the scope of this study, yet will undoubtedly allow us to further understand how lncRNAs function in regulating development and disease. Finally, the study restricted its analysis to coding regions that were in close proximity to promoter regions and lncRNAs, and may have thus overlooked important relationships that are distal to protein-coding regions. As the tools to investigate trans noncoding elements improve, so too will the understanding of how fetal versus adult lncRNAs may be contributing to cardiac physiology and ultimately disease.

Nevertheless, this study provides a valuable resource for the in-depth investigation of lncRNAs in human cardiovascular health and disease. The investigators are particularly lauded for posting and sharing their data on Heart Development Associated lncRNA Database (http://210.42.113.162/Heart/index.php) to encourage collaboration among other interested investigators of the scientific community. This will help to further experimental explorations of how RNAs specifically regulate human heart development and shed light on the pathogenesis of disease. Working as a community to coordinate and unravel the program represents a new way to move science forward for the benefit of understanding ourselves, our patients, and society at large.

Acknowledgments
Dr Rayner is supported by a New Investigator Career Award from the Canadian Institutes of Health Research.

Sources of Funding
This study was supported, in part, by grants from the Canadian Institutes of Health Research, Heart and Stroke Foundation, and Genome Canada.

Disclosures
None.

References

Key Words: editorials ■ bioinformatics ■ cardiomyopathy ■ development genes ■ genetics ■ long noncoding RNA
Long Noncoding RNAs in the Heart: The Regulatory Roadmap of Cardiovascular Development and Disease
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doi: 10.1161/CIRCGENETICS.116.001413
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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