Decoding the Non-Coding Transcripts in Human Heart Failure

Running title: Xiao et al.; RNA-seq profiling in human failing heart

Xinshu (Grace) Xiao, PhD\textsuperscript{1,2}; Marlin Touma, MD\textsuperscript{3}; Yibin Wang, PhD\textsuperscript{1,4}

\textsuperscript{1}Molecular Biology Institute; \textsuperscript{2}Dept of Integrative Biology and Physiology, College of Life Sciences; \textsuperscript{3}Dept of Pediatrics, David Geffen School of Medicine; \textsuperscript{4}Dept of Anesthesiology, Medicine and Physiology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA

Address for Correspondence:
Yibin Wang, PhD
Division of Molecular Medicine, Department of Anesthesiology
David Geffen School of Medicine, University of California at Los Angeles
650 Charles E. Young Drive, Room BH 569, CHS
Los Angeles, CA 90095
Tel: 310 206-5197
Fax: 310-206-5907
E-mail: yibinwang@mednet.ucla.edu

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Heart failure (HF) is a complex disease with a broad spectrum of pathological features. Despite significant advancement in clinical diagnosis through improved imaging modalities and hemodynamic approaches, reliable molecular signatures for better differential diagnosis and for better monitoring of heart failure progression remain elusive. The few known clinical biomarkers for heart failure, such as plasma BNP and Troponin, have been shown to have limited use in defining the etiology or prognosis of the disease\(^1,2\). Consequently, current clinical identification and classification of heart failure remain descriptive, largely based on functional and morphological parameters. Therefore, defining the pathogenic mechanisms for hypertrophic vs. dilated or ischemic vs. non-ischemic cardiomyopathies in the failing heart remain a major challenge to both basic science and clinic researchers. In recent years, mechanical circulatory support using left ventricular assist devices (LVAD) has assumed a growing role in the care of patients with end-stage HF\(^3\). During the earlier years of LVAD application as a bridge to transplant, it became evident that some patients exhibit substantial recovery of ventricular function, structure and electrical properties\(^4\). This led to the recognition that reverse remodeling is potentially an achievable therapeutic goal using LVAD. However, the underlying mechanism for the reverse remodeling in the LVAD treated hearts is unclear and its discovery would likely hold great promise to halt or even reverse the progression of heart failure.

During cardiac development, gene expression dictates the cellular differentiation of cardiac cells, including cardiomyocytes, endothelial cells, smooth muscle cells and fibroblasts, each driven by cell-type specific regulatory circuits of transcription\(^5\). Significant changes in cardiac gene expression have also been extensively studied in diseased hearts, leading to the identification of the so-called "fetal-like" gene expression profile associated with the onset of cardiac pathology\(^6\). These insights allow us to hypothesize that specific changes in gene
expression profiles may reveal the underlying etiology and the pathological features of specific forms of cardiomyopathy, and suggest that normalization of gene expression is both the signal of as well as a molecular mechanism for the reverse remodeling in LVAD treated failing hearts. Using GeneChip (a cDNA microarray platform), Margulies et al performed a comprehensive transcriptome analysis of 199 human myocardial samples from non-failing, failing, and LVAD-supported human hearts. The study revealed significant HF-related changes in over 3088 transcripts, of which 11% exhibited partial recovery after LVAD and only 5% showed normalization. Most notably, the normalization of gene expression, like the functional recovery following LVAD implantation is highly heterogeneous among patients. In a similar microarray study by Hall et al., 6 paired human heart specimens were harvested at the time of LVAD implant as well as from explants after a significant recovery of the ventricular function. In this cohort, significant changes in integrin signaling and several new target genes, including EPAC2, were found to be associated with post-LVAD reverse remodeling. By comparing the gene expression profiles between the LVAD responsive hearts and the non-responsive hearts, Birks et al also found distinct differences in the expression of sarcomeric and cytoskeletal proteins between the two patient groups. Finally, Kittleson et al used a similar genechip approach to profile differentially expressed genes in non-ischemic cardiomyopathy vs. ischemic cardiomyopathy hearts, and identified a pattern of gene expression which differentiated these two forms of cardiomyopathy. These earlier studies demonstrate that changes in mRNA expression profile are very complex in the failing hearts. Despite some tantalizing new insights, the original hypothesis that mRNA profiles could be used to define the pathological nature of patient's heart failure as well as its recovery following mechanical unloading appears to be overly simplistic.
It is increasingly clear that the cellular transcriptome is populated not only by protein coding mRNAs, but also non-coding (nc)RNAs beyond the familiar rRNAs and tRNAs species. These non-coding transcripts are categorized into short/small non-coding RNAs, including micro(mi)RNAs, snoRNAs, piRNAs etc.; as well as long non-coding (lnc) RNAs. Many of them, such as miRNA and piRNAs, are generated from dedicated biosynthesis machinery. The landscape of the eukaryotic transcriptome has expanded significantly thanks to the application of RNA sequencing integrated with bioinformatic approaches\textsuperscript{11,12}. ncRNA species are now recognized as a significant component in the total complexity of the transcriptome. The emerging evidence suggest that these abundant ncRNAs have a major role in the regulation of almost all important gene expression processes, from epigenetic modification to transcription, mRNA splicing and processing and translation\textsuperscript{13,14}. A number of RNA-seq based transcriptome profiling studies have been performed in the experimental models of heart failure, revealing dramatic changes of transcriptome for both mRNA and ncRNAs during pathogenesis\textsuperscript{15-17}. In this issue, Yang et al led by Jeanne M. Nerbonne and her team report a comprehensive RNA-seq based transcriptome analysis in failing human hearts that expands our current knowledge of transcriptome complexity and dynamics in human heart failure\textsuperscript{18}.

The study utilized the standard Illumina TruSeq method and the well-established TopHat and Cufflinks suite to map RNA-Seq reads and to reconstruct transcript isoforms. In addition to mRNA and miRNA, a more comprehensive profiling of lncRNAs was performed by taking advantage of the recently updated lncRNA databases and improved de novo Cufflinks prediction. As expected from other RNA-seq studies, the increased detection sensitivity afforded by RNA-seq uncovered a large number of lncRNA transcripts (nearly 18,000) in the human myocardium\textsuperscript{18}. Somewhat unexpectedly, however, the clustering analyses showed that
differences in expression signatures of the lncRNAs were able to distinguish between cardiomyopathy of ischemic and non-ischemic origin and, furthermore, discern between before and after LVAD treatment for either type of cardiomyopathy. In contrast, the profiles of miRNA and mRNA expression did not have similar discriminatory power. It should be noted that clustering results are highly sensitive to the specific set of genes selected for the analysis. Better clustering can be achieved when more genes are differentially expressed across samples. Even in their own analysis, the discrimination between ischemic cardiomyopathy and non-ischemic cardiomyopathy samples was improved when only the 100 most differentially-expressed mRNAs were used to cluster the samples. Nonetheless, the finding that clustering was able to achieve highly discriminative results using all expressed lncRNAs without stringent selection criteria suggests that there is a larger fraction of lncRNAs that are dynamically regulated during heart failure or in response to LVAD treatment. However, the overall sample sizes in this study (8 pairs of pre- and post-LVAD samples) are low and this conclusion would need to be validated in a larger independent cohort.

The majority of the reported lncRNAs from this study have relatively low abundance in the human hearts (RPKM<1), raising questions about their possible biological roles in cardiomyopathy. Compared to our current knowledge of mRNAs and miRNAs, our understanding of lncRNA function remains extremely primitive at this time. In Yang's report, the authors made an interesting observation that the expression of lncRNAs and their neighboring coding genes were more correlated on average than between mRNAs and their neighbors. Based on this relatively weak association alone, it is hard to determine whether widespread positive/negative regulatory potentials of lncRNAs on neighboring mRNAs exist since the significant correlation may simply reflect common regulatory elements shared by
neighboring lncRNAs and mRNAs, as demonstrated in the case study for Rara and n340651. In fact, many lncRNAs are reported to have anti-sense activities to their overlapping mRNAs which would have yielded an inverse correlation between lncRNA and their cis-mRNA partners\textsuperscript{11}. Thus, it would have been informative to expand this study and examine such relationships among the lncRNA/cis-mRNA pairs with highly correlated expression patterns (e.g., the ~20% lncRNAs with Pearson's $r > 0.8$). Finally, as the authors alluded to, lncRNAs can also function beyond the mode of cis-regulation, and extends their impact on post-transcriptional regulation to mRNA splicing, decay and translation.

In summary, RNA-seq based transcriptome profiling in the failing human heart revealed additional transcriptional complexity associated with the disease and recovery following mechanical unloading. The newly uncovered importance of lncRNAs in disease pathogenesis highlights their potential value as potential diagnostic and therapeutic targets. Decoding the function of lncRNAs in cardiac physiology and diseases is both a major challenge as well as an exciting new opportunity for future studies.

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