Ingenuity pathway analysis of the human cardiac cell Atlas identifies differences between right and left ventricular cardiomyocytes

Sasha Z. Prisco | Felipe Kazmirczak | Thenappan Thenappan | Kurt W. Prins

Department of Medicine, Cardiovascular Division, Lillehei Heart Institute, University of Minnesota, Minneapolis, Minnesota, USA

Correspondence
Kurt W. Prins, Department of Medicine, Cardiovascular Division, Lillehei Heart Institute, University of Minnesota, Minneapolis, MN 55455, USA.
Email: prin0088@umn.edu

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Abstract
Pharmaceuticals for left ventricular (LV) dysfunction do not have similar success in right ventricular (RV) failure, which may reflect biological differences between the ventricles. In this study, we performed Ingenuity Pathway Analysis of the Human Cell Atlas to understand how the transcriptomic signatures of the RV and LV differ.

KEYWORDS
heart diseases, pulmonary heart disease, right ventricle function and dysfunction

Right ventricular failure (RVF) is a leading cause of morbidity and mortality in pulmonary arterial hypertension (PAH) and multiple other cardiovascular diseases, but no currently available pharmaceutical targets RV pathophysiology directly.1 Unfortunately, therapies proven to be effective in left heart dysfunction are not usually beneficial in RVF,1 and thus there is an unmet need to identify mechanisms that can be targeted to either slow or reverse RVF to improve outcomes in multiple disease states. Certainly, there are developmental, anatomical, and physiological differences between the left ventricle (LV) and the RV that may explain why LV-directed therapies have not yielded similar success in RVF.1 In addition, there may be proteins or molecular pathways that are more important for proper RV function than LV function, and identification of these molecules may allow for the development of RV-directed therapies. Therefore, we performed Ingenuity Pathway Analysis (IPA) of single-nucleus RNA-sequencing data from the Human Heart Cell Atlas2 to define how the healthy RV and LV differ at the molecular level.

IPA is a bioinformatics application that integrates high-throughput omics results with curated genomic and clinical data to identify molecular interactions and upstream regulators and generate hypotheses about disease processes.3 IPA can be used for any comparisons of interest (between disease states, different tissues, etc.). The Human Heart Cell Atlas recently described five distinct ventricular cardiomyocyte populations,2 and we performed IPA on all five data sets. Description of the donor population and research ethics were delineated in Litvinukova et al.2 P-values for the predicted upstream regulators of the differentially expressed genes were calculated in IPA. Hierarchical cluster analysis and principal component analysis of the different LV and RV cardiomyocyte types were completed in MetaboAnalyst.
The upstream regulator analysis identified that p53 and β-estradiol modulate gene expression in all five cardiomyocyte types (Table S1). Certainly, there are strong data supporting the role of β-estradiol, the female predominant sex hormone, in RV physiology as there are clear sex-differences in RV function with women having superior RV performance than men in both healthy and diseased states. Perhaps, β-estradiol regulated genes are disproportionately important for the RV. Consistent with this hypothesis, our canonical pathway analysis revealed estrogen receptor (ER) pathway proteins are frequently expressed at higher levels in the RV than the LV (Figure 1). A recent manuscript clearly describes the importance of ER signaling in RVF as genetic deletion of ERα causes RVF in multiple forms of rodent models.
Interestingly, many CPVT arrhythmias originate catecholaminergic polymorphic ventricular tachycardia (CPVT). Moreover, RV ERα levels are reduced in PAH-mediated RVF, further confirming the crucial role of ERα in proper RV physiology. Tumor protein p53 was also identified to regulate differential gene expression between the RV and LV in all five cardiomyocyte populations (Table S1). In contrast to ER signaling, the effect of p53 on the RV is not clearly delineated. However, p53 is important for proper cardiac function as knockout of p53 leads to age-dependent heart failure in mice. In addition, loss of p53 protects against pressure overload. Thus, clearly the role of p53 in cardiac physiology is complex and requires further investigation. In conclusion, the upstream regulator analysis identified p53 and β-estradiol as modulators of gene expression in all five ventricular cardiomyocyte populations and pathway analysis revealed that proteins in the ER pathway are more highly expressed in RV than LV, suggesting a more prominent role of estrogen signaling in RVF. Existing data support these observations, and perhaps modulating the effects of these molecules could combat RVF.

When we examined the absolute differences in transcript abundance, we noted the RV has higher levels of the sarcomeric proteins titin, myosin binding protein C, and multiple troponin isoforms (Table S2). Emerging data identify sarcomeric dysfunction as an etiology of RVF in both scleroderma-associated PAH and left heart failure. In the scleroderma RV and the failing RV of left heart failure patients, maximal calcium force production is reduced, and perhaps differential regulation of sarcomeric proteins in the RV contributes to these findings. In addition, ryanodine receptor 2 (RYR2) abundance is greater in the RV. This may be clinically relevant because mutations in RYR2 cause catecholaminergic polymorphic ventricular tachycardia (CPVT). Interestingly, many CPVT arrhythmias originate from the RV, and thus the increased expression of RYR2 in the RV may provide a molecular explanation for this clinical observation. Moreover, in PAH-induced RV dysfunction, there is increased calcium sparks and prolonged calcium transients in RV cardiomyocytes, possibly due to abnormal gating of the RYR2.

Canonical pathway analysis of the five cardiomyocyte populations revealed eukaryotic initiation factor 2 (EIF2) signaling as an important differentiator between the RV and LV (Figure 1). EIF2 integrates a diverse set of signals including inflammation, stress, and nutritional states to modulate protein synthesis. The LV and RV are exposed to different loads and stresses under normal physiological conditions, and the EIF2 pathway may explain how each ventricle fine tunes their physiological function.

Our analysis highlighted differentially regulated long noncoding ribonucleic acids (lncRNAs) between the two ventricles (Table S2). The importance of lncRNAs in cardiac dysfunction is gaining attention, and a recent manuscript showed the lncRNA H19 causes RVF in rodent PAH. At the molecular level, antagonism of H19 prevents RV hypertrophy, fibrosis, and dysfunction via modulation of zeste homolog 2, a protein known to silence certain genes that lead to cardiomyocyte hypertrophy and fibrosis. Omura et al. showed that H19 inhibition leads to zeste homolog 2 upregulation. In human PAH, elevated serum levels of lncRNA H19 are associated with markers of RVF and predict long-term outcomes in two independent cohorts of PAH patients. Perhaps future studies altering the expression of the identified lncRNAs with divergent ventricular expression will delineate novel means to target RVF more selectively.

Our study has important limitations that must be acknowledged. First, this study used data from a mixture of male and female samples to define the RV and LV transcriptomic signature. Unfortunately, we were unable to dissect the biological effects of sex in this analysis. In addition, the divergent expression of transcripts in healthy RV and LV may not be as important as changes observed in disease states, and therefore future research will be needed to delineate which of these pathways are directly relevant to RVF. Single-nucleus RNA-sequencing quantifies RNA levels in the nucleus, but these messenger RNA (mRNA) changes may not equate to a 1:1 change in protein expression as there may be differences in mRNA handling/stability or translational efficiency. Finally, the pathways in cardiomyocyte 1 (CM1) and CM2 populations may be the most important for differentiating the RV from the LV as the LV is more enriched in CM1 compared to the RV (63.86 vs. 36.71%) while the RV has a higher proportion of CM2 compared to the LV (39.91 vs. 9.12%). Notably, unbiased assessment of clustering between the RV and LV cardiomyocyte populations identified that the same cardiomyocyte type (CM1–5) between the RV and LV group together and there is overlap between the RV and LV cardiomyocyte populations on principal component analysis (Figure S1). As there are differences in expression in each cardiomyocyte population between the RV and LV, we thus conducted IPA to help define how these changes may be important for the RV.

In conclusion, IPA of the Human Heart Cell Atlas identifies pathways that may mediate some of the important physiological differences between the RV and LV. Interestingly, our analysis highlights the contributions of β-estradiol, the sarcomere, and RYR2 as key molecular differences between the RV and LV, and existing data supports these RV-centric observations. Certainly, future studies are needed to understand how the nominated pathways may preferentially modulate RV function in an attempt to define molecular targets for RV-directed therapies.

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CONFLICT OF INTERESTS
Kurt W. Prins served on an advisory board for Actelion and Edwards and receives grant funding from United Therapeutics. Thenappan Thenappan served on an advisory board for Actelion, United Therapeutics, Altavant Sciences, and Aria CV. Thenappan Thenappan receives research funding for clinical trials from United Therapeutics, Aria CV, Gossimer Bio, and Acceleron. The other authors declare that there are no conflict of interests.

ETHICS STATEMENT
The ethics statement is not available.

AUTHOR CONTRIBUTIONS
Sasha Z. Prisco and Kurt W. Prins analyzed data and prepared the manuscript. All authors revised the manuscript and approved the submitted version.

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SUPPORTING INFORMATION
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