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Defining the molecular signatures of human right heart failure

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ABSTRACT

Aims: Right ventricular failure (RVF) varies significantly from the more common left ventricular failure (LVF). This study was undertaken to determine potential molecular pathways that are important in human right ventricular (RV) function and may mediate RVF.

Materials and methods: We analyzed mRNA of human non-failing LV and RV samples and RVF samples from patients with pulmonary arterial hypertension (PAH), and post-LVAD implantation. We then performed transcript analysis to determine differential expression of genes in the human heart samples. Immunoblot quantification was performed followed by analysis of non-failing and failing phenotypes.

Key findings: Inflammatory pathways were more commonly dysregulated in RV tissue (both non-failing and failing phenotypes). In non-failing human RV tissue we found important differences in expression of FIGF, TRAPPAC, and CTGF suggesting that regulation of normal RV and LV function are not the same. In failing RV tissue, FBN2, CTGF, SMOC2, and TRAPP6AC were differentially expressed, and are potential targets for further study.

Significance: This work provides some of the first analyses of the molecular heterogeneity between human RV and LV tissue, as well as key differences in human disease (RVF secondary to pulmonary hypertension and LVAD mediated RVF). Our transcriptional data indicated that inflammatory pathways may be more important in RV tissue, and changes in FIGF and CTGF supported this hypothesis. In PAH RV failure samples, upregulation of FBN2 and CTGF further reinforced the potential significance that altered remodeling and inflammation play in normal RV function and failure.

1. Introduction

Heart failure (HF) currently affects 5.7 million adults in the United States [1], with nearly 1 million new cases diagnosed each year. Further, this number is projected to increase by nearly 50% by the year 2030 [2]. Coronary heart disease accounts for more than half of all cardiovascular events in those under age 75 [3], and is largely characterized by acute coronary syndromes that occur at a rate of 750,000 annually in the United States [1]. Coronary disease predominantly affects the left heart, and given the prevalence, it is not surprising that the study of HF has primarily involved the left ventricle (LV). Subsequently, diseases that affect the right ventricle (RV), such as pulmonary hypertension, congenital heart disease, and right-sided valvular disease, are less studied and understood.

In the past two decades, advances in medicine have highlighted the importance of the RV. The two largest groups of patients that have brought attention to the RV are congenital heart disease (CHD) survivors and those with pulmonary vascular disease, known as pulmonary arterial hypertension (PAH). Each of these conditions underscores the importance of right ventricular function in chronic disease. In fact, given the increasing recognition of this, the National Heart, Lung, and Blood Institute organized a working group to define understanding of RV disease and proposed areas of importance for investigation in an effort to improve knowledge [4]. While it has been a decade since this group organized, due to lack of translational animal models and data on specific molecular pathways altered in tissue from patients with RV failure, we unfortunately still lack knowledge of the molecular pathophysiology of these diseases. Further, we lack even a fundamental baseline of how RV dysfunction differs from LV dysfunction in the human heart. To provide new information on the pathways associated

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with RV dysfunction, we performed transcriptional profiling on tissue from hearts with LV failure, hearts with RV failure, and non-failing hearts. We report that the molecular pathways associated with RV dysfunction are significantly different from LV failure. Further, we validate key surrogate pathways unique to RV failure by protein analyses. Our findings provide new data on the unique pathways associated with human RV failure as well as identify potential markers for disease assessment/progression and possible future therapeutic avenues.

2. Materials and methods

2.1. Human tissue acquisition

Non-failing non-transplantable donor hearts (control) were acquired through Lifeline of Ohio Organ Procurement, and end-stage diseased hearts were obtained at the time of cardiac transplantation, as described by our group [5]. Samples were obtained from the free wall of the LV and RV so that septal tissue, which contains myocytes from both the LV and RV could be avoided, thereby eliminating the possibility of "cross-contamination" between left and right ventricular tissue. Informed consent was obtained from transplant and left ventricular assist device (LVAD) patients and the institutional review board approved this study. Based upon available phenotypes within the repository, we included normal RV ($n = 5$) and LV samples ($n = 5$), RV samples from patients with PAH ($n = 2$), and both normal and RV failure samples from patients post-LVAD implantation (non-failing RV $n = 2$, mild RV failure $n = 2$, severe RV failure $n = 2$). We conducted 3 experiments evaluating the relationship between: 1) normal RV and LV tissue, 2) normal RV tissue and failing RV-PAH tissue, and 3) RV tissue from LVAD patients with normal systolic function, mild-RV failure and severe-RV failure.

2.2. Phenotype definitions

Within our institutional biorepository we searched for samples with phenotypes representative of 2 right heart failure disease states: PAH with RV failure (RVF) and post-LVAD with RVF, in addition to non-failing control RV and LV samples (Table 1). Using a de-identified dataset, patients with right heart dysfunction due to pulmonary hypertension out of proportion to left heart failure were identified by evaluating standard invasive hemodynamic criteria (mean pulmonary artery pressure (mPAP) $\geq$25 mmHg, pulmonary capillary wedge pressure (PCWP) $\geq$15 mmHg, pulmonary vascular resistance (PVR) $\geq$3 Wood units, and transpulmonary gradient $\geq$12 mmHg) [6]. Post-LVAD patients with right heart failure were identified by applying the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) diagnostic criteria for RVF and severity score [7,8]. The LVAD-RVF group was further subdivided into those with mild (LVAD-mild-RVF) and severe (LVAD-sev-RVF) right heart failure (Tables 1, 2).

2.3. mRNA and immunoblot experiments

mRNA isolation and analysis was performed according to methods previously described [9]. Briefly, tissue is flash frozen at the time of acquisition. RNA was extracted using the Qiagen RNeasy Mini Kit and RNA yield was measured using a Nanodrop 1000 Spectrophotometer. RNA was then converted to cDNA using a reverse transcription kit. Custom-designed gene arrays were used to probe for a number of targets using the Affymetrix system as previously described [9]. We then used analysis of variance (ANOVA) to measure differences ($>$ 2 fold differences, both positive and negative) in expression between the listed groups (Fig. 1). Immunoblots were performed as described [9–11]. All proteins were normalized to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Antibodies utilized include (in albumin, or blotting-grade blocker): Anti-CTGF (Abcam, ab6990, 1:1000), Anti-Fibrillin 2 (Abcam, ab128026, 1:1000), Anti VEGFD (Bosterbio, PA1332, 1:4000), Anti-MYBPC2 monoclonal antibody (Invitrogen,MA1-26180, 1:4000), Anti-AACT antibody (Origene, TA323307, 1:1000), Anti-SMOC2 antibody (Novus Biologicals, NBP2-20425, 1:1000), Anti-S1PR3 antibody (Sigma-Aldrich, HPA059513, 1:1000) and Anti-TRAPP6AC antibody (Novus Biologicals, NBP1-83167, 1:1000). Secondary antibodies (Peroxidase AffiniPure Donkey Anti-Mouse IgG and Peroxidase AffiniPure Donkey Anti-Rabbit IgG both from Jackson Immunoreserach Laboratories, 715-035-150 and 711-035-152 respectively) were all prepared at concentrations of 1:10,000.

2.4. Statistical analysis

Clinical data were reviewed and basic demographic information and descriptive statistics were generated. Data is expressed as mean $\pm$ SEM with significance occurring at an α level $<$ 0.05. To better understand the basic differences between human left and right ventricular tissue, we identified transcripts up or down-regulated in non-failing LV and RV samples (Fig. 2) [12]. mRNA was analyzed for $\geq$2 fold change between groups by ANOVA. From this list, key transcripts of interest were identified (Table 3).

Table 2
Diagnostic criteria for RVF and severity score of RVF.

Diagnostic criteria for RV failure
Symptoms and signs of persistent right ventricular dysfunction, CVP > 18 mmHg with a CI < 2.0 L/min/m^2
In the absence of elevated left atrial/pulmonary capillary wedge pressure > 18 mmHg, tamponade, ventricular arrhythmias or pneumothorax
Requiring RVAD implantation; or requiring inhaled nitric oxide or inotropic therapy for duration of more than one week at any time after LVAD implantation
Severity scale
Severe: RVAD implantation
Moderate: inotropes or use of IV or inhaled pulmonary vasodilator (iNO or prostaglandin E)
Mild: 2 of the 4 following criteria
CVP > 18 mmHg or mean RA pressure > 18 mmHg
CI < 2.3 L/min/m^2 (using a pulmonary artery catheter)
Ascites or evidence of moderate to worse peripheral edema
Evidence of elevated CVP by echocardiogram (dilated inferior vena cava without collapse), and in physical exam (signs of increased jugular venous pressure).

CI: cardiac index; CVP: central venous pressure; LVAD, left ventricular assist device; RV: right ventricle; RVAD: right ventricular assist device; RVF: right ventricular failure. Modified from Argiriou M, et.al.[8]

Table 1
Experimental samples.

Control	Non-failing tissue (RV and LV)	Lifeline of Ohio, no known cardiovascular disease
RVF groups	PAH with RVF	• Tissue from patients with known PAH (PH out of proportion to LV dysfunction), explanted at time of OHT[6]
	LVAD control (normal RV function)	• Post-LVAD with normal right heart function, explanted at time of OHT
	LVAD-mild-RVF	• Post-LVAD with INTERMACS criteria for mild RVF, explanted at time of OHT[8]
	LVAD-sev-RVF	• Post-LVAD with INTERMACS criteria for severe RVF, explanted at time of OHT[8]

LV: left ventricle, LVAD: left ventricular assist device, OHT: orthotopic heart transplant, RV: right ventricle, RVF: right ventricular failure.