Right Ventricular Myocardial Biomarkers in Human Heart Failure

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ABSTRACT

Background: Right ventricular (RV) dysfunction contributes to mortality in chronic heart failure (HF). However, the molecular mechanisms of RV failure remain poorly understood, and RV myocardial biomarkers have yet to be developed.

Methods and Results: We performed RNA sequencing (RNA-seq) on 22 explanted human HF RVs and 5 unused donor human heart RVs (DON RV) and compared results to those recently reported from 16 explanted human LVs We used Bowtie-Tophat for transcript alignment and transcriptome assembly, DESeq for identification of differentially expressed genes (DEGs) and Ingenuity for exploration of gene ontologies. In the HF RV, RNA-seq identified 130,790 total RNA transcripts including 13,272 protein coding genes, 10,831 long non-coding RNA genes and 8,605 pseudogenes. There were 800-1000 DEGs between DON and HF RV comparison groups with differences concentrated in cytoskeletal, basement membrane, extracellular matrix (ECM), inflammatory mediator, hemostasis, membrane transport and transcription factor genes, lncRNAs and pseudogenes. In an unbiased approach, the top 10 DEGs SERPINA3, SERPINA5, LCN6, LCN10, STEAP4, AKR1C1, STAC2, SPARCL1, VSIG4 and F8 exhibited no overlap in read counts between DON and HF RVs, high sensitivities, specificities, predictive values and areas under the receiver operating characteristic curves. STEAP4, SPARCL1 and VSIG4 were differentially expressed between RVs and LVs, supporting their roles as RV-specific myocardial biomarkers.

Conclusions: Unbiased, comprehensive profiling of the RV transcriptome by RNA-seq suggests structural changes and abnormalities in inflammatory processes and yields specific, novel HF RV vs HF LV myocardial biomarkers not previously identified by more limited transcriptome profiling approaches. (*J Cardiac Fail 2015;21:398–411*)

Key Words: Human heart failure, right ventricle, RNA-seq, transcriptome, myocardial, biomarkers.

Heart failure (HF) biomarkers have evolved to inform myocardial function and fate and predict clinical outcomes.[1–3] Biomarkers specifically informing right ventricular (RV) function and fate have yet to evolve.[4] RV function is an independent predictor of survival in left ventricular (LV) HF, a critical determinant of the outcome of LV mechanical circulatory support, and a more potent predictor of mortality than pulmonary arterial pressure in patients with primary pulmonary hypertensive disorders.[4] RV-specific biomarkers would therefore be applicable in a wide variety of cardiopulmonary diseases.

Via high-throughput sequencing of fractionated RNA, RNA sequencing (RNA-seq) provides an unbiased, comprehensive profile of polyadenylated [poly(A)] mRNA, including poly(A) noncoding RNA transcripts.[5,6] We hypothesized that an unbiased RNA-seq approach might yield novel candidate myocardium-specific human HF RV biomarkers. We therefore used RNA-seq to generate the

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transcriptional profile of end-stage human HF RV myocardium and unused donor RV myocardium to identify candidate RV myocardial biomarkers in an unbiased approach. We then reviewed the transcriptomes of HF RVs with echocardiographic dysfunction versus echocardiographic normal function to identify candidate biomarkers of RV dysfunction in end-stage human HF. Finally, to identify RV-specific biomarkers, we compared our findings in the end-stage HF RV transcriptomes with the RNA-seq findings recently published from a study of end-stage human HF LVs.[6]

Materials and Methods

RV Tissue Acquisition

After Institutional Review Board approval of the study, all subjects before transplantation were enrolled in the Vanderbilt Heart and Vascular Institute Biorepository after signing informed consents. We identified 5 unused donor hearts and 22 end-stage LV HF hearts (11 ischemic, 11 nonischemic) explanted at the time of cardiac transplantation. Immediately after explantation, a small piece of RV tissue free of macroscopic fibrosis or infarction was dissected from a cross-section 5−7 cm above the apex, flash frozen in liquid nitrogen, and stored in −80°C till use.

Clinical Characterization, Including the Definitions of RV Failure and RV Dysfunction

Deidentified clinical data were abstracted for all subjects. We defined RV failure as right atrial pressure >8 mm Hg and cardiac index <2.2 L min^{-1} m^{-2},[7] recorded at the right heart catheterization performed closest to the time of transplantation. As discussed below, we elected not to analyze the RV RNA-seq results based on the presence or absence of RV failure owing to the small number of subjects who satisfied this hemodynamic definition of RV failure.

The presence or absence of RV dysfunction was determined solely by the 2-dimensional Doppler echocardiogram performed closest to the time of transplantation. The American Society of Echocardiography (ASE) consensus guidelines for RV echocardiographic function assessment[8] recommends both a qualitative assessment of RV function based on inspection of all conventional RV imaging planes and performance of ≥1 specialized RV quantitative functional assessment, such as tricuspid annulus position systolic excursion (TAPSE), RV fractional systolic area change (FAC), or RV index of myocardial performance (RIMP). Each of the recommended individual quantitative echocardiographic measures of RV dysfunction (eg, TAPSE <16 mm, FAC <35%, or RIMP >0.4) is potentially limited by available image acquisition planes, sonographic resolution, pre- and afterload dependency, and earlier cardiac surgery.[9,10] For example, earlier coronary artery bypass graft surgery (CABG) or aortic valve replacement (history of pericardiotomy or open heart surgery) lowers TAPSE even in the setting of preserved global RV function.[11] Given the inherent limitations of each of these quantitative echocardiographic measures of RV function, in accord with the ASE guidelines we used a 2-step method to define echocardiographic RV dysfunction. First, an experienced multimodality coinvestigator (E.B.), blinded to the quantitative echocardiographic measures, hemodynamics, and clinical history, provided a provisional qualitative determination of echocardiographic RV function. Second, this provisional qualitative determination was refined after an unblinded review of the 3 ASE-recommended quantitative echocardiographic measures, TAPSE, FAC, and RIMP. RV dysfunction was then defined as the presence of both (1) dysfunction by qualitative assessment and (2) ≥2 abnormal quantitative assessments (TAPSE <16 mm, FAC <35%, or RIMP >0.4). Review of the aggregate quantitative RV echocardiographic measures did not result in reclassification of any provisional qualitative RV functional determinations.

RV RNA Preparation

Ninety to 120 mg of tissue per study subject was used for RNA extraction. Total RNA was isolated using RNeasy Mini Kit (Qiagen) following the manufacturer's instructions.

RV RNA-seq

We first performed a quality check of the input total RNA by running an aliquot with the use of the Agilent Bioanalyzer to confirm integrity, and a Qubit RNA fluorometry assay was used to measure concentration. For each library, 100 ng total RNA underwent enrichment for poly(A)-containing mRNA with the use of poly-T oligo-attached magnetic beads.[12] After purification, eluted poly(A) RNA was cleaved into small fragments of 120−210 bp (divalent cations, elevated temperature). The cleaved RNA fragments were copied into 1st-strand cDNA with the use of Superscript II reverse transcriptase and random primers. This was followed by 2nd strand cDNA synthesis with the use of DNA Polymerase I and RNase H. The cDNA fragments then underwent an end-repair process, the addition of a single "A" base, and ligation of the Illumina multiplexing adapters. The products were purified and enriched with the use of polymerase chain reaction to create the final cDNA sequencing library. The cDNA library underwent quality control check with the use of an Agilent Bioanalyzer HS DNA assay to confirm final library size and with the use of an Agilent Mx3005 P qPCR machine using the KAPA Illumina library quantification kit to determine concentration. From a 2-nmol stock, samples were pooled by molarity for multiplexing. From the pool, 12 pmol was loaded into each well for the flow cell with the use of the Illumina cBot for cluster generation. The flow cell was loaded into the Illumina HiSeq 2500 using v3 chemistry and HTA 1.8, and sequenced at paired-end 50 bp with a target of 30 million pass filter reads per library. The raw sequencing reads in BCL format were processed with the use of Casava-1.8.2 for Fastq conversion and demultiplexing. The RTA chastity filter was used and only the pass filter reads were retained for further analysis.

Analysis of RV Transcript Expression

Raw sequence-derived data underwent quality controls with the use of tools such as Fastx Toolkit and Fastqc to identify potential outliers before undergoing any advanced analysis. RNA read alignment and mapping was performed with the use of Bowtie/Tophat, and transcriptome reconstruction was performed with the use of Cufflinks for both mRNA and long noncoding RNA (lncRNA).[13] For Cufflinks, a minimum reads per kilobase of exon model per million mapped reads (RPKM) value of ≥1 was required for further analysis.[12] Cufflinks was used also to detect and quantitate alterative spliced transcripts and isoforms.[14]

To identify biomarkers discriminating unused donor RVs versus HF RVs, we explored differential gene expression[15,16] with the use of the negative binomial method DESeq[17] and compared unused donor RVs (n = 5) with 4 HF RV comparison groups: (1) ischemic