Sarcomeric Genes Involved in Reverse Remodeling of the Heart During Left Ventricular Assist Device Support

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Background: Left ventricular assist devices (LVADs) implanted in patients with severe congestive heart failure (CHF) as a bridge to transplantation have been shown to reverse chamber enlargement, regress cellular hypertrophy, and increase contractility. The purpose of this study was to gain a better understanding of the molecular changes associated with increased contractility after LVAD support.

Methods: We took tissue sections from the left ventricular apex of 12 patients with CHF who were undergoing LVAD insertion (pre-LVAD) and from the LV free wall of those same patients before transplantation (post-LVAD). To control for sample-site differences, we obtained samples from the same regions in 7 patients with CHF who were undergoing transplantation without LVAD support and in 4 non-failing donor hearts. Gene expression was then probed on a custom DNA array containing 2,700 cardiac-enriched cDNA clones.

Results: Calcium-handling genes were up-regulated by LVAD support, as previously reported. Sarcomeric genes were the other principle class of genes up-regulated by LVAD support, consistent with a possible restoration of sarcomere structure in reverse ventricular remodeling. However, a decrease in the fibrous component of the myocardium, also potentially involved in reverse remodeling, was not evident at the level of gene transcription because fibroblast markers were either unchanged or up-regulated. The remaining regulated genes did not fall into any defined functional class.

Conclusions: Changes in the regulation of sarcomeric, calcium-handling, and fibroblast genes during LVAD support indicate a cardiac molecular adaptation to mechanical unloading. These molecular changes may play a role in the observed increase in contractile function during reverse remodeling. J Heart Lung Transplant 2005;24:73–80. Copyright © 2005 by the International Society for Heart and Lung Transplantation.

Congestive heart failure (CHF) represents the final manifestation of a number of cardiovascular diseases, such as ischemic heart disease, hypertension, valvular lesions, primary cardiomyopathies, and viral infections. Cardiac remodeling occurs during progressive heart failure and involves adverse changes at the molecular, cellular, and interstitial level that result in the alteration of heart size, shape, and function. These changes include hypertrophy, chamber dilation, wall thinning, hypocontractile function, myofibrillar disarray, and increased fibrosis, all of which compromise cardiac function.

Reverse remodeling in turn is defined as a decrease in left ventricular (LV) chamber dimension and improved function.[1] During support of critically ill patients with left ventricular assist devices (LVADs), the decrease in ventricular pressure and volume load results in a reversal of chamber enlargement, normalization of cardiac structure,[1–3] and improved myocyte function.[4,5] Studies have shown a decrease in neuroendocrine activation during LVAD support, including a decrease in angiotensin II, plasma epinephrine, norepinephrine, and arginine vasopressin levels, and plasma renin activity.[6] Tumor necrosis factor-α protein, increased in heart failure, also is decreased in the myocardium after LVAD support.[7,8] Numerous other studies have reported down-regulation of natriuretic factors,[9,10] up-regulation of calcium-handling proteins,[5,11,12] reversal of the down-regulation of β-adrenergic receptors associated with heart failure,[13] and down-regulation of matrix metalloproteinases.[14] Because of the documented dramatic changes

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in cardiac geometry and the increase in contractile function after LVAD support,[4,5] we hypothesized that additional transcriptional changes would participate in this reverse-remodeling process.

METHODS

Heart and Tissue Harvest

Under protocols approved by the institutional review boards of the New York Presbyterian Hospital and Temple University, we used for this study LV myocardium obtained from 19 patients with end-stage heart failure (CHF) and myocardium from 4 non-failing donor hearts. Of the CHF hearts, 12 had been supported with LVADs for an average of 59.7 ± 36.1 (range, 17–120) days. The non-failing hearts were unsuitable for transplantation for various reasons unrelated to hypertrophy. For patients with LVAD support, insertion and operation of the devices (Heartmate I, Thoratec; Woburn, MA) was performed as previously described.[15] We excluded regions of apparent infarct during sampling. Inflow to the LVAD is provided through a conduit inserted into the LV through an ~1-inch hole made in the apex. All samples were taken transmurally so that endocardial and epicardial differences would not contribute to the differences seen here. Tissue removed in making this hole was immediately frozen in liquid nitrogen and subsequently used for "pre-LVAD" analyses. At the time of transplantation, all hearts were perfused with 4°C hypocalcemic, hyperkalemic cardioplegia solution. Tissue taken after LVAD support and removal of the heart for transplantation was from the LV free wall (post-LVAD sample). To determine potential regional differences in gene expression unrelated to LVAD support, we analyzed free-wall and apex samples for gene expression levels from patients with CHF who were undergoing transplantation without LVAD support. In all cases, we froze tissue samples in liquid nitrogen immediately after excision. Normal human left ventricular cardiomyocytes and cardiofibroblasts were isolated as previously described.[16] Total RNA was extracted using RNA-STAT (Tel-test; Friendswood, TX).

cDNA Array Construction, Hybridization, and Data Analysis

Bacterial cultures of 2,700 cDNA pre-selected clones from 2 heart libraries, 1 heart subtracted library, various IMAGE clones (ResGen, an Invitrogen Corporation; Huntsville, AL), and 300 other relevant known gene clones were consolidated into 96-well plates. We performed custom array preparation, sequence verification, hybridization of labeled probes, and all subsequent data capture as previously described.[17] Briefly, ^{33}P-labeled cDNA was generated from total RNA[17] and hybridized to triplicate nylon arrays containing spotted polymerase-chain-reaction products. After hybridization and washing, we detected the signals using a phosphoimager and digitized signals using data-capture software. To normalize expression distributions across arrays, we transformed the data using a moving window that mean-normalized the expression levels within ranked bins of 100 clones. We used a permutation test that incorporated a global noise model to score each clone for statistical significance. To construct the global noise model, expression data were partitioned into ranked bins, based on the clone mean-expression level. We used a function relating expected variance to mean expression level as the global noise model. For each clone in each set of comparisons, the means of the samples compared were subtracted pair-wise. We determined within-group variance for each clone to model local noise of the data. To improve the accuracy of the noise estimation, we combined local and global expected variance estimates in a weighted function (0.8 and 0.2, respectively.) For each test, 100 random permutations (permuted across arrays) of the data were constructed for comparison with the observed data. We calculated the test statistic as the ratio of the difference of means and the combined noise estimates. For each of these 100 randomized data sets, a test statistic for each clone was computed, as was computed for the original data. For each clone, by comparing observed randomized data, we obtained false-positive rates as described elsewhere.[18] Pair-wise comparisons within the same patient were chosen to eliminate as many variables as possible. To determine fold change, we calculated the post/pre or free-wall/apex ratio for each patient and determined the median of all fold changes in each group. For genes that were represented by more than 1 clone, we report the median fold change and an adjusted p value.

To address the reproducibility of the results, we performed a transcription-profiling study of a smaller pre- and post-LVAD group and obtained results similar to the those presented here.

Slot Blot Northern Confirmation

We confirmed regulation using a slot blot Northern analysis. A dilution series of total RNA (1.8 μg–28 ng) was loaded directly onto a nylon membrane using a slot blot apparatus. The membrane was then pre-incubated with a sodium dodecylsulfate/Triton X-100–based nylon wash solution for 4 hours at 65°C and then hybridized overnight with a ^{33}P-labeled probe. After washing, the blot was exposed to a phosphoimager screen for 4 to 64 hours, depending on intensity detection levels, and then quantitated with a phosphoimager apparatus.