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Original article

PGC-1α and *ERRα* target gene downregulation is a signature of the failing human heart

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ABSTRACT

Heart failure is a cause of significant morbidity and mortality in developed nations, and results from a complex interplay between genetic and environmental factors. To discover gene regulatory networks underlying heart failure, we analyzed DNA microarray data based on left ventricular free-wall myocardium from 59 failing (32 ischemic cardiomyopathy, 27 idiopathic dilated cardiomyopathy) and 33 non-failing explanted human hearts from the Cardiogenomics Consortium. In particular, we sought to investigate cardiac gene expression changes at the level of individual genes, as well as biological pathways which contain groups of functionally related genes. Utilizing a combination of computational techniques, including Comparative Marker Selection and Gene Set Enrichment Analysis, we identified a subset of downstream gene targets of the master mitochondrial transcriptional regulator, peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), whose expression is collectively decreased in failing human hearts. We also observed decreased expression of the key PGC-1α regulatory partner, estrogen-related receptor α (ERRα), as well as *ERRα* target genes which may participate in the downregulation of mitochondrial metabolic capacity. Gene expression of the antiapoptotic Raf-1/extracellular signal-regulated kinase (ERK) pathway was decreased in failing hearts. Alterations in *PGC-1α* and *ERRα* target gene sets were significantly correlated with an important clinical parameter of disease severity — left ventricular ejection fraction, and were predictive of failing vs. non-failing phenotypes. Overall, our results implicate PGC-1α and ERRα in the pathophysiology of human heart failure, and define dynamic target gene sets sharing known interrelated regulatory mechanisms capable of contributing to the mitochondrial dysfunction characteristic of this disease process.

1. Introduction

Congestive heart failure is a cause of significant morbidity and mortality in developed nations. For individuals free of heart failure at age 40, data from the Framingham Heart Study indicates that the lifetime risk of developing heart failure is 20% [1]. Among the most common etiologies of end-stage human heart failure are idiopathic dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM). To investigate pathophysiologic mechanisms of heart failure, microarray technology has been utilized extensively in the characterization of global gene expression changes in both ischemic and idiopathic dilated (or non-ischemic) cardiomyopathy [2,3]. A number of prior microarray studies of heart failure have noted no significant gene expression changes between DCM and ICM [4] or have grouped ischemic and non-ischemic samples into a single failing category when comparing against non-failing controls [5,6]. Although end-stage heart failure may represent a common clinical endpoint with a uniform gene expression signature irrespective of etiology, additional studies have identified both shared and distinct gene expression changes between ischemic and non-ischemic heart failure [7–9].

Gene regulatory networks, or pathways, previously associated with heart failure include immune and inflammatory response [10], cell signaling and metabolism [4,6,8], sarcomere and cytoloskeletal organization [6,9,11], transcription/translation [12], and apoptosis [13,14]. Conventional methods used to discover dysregulated pathways in heart failure have relied fundamentally on single-gene approaches combined with Gene Ontology or self-defined functional annotations [7,10,11,15]. Our current study differs in that it also employs biological pathway-based analytic techniques to detect overarching patterns of gene expression in relatively large populations of failing (DCM/ICM) vs. non-failing (NF) human hearts, as well as in individual comparisons of DCM and ICM groups.

Given that insufficient energy delivery and altered substrate utilization occur in the failing heart, gene regulatory networks modulating mitochondrial function have been implicated in the pathogenesis of heart failure [16–19]. In particular, studies investigating mechanisms of defective energy transduction in heart failure have

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defined a role for the transcriptional coactivator, peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), a key regulator of post-natal cardiac mitochondrial function [16,20–22]. In the mouse heart, PGC-1α is essential for maximal and efficient cardiac mitochondrial fatty acid oxidation, ATP synthesis, and lipid homeostasis, especially in the setting of increased work [23]. Forced expression of PGC-1α in hearts of transgenic mice increases mitochondrial number and stimulates respiration via several downstream transcriptional regulatory circuits, one of which involves the nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) [24]. Additionally, the ability of PGC-1α to coactivate the peroxisome proliferator-activated receptors (PPARs) and estrogen-related receptor α (ERRα) gives it a critical role in the regulation of genes central to the mitochondrial β-oxidation of fatty acids as well as overall mitochondrial oxidative phosphorylation [16,25–30].

Reduced PGC-1α activity has been observed in the evolution of several important diseases associated with metabolic derangement, including diabetes mellitus, pathologic cardiac hypertrophy, and heart failure. Oxidative phosphorylation genes regulated by PGC-1α are coordinately depressed in human skeletal muscle with diabetes mellitus type 2 [28,31]. In pressure-overload cardiac hypertrophy in mice, *PGC-1α* gene expression is decreased [21], and its level of expression correlates with mitochondrial oxidative capacity in both healthy and failing rat hearts subjected to chronic pressure overload [32]. Consistent with involvement of PGC-1α in the maintenance of cardiac function following the stress of pressure overload, mice with generalized loss of PGC-1α develop accelerated heart failure two months following transverse aortic constriction [33]. The current study attempts to extend to human heart failure a role for mitochondrial transcriptional regulatory circuits characterized predominantly in normal and failing mouse hearts. This work implicates the PGC-1α/ERRα regulatory circuit in the pathophysiology of human heart failure, suggesting transcriptional regulatory mechanisms involving PGC-1α and its key partners which may contribute to the mitochondrial dysfunction characteristic of the failing heart.

2. Methods

2.1. Source of microarray data

Publicly available microarray data was obtained from the Cardiogenomics Consortium at Brigham and Women's Hospital in Boston, Massachusetts (http://cardiogenomics.med.harvard.edu), an NHLBI sponsored Program in Genomic Applications. RNA was isolated from left ventricular free-wall myocardium extracted from 92 explanted human hearts and hybridized to the Affymetrix HG-U133 plus 2.0 oligonucleotide microarray (54,675 probe sets) according to the guidelines and protocols as outlined on the website. (The 'Quality Control' section of the Cardiogenomics Consortium website (above) offers further details.) These studies were approved by the Committee for the Protection of Human Subjects at Brigham and Women's Hospital and Harvard Medical School. The gene expression analysis component reported in this paper was further reviewed by the Washington University Human Research Protection Office. Fifty-nine of the 92 explanted hearts were from patients with end-stage heart failure at the time of cardiac transplantation, where the etiology of failure was ischemic cardiomyopathy (ICM) in 32 patients and idiopathic dilated cardiomyopathy (DCM) in 27 patients. The remaining 33 samples were from normal, non-failing (NF) cardiac transplant donors whose cause of death was non-cardiac in origin, but whose heart could not be used for transplantation. Left ventricular tissue was harvested intraoperatively at the time of transplantation and snap frozen immediately (within 1 min) in liquid nitrogen, then stored at −80 °C until RNA was isolated. Demographics and limited clinical parameters were available for the majority of patients. For the non-failing group, data on body mass index, hypertension, diabetes, and left ventricular ejection fraction were available for less than 50% of patients, while pulmonary capillary wedge pressures were not available for any of the patients in the non-failing group.

2.2. Data scaling and processing

The data were subjected to global scaling to correct for intensity-related biases as described on the website. Only scans with a 3′/5′ ratio <1.33 were included in our study. A single ICM sample was eliminated by this criterion, leaving a total of 91 samples — 27 DCMs, 31 ICMs, and 33 NFs.

All probe sets which were found to have greater than 93% absent calls across 91 samples were eliminated. These probe sets were considered absent, or under-expressed, in our study and were designated as 'filtered'. Of 54,675 total probe sets on the HG-U133 plus 2.0 array, 30,608 probe sets subsequently remained after filtering. We further collapsed the data from 30,608 probe sets to 17,419 non-redundant genes with distinct HUGO gene symbols, since multiple probe sets often map to a single gene. In this instance, the probe set with the maximum absolute expression value was uniformly assigned to the gene.

In order to reduce the age variability in our population and given the imbalanced number of samples derived from female subjects, all females and marked age outliers were eliminated from our study. A simple algorithm was constructed to remove age outliers in an objective fashion, such that the oldest and youngest were systematically subtracted from each population until the mean age of the three populations was not significantly different. In effect, an age and gender 'matched' subset was created, consisting of 49 samples — 13 DCMs, 20 ICMs, and 16 NFs, while still preserving sample size and power. This subset is referred to as the 'matched' population. The remaining 42 samples were defined as the 'unmatched' population and used for independent validation of our class prediction model.

2.3. Single-gene analysis

The Comparative Marker Selection algorithm from the GenePattern 2.0 software package [34,35] was employed to identify individual genes that were differentially expressed in all four pair-wise comparisons (DCM/ICM vs. NF, DCM vs. NF, ICM vs. NF, DCM vs. ICM). A 2-sided *t*-test followed by 1000 permutations of phenotype labels was performed for this analysis. A false discovery rate *q* value (FDR)<0.05, accounting for multiple hypothesis testing [35], and a fold change (FC)>1.2 served as a cutoff for significant genes. A family-wise error rate (FWER)<0.05 was used as another, more stringent statistic to discover highly significant genes.

2.4. Pathway analysis

Gene Set Enrichment Analysis (GSEA) was utilized to probe pathways, or groups of functionally related genes, dysregulated in heart failure [36]. In discovery mode, gene sets with an FDR<0.25 and a nominal *p* value<0.05 were considered significant [31,36]. The gene ranking metric was a signal-to-noise ratio and the number of permutations specified was 1000.

502 curated gene sets representing generic biological pathways were downloaded from the Broad Institute Molecular Signature Database (MSigDb) (http://www.broad.mit.edu/gsea). The original sources of these pathways include BioCarta, GenMapp, Kyoto Encyclopedia of Genes and Genomes (KEGG), and the Broad Institute. The 'PGC-1α targets' gene set was derived from the MSigDb pathway annotated as 'PGC', and is based on genes responsive to adenoviral-mediated PGC-1α gain-of-function in cultured mouse myoblasts (C2–C12 cells) [31]. The 'ERRα targets 1' and 'ERRα targets 2' gene sets were mined from the literature, and are based on genes influenced by adenoviral-mediated ERRα gain-of-function in primary rat neonatal cardiac