Gene Expression Profile of the Transforming Growth Factor-Beta/Smad3 Pathway in Systolic Heart Failure

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A. Study Purpose and Rationale

Goal

The purpose of this study is to determine if the expression profile of genes found in the transforming growth factor-beta (TGF-β)/Smad3 signaling pathway differs between patients with systolic heart failure and patients without heart failure.

Heart Failure Prevalence

Heart failure (HF) affects more than 5 million Americans, representing nearly 2.5% of the population, with an incidence of approximately 10 per 1000 in persons older than 65 years of age.[1] HF represents an enormous disease burden: over the past 10 years, hospitalizations for HF have increased by 159 percent, and it is estimated that 2% of all hospital admissions in the United States are for decompensated HF. The estimated direct and indirect cost for HF in the United States in 2008 was $34.8 billion, underscoring the extent and gravity of this disease's impact in this country.[2]

The Syndrome of Heart Failure and Cardiac Remodeling

Heart failure is a clinical syndrome that is thought to represent a final common phenotype for an array of disease processes. Traditionally, the signs and symptoms of heart failure were thought to be secondary to the inability of the heart to maintain normal blood flow to meet the body's hemodynamic demands. However, over the past 10 to 15 years, it has become clear that a number of processes, such as structural cardiac abnormalities, neurohormonal dysregulation, and cytokine/growth factor expression, work in concert to cause cardiac remodeling, which eventually results in heart failure. Cardiac remodeling refers to the process by which mechanical, neurohormonal, and genetic factors cause pathologic changes in ventricular size, shape, and function.[1] The detrimental changes seen in cardiac remodeling include ventricular hypertrophy which often coincides with or progresses to ventricular dilatation and fibrosis. Further progression of remodeling involves the alteration of myocardial contractile proteins and derangements in calcium handling in the myocardium.[1, 3] Remodeling occurs in many clinical conditions, including myocardial infarction, hypertension, valvular disease, and myocarditis. After the initial insult to the heart, remodeling can continue for months to years, and results in the clinical syndrome of heart failure.[4] Underscoring the importance of remodeling, many of the current treatments for heart failure (beta-blockers, ACE inhibitors, ARBs, cardiac resynchronization) are aimed to slow or even reverse the deleterious changes that occur in myocardium that has been exposed to stress.[1]

Data demonstrates that increased neurohormonal regulation, which initially was thought to be a compensatory response to an improperly functioning myocardium, actually changes cardiac myocyte gene expression, leading to detrimental alteration in vasculature and myocytes, culminating in the "remodeled" dysfunctional ventricle and worsening HF.[5] Over the past 15 years, therapy for heart failure has undergone a major paradigm shift, moving from a focus solely on correcting hemodynamics to treating specific disease mechanisms. For example, the use of β-blockers, a negative ionotrope, improves mortality in heart failure, not due to their

effects on hemodynamics, but rather due to their antagonism of sympathetic nervous system signaling in cardiac myocytes.[6-8] The use of β-blockers, angiotensin-converting enzyme (ACE) inhibitors, and ventricular assist devices (when indicated) have been shown to be able to reverse some of the hypertrophy and structural changes that occur in chronic heart failure; hence, these beneficial changes have been called "reverse remodeling."[3] The hypothesis is that the myocardium retains some plasticity and may be amenable to therapies that block the pathological effects of sustained neurohormonal signaling.

Studying Gene Expression Profiles in Heart Failure

The progression of HF is associated with derangements in calcium handling, neurohormonal activation, and cytokine and growth factor signaling.[9] Despite multiple cellular mechanisms, HF is a clinical syndrome that in many patients manifests with a common phenotype of ventricular dilatation and reduced contractility. Recently, microarray data has been published by multiple groups in an effort to determine if the gene expression profiles of failing versus non-failing myocardial tissue differ. These studies have used endomyocardial biopsy samples obtained from HF patients during left-ventricular assist device (LVAD) placement or at heart transplant and compared them to myocardium from non-failing ventricles. Data from these studies demonstrate that the expression profiles of genes involved in cell growth, calcium handling, metabolism, and inflammation differ among failing and non-failing myocardium and among myocardium that showed improved function following LVAD placement. The long term goals of these studies are to gain a better understanding of the molecular pathogenesis of remodeling, to develop novel biomarker assays to help determine HF prognosis, and to identify new targets for heart failure therapeutics. The use of DNA microarray data to quantify gene expression represents a powerful tool in understanding the pathogenesis and progression of heart failure.[9-13]

The TGF-β/Smad3 Pathway in Cardiac Fibrosis

Cardiac fibrosis is a common finding in remodeling associated with heart failure, characterized by the disruption of normal myocardial structure and function. Fibrosis can be caused by myocardial ischemia and infarction, myocarditis, pressure overload, and idiopathic cardiomyopathies. On a molecular level, fibrosis is caused by excessive deposition of extracellular matrix proteins and is mediated by fibroblasts. Recent data suggests that fibroblasts involved in cardiac remodeling are derived from endothelial cells, indicating that an epithelial-to-mesenchymal transition can occur in the heart.[14] Furthermore, fibrosis in the heart via this epithelial-to-mesenchymal transition appears to be dependent on the TGF-β signaling pathway,[15] which has also been implicated in fibrosis in the lung and kidney.[16, 17]

The TGF-β pathway is implicated in vascular fibrosis, hypertension, heart failure, and atherosclerosis.[18, 19] On a broader scale, TGF-β is a cytokine that exerts pleiotropic effects on cell development, proliferation, inflammation, and wound healing. Members of the TGF-β pathway are involved in fibrosis in the heart, liver, lungs, and kidney. TGF-β1 is the predominant TGF-β isoform found in the heart, and through its signaling pathway can activate effector proteins called Smads. Recent data has shown that the TGF-β/Smad3 pathway is a key mediator in the fibrotic response in myocardium following ischemic insult.[20, 21] Additionally,

expression levels of TGF-β1 and Smad3 increase dramatically in mouse myocardium after aortic banding (pressure overload model) or ischemic insult, underscoring these proteins' importance in cardiac remodeling.[14, 21] Small molecule inhibitors of the TGF-β1/Smad3 signaling pathway may represent attractive therapeutic targets in heart failure.[22] However, there are currently no data that establish that *TGF-β1* or *Smad* gene expression is upregulated in heart failure in humans.

This study aims to examine the expression level of proteins in the TGF-β1 signaling pathway in patients with systolic heart failure to patients with normal cardiac function without clinical evidence of heart failure.

B. Study Design and Statistical Analysis

This is a case-control study comparing the expression levels of various proteins in the TGF-β1 signaling pathway in ventricular endomyocardial biopsy (EMB) tissue obtained from two groups of patients: (1) patients with chronic systolic heart failure and (2) patients with normal cardiac function without evidence of heart failure.

Group 1 will consist of patients with chronic systolic heart failure. Inclusion criteria for group 1 include age > 21 years, a left ventricular ejection fraction < 40% as determined by echocardiography, combined with a clinical diagnosis of Stage C or D heart failure.[23, 24] Left ventricular EMB will be obtained either by left-heart catheterization, at the time of LVAD placement, or at the time of heart transplant.

Group 2 will consist of patients with normal cardiac function. Inclusion criteria for group 2 are age >21, left ventricular ejection fraction >55% and normal right ventricular function as determined by echocardiography, combined with no clinical evidence of heart failure or structural heart disease. Left or right ventricular EMB will be obtained from patients while undergoing a heart catheterization for non-cardiac indications (e.g. non-heart transplantation workup).

Exclusion criteria for this study include heart failure with preserved ejection fraction[25] and an acute coronary syndrome within 8 weeks prior to enrollment into the study.

Controls (Group 2) will be recruited first and will be matched to cases on the basis of age, sex, race, and presence of diabetes and hypertension.

The outcome will be expression levels of the following genes, as measured quantitatively using the Affymetrix Human Gene 1.0 ST GeneChip Microarray (Santa Clara, CA) as per published protocols.[26]

TGF-β1	*Smad2*	*Smad3*
Smad4	*Smad7*	*Bone Morphogenic Protein 7 (BMP7)*
DAP Kinase	*Angiotensin 2*	*Angiotensin Converting Enzyme*

Data will be presented as the expression level $\pm$ standard deviation. Expression levels are continuous variables, and will be compared using an unpaired t-test. Effect size, based on the literature,[10-13] is estimated to be an expression level of 1.0 $\pm$ 0.5 for non-heart failure patients

(group 2), and 1.5 $\pm$ 0.5 for heart failure patients (group 1). Given these estimations, and using the Bonferroni Correction for a predetermined α of 0.005, with a β of 0.20 (power of 80%), the study requires 60 patients, with 30 in each group.

C. Study Procedure

Endomyocardial biopsies will be obtained from patients either in the cardiac catheterization laboratory or while patients are in the operating room for either LVAD implantation or heart transplant. Levels of gene expression will be determined using the commercially available Affymetrix Human Gene 1.0 ST GeneChip Microarray (Santa Clara, CA) as per manufacturer's protocols.[26] The laboratory researcher analyzing the specimens will be blinded to the status of the samples.

D. Study Drugs

No drugs are being studied.

E. Medical Device

No medical devices are being studied

F. Study Questionnaires

No questionnaires are planned for this study.

G. Study Subjects

Study subjects are patients referred for heart transplantation, LVAD placement, cardiac catheterization, or organ donation.

Group 1 inclusion criteria are age > 21 years, left ventricular ejection fraction < 40% as determined by echocardiography, combined with a clinical diagnosis of Stage C or D heart failure.[23, 24] Group 2 inclusion criteria are age > 21 years, normal cardiac function, defined as a left ventricular ejection fraction >55% and normal right ventricular function as determined by echocardiography, combined with no clinical evidence of heart failure or structural heart disease.

Exclusion criteria are age < 21 years, heart failure with preserved ejection fraction[25] and an acute coronary syndrome within 8 weeks prior to enrollment into the study.

H. Recruitment of Subjects

Patients referred for heart transplantation, LVAD placement, or left heart catheterization will be approached for enrollment in this study by the study coordinator and will subsequently be screened for eligibility. Patients will not be enrolled in the study without prior discussion with the patient's primary physician, who must agree that the patient may be suitable for the study and allow to the research coordinator to approach the patient. All patients must have capacity to sign

informed consent and release of medical information forms. The study purpose, risk, and benefits will be described to the patient.

I. Confidentiality of Study Data

All samples used will be numerically coded with a randomly generated number in order to protect the identity of the subjects. All study data will be stored in a central secure location according with CUMC IRB regulation. Data will only be available to the investigators of this study.

J. Potential Conflict of Interest

None

K. Location of Study

Patient enrollment will take place at New York Presbyterian Hospital Columbia University Medical Center. Endomyocardial biopsy specimens will be stored in laboratory research facilities at Columbia University Medical Center. All laboratory assays will be performed in research laboratories at Columbia University Medical Center.

L. Potential Risks

There is no additional risk to the patient beyond that of the described procedures for obtaining endomyocardial biopsy tissue in the case of heart transplant (where the biopsy will be taken from the explanted heart) or LVAD placement (where the biopsy will be taken from left ventricle tissue already removed for the procedure).

In the case of LV or RV biopsy during cardiac catheterization, there are risks associated with the procedure, including infection, pain and bruising at the site of venous and arterial access and in rare cases, hematoma and/or aneurysm formation. In addition, there is a small risk of pneumothorax or hemothorax during cannulation of the internal jugular vein should this approach be used. Specific to the endomyocardial biopsy, rare but possible risks include perforation with pericardial tamponade, arrhythmias (VT, SVT, heart block), or creation of an arterial-venous fistula within the heart.

M. Potential Benefits

Study subjects are unlikely to benefit from this study. Information obtained through this study may increase our understanding of the pathophysiology of remodeling in heart failure and may benefit future patients.

N. Alternative Therapies

Not Applicable

O. Compensation to Subjects

No Compensation will be provided

P. Costs to Subjects

This study will incur no additional costs to the subjects.

Q. Minors as Research Subjects

This study will not include minors.

R. Radiation or Radioactive Substances

Study subjects undergoing right or left heart catheterization will be exposed to ionizing radiation as part of this procedure. The amount of additional ionizing radiation required to obtain an endomyocardial biopsy is minimal.

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