HEART FAILURE (HF) is the leading cause of morbidity and mortality in the industrialized world, with an estimated 5 million HF patients in the United States alone (5). Virtually any form of cardiovascular disease may culminate in HF, a final common pathway to mortality, through maladaptive left ventricular remodeling, which may begin before clinical symptoms become apparent (14, 29). Hence, early diagnosis of asymptomatic left ventricular dysfunction and timely intervention are critical to slowing or arresting the development of HF. While the clinical symptoms of decompensated HF are well described, it is difficult, if not impossible, for a physician to make a diagnosis when left ventricular dysfunction is in its earliest stages. Limited understanding of the molecular systems underlying ventricular dysfunction and the evoked systemic responses has hampered the early diagnosis of asymptomatic left ventricular dysfunction, timely intervention, and development of novel therapies for slowing HF development. Therefore, understanding of the molecular systems underlying the progression of left ventricular dysfunction to HF is essential to improvement of HF treatment.

One of the goals of functional genomics is to reveal the molecular systems responsible for development of multifactorial complex syndromes such as HF. Gene expression profiling has been used to identify differentially expressed genes between normal and end-stage failing human hearts (8, 27, 33). While such studies have improved our knowledge of transcriptional differences between normal and end-stage failing hearts, it remains largely unknown how the cardiac transcriptome responds to an index event and changes with progressive remodeling in HF development. Here, we aim to identify key molecular systems contributing to the development of HF by analyzing gene networks that are temporally correlated with remodeling of cardiac function initiated by rapid ventricular pacing, an intervention that induces LV dysfunction and HF. It is not possible to conduct controlled studies of HF progression in human subjects; thus we utilize a large mammalian HF model induced by rapid ventricular pacing in dogs that reproduces mechanical, electrophysiological, biochemical, and molecular changes similar to human HF (24).

MATERIALS AND METHODS

Overall experimental design. Transmural tissue sections from the anterior left ventricular wall in the left anterior descending coronary artery distribution were collected at different time points after initiation of rapid pacing to represent various stages of LV dysfunction, i.e., tachypaced for 3 days (D3), 1 wk (W1), 2 wk (W2), 3–4 wk (HF), and unpaced controls (D0). As biological replicates, myocardial specimens were collected separately from three individual dogs at each of the time points. In total, we collected 15 tissue samples for all five time points. Three RNA samples were independently isolated from each of the 15 tissue samples as technical replicates. The resulting 45 RNA samples were used for microarray experiments and subsequent data analysis. Cardiac functional measurements of action potential duration (APD), conduction velocity (CV) and left ventricular end diastolic pressure, and dP/dt(max) over the time course of rapid ventricular pacing. As a result, we present a phenotype-centered gene association network, defining molecular systems that correspond temporally to hemodynamic and electrical remodeling processes. Gene Ontology analysis revealed an orchestrated regulation of oxidative phosphorylation, ATP synthesis, cell signaling pathways, and extracellular matrix components, which occurred as early as 3 days after the initiation of ventricular pacing, coinciding with the early decline in left ventricular pump function and prolongation of action potential duration. The development of clinically overt left ventricular dysfunction was associated with few additional changes in the myocardial transcriptome. We conclude that the majority of tachypacing-induced transcriptional changes occur early after initiation of rapid ventricular pacing. As the transition to overt HF is characterized by few additional transcriptional changes, posttranscriptional modifications may be more critical in regulating myocardial structure and function during later stages of HF.

Microarray analysis. Heart samples were collected at multiple time points from the anterior left ventricular wall in the left anterior descending coronary artery distribution. RNA samples were used for microarray experiments and subsequent data analysis. Cardiac functional measurements of action potential duration (APD), conduction velocity (CV) and left ventricular end diastolic pressure, and dP/dt(max) over the time course of rapid ventricular pacing. As a result, we present a phenotype-centered gene association network, defining molecular systems that correspond temporally to hemodynamic and electrical remodeling processes. Gene Ontology analysis revealed an orchestrated regulation of oxidative phosphorylation, ATP synthesis, cell signaling pathways, and extracellular matrix components, which occurred as early as 3 days after the initiation of ventricular pacing, coinciding with the early decline in left ventricular pump function and prolongation of action potential duration. The development of clinically overt left ventricular dysfunction was associated with few additional changes in the myocardial transcriptome. We conclude that the majority of tachypacing-induced transcriptional changes occur early after initiation of rapid ventricular pacing. As the transition to overt HF is characterized by few additional transcriptional changes, posttranscriptional modifications may be more critical in regulating myocardial structure and function during later stages of HF.

Key pathways associated with heart failure development revealed by gene networks correlated with cardiac remodeling

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Hemodynamic variables, LVEDP and dP/dt(max) were assessed on each animal (Suppl. Table S1).

Canine pacing-induced HF protocol. HF was induced by rapid pacing from the right ventricle (RV) following the protocols approved by Johns Hopkins University Animal Care and Use Committee (IACUC no. DO04M538) (3). In brief, animals were intubated and anesthetized; blood oxygen saturation and expired CO2 were kept within the physiological range throughout the procedure. A VVI pacemaker (Medtronic) was implanted via a left lateral thoracotomy and the pacing lead was fixed to the RV epicardium under sterile conditions. Rapid pacing at 240 beats per min was initiated 1 to 2 days after surgery. Rapid pacing was terminated at different time points, i.e., 3 days, 1 wk, and 2 wk for intermediate stages of HF progression. End stage HF was verified by hemodynamic measurements (LV end diastolic pressure, dP/dtmax and -min) typically at 3–4 wk of rapid pacing. Transmural left ventricular tissues were harvested by left lateral thoracotomy and immersed in ice-cold saline. Tissue sections were quickly frozen in liquid nitrogen and were kept in –80°C. All the dogs in this experiment were male and age-matched.

Hemodynamic and electrophysiological measurement and analysis. Hemodynamic variables, LVEDP and dP/dt(max) were recorded for all the dogs in the study as previously described at the time of death (3). AVC and CV were measured over the time course using optical action potential mapping (2). Biological replicates of three dogs were measured at each of the five time points. Principle component analysis (PCA) was conducted on the time course measurements of the hemodynamic and electrophysiological variables to characterize the relationship of the five stages as described below.

RNA isolation, microarray hybridization, and data acquisition. Myocardial RNA was prepared from canine ventricular tissues using TRIzol reagent (GIBCO BRL) and Qiagen RNeasy column (Qiagen). The processing of the samples and Affymetrix chip hybridization were performed following the manufacturer’s instructions (1). We used 5 μg of total RNA to synthesize first-strand cDNA using oligo-nucleotide probes with 24 oligo-DT plus T7 promoter as a primer (Proligo LLC, Boulder, CO) and the SuperScript Choice System (Invitrogen, Carlsbad, CA). Following purification, biotinylated antisense cRNA was generated through in vitro transcription using the BioArray RNA High Yield Transcript Labeling kit (ENZO Life Sciences, Farmingdale, NY). After fragmentation, 10 μg of fragmented, biotin-labeled cRNA from each sample was hybridized to an individual Affymetrix Canine Genome Array (Affymetrix, Santa Clara, CA). The Affymetrix GeneChip Canine Genome Array contains over 21,700 transcripts of 137.0, August 2003), dbEST (October 2003), and proprietary beagle sequence content licensed from LION Bioscience AG. Fluorescence was detected using the Affymetrix G3000 GeneArray Scanner and image analysis of each GeneChip was conducted through the GeneChip Operating System 1.1.1 (GCOS) software (Affymetrix) using the standard default settings. Quality control was performed on samples, hybridization, and chip images according to Affymetrix requirements (1).

Microarray data analysis. Microarray data were normalized using the MAS5.0 algorithm. Probe sets with “absent” calls in >50% of all tissue samples were filtered out. We used the significance analysis of microarrays software (SAM, two-class or multiclass mode) to identify genes differentially expressed over the time course of HF progression with high statistical significance (31). The SAM method provides the false discovery rate (FDR) estimate, using permutations of repeated measurements, to a set of gene-specific t-tests (http:// www-stat.stanford.edu/~tibs/SAM). Hierarchical clustering was performed by using an average linkage algorithm with Euclidean distance measure incorporated in the Genesis software package (28).

PCA was conducted on the time course of the gene expression profiles of 1,185 genes identified by SAM to characterize the relationship of the five stages described previously. PCA was implemented using PROC PRINCOMP of SAS software (SAS Institute, Cary, NC). To characterize patterns of the five stages, the stages were plotted in two-dimensional spaces defined by PC1 vs. PC2 and PC2 vs. PC3, respectively. Functional classification was based on KEGG pathways and the “cellular component” and “biological process” of Gene Ontology (GO) (7) implemented in “FatiGO+,” a publicly available web tool for finding significant associations of GO terms within groups of transcripts (4).

The transcriptomic profiles of rapid ventricular pacing-induced HF of the current study were compared with a recent microarray study examining the myocardial gene expression during the transition from compensated to decompensated heart failure in canines (23). This study was downloaded from Gene Expression Omnibus (GEO accession number GSE5247) and analyzed similarly to the current study (GSE9794).

Construction of APD-centered gene association network for HF progression. An APD-centered gene association network was constructed, using a two-step approach, to explore relations between genes whose expression shares a temporal association with the APD over the development of pacing-induced HF. First, pairwise correlation coefficients were calculated to measure associations between expression changes of a gene and APD changes over the time course. With a correlation coefficient threshold r > 0.90 or r < −0.90, a total of 317 probe sets, including 105 known genes, were identified to be temporally correlated with APD changes in HF progression (Suppl. Table S4). Here we focus on analysis of the 105 genes of known function; these transcripts compose the APD-centered gene association network for further analysis. In the second step, pairwise correlation coefficients were computed for all pairs of transcripts in the network based on time course changes. Associations between the genes within the network were identified based on correlation coefficient thresholds >0.95 or < −0.95; we assume that a temporally correlated gene pair signifies a potential functional relationship. A more stringent criterion was applied in the second step resulting from a scaling up of the number of comparisons between gene pairs. This was implemented with SAS statistical software (SAS Institute). The generated network is represented by an association matrix with each element being the association of a pair of genes (1 for positive correlation, −1 for negative correlation, 0 for no correlation). Two-dimensional clustering on rows and columns of the network matrix was conducted to identify network modules based on the topology of the network, using Cluster 3.0 and Java Treeview (10).

Western blotting. Protein preparation and Western blotting were conducted as previously described (12, 18). Incubations with primary antibody against SERCA2 (1:1,000 dilution; Affinity Bioreagents, Golden, CO) were performed overnight at 4°C to measure total SERCA2 protein.

Simulation of APD using a computational canine HF model. The impact of SERCA2 reduction on APD was simulated using a previously developed computational model of the canine ventricular myocyte action potential and intracellular Ca2+ handling (the code for the model is available at http://www.ccbm.jhu.edu/software/models.php) (32). This model was developed to reconstruct action potential and Ca2+ transients of both normal and failing canine ventricular myocytes and was constrained by cellular experimental data (32). In this study, reconstruction of APD prolongation at end-stage HF was done with the following model parameters: 50% downregulation of SERCA2 protein that was observed in this study and default changes for the other proteins in the original model, i.e., 62% downregulation of Ca2+-independent 4-aminopyridine-sensitive current iNa, 32% downregulation of the delayed rectifier current iKr, and 75% upregulation of current through the Na+-Ca2+ exchanger (NCX1) (32).
RESULTS

Functional gene classes associated with tachypacing-induced HF. Based on KEGG pathways, distinct functional classes were identified for transcripts differentially expressed between D0 and various pacing time points. Figure 1 shows the relative distribution of 9 KEGG pathways as the percentage of up- and downregulated transcripts that were statistically significant between D0 and D3 and W1+W2+HF hearts, respectively. It becomes evident that energy-deriving processes including oxidative phosphorylation and tricarboxylic acid (TCA) cycle were dramatically downregulated as early as D3, while cell signaling pathways, and extracellular matrix components were upregulated.

To identify a robust gene expression profile of canine tachypacing-induced HF, we performed sequential analysis of different time points and noted that the number of differentially expressed transcripts varied widely (Fig. 2B). Remarkably, pair-wise comparisons of nonfailing (D0) to various pacing stages (D3, W1, W2, and HF) resulted in a 5- to 10-fold greater number of differentially expressed transcripts than pair-wise comparison of time-points after pacing had been initiated (Fig. 2B and Suppl. Table S1), suggesting that the majority of transcriptional changes occur at very early stages of the disease. In agreement with this finding, a stage-specific Gene Ontology classification revealed that the gene expression changes characteristic of pacing-induced HF occurred as early as D3. Concordant with the marked downregulation of the KEGG pathways of “oxidative phosphorylation” and “TCA cycle” (Fig. 1), GO analysis based on biological processes confirmed a significant downregulation of transcripts encoding “cellular metabolic processes” and “catabolic processes.” Likewise, the functional gene classes of “response to stress,” “cell communication,” and “cell death” showed a marked dysregulation between failing and nonfailing hearts, but not when paced animals were compared with each other. Surprisingly, the dysregulation of these gene classes occurred shortly after pacing was initiated and reached a steady state after only 3 days of tachypacing. In contrast, comparison within the group of paced animals showed a 5- to 10-fold smaller number of changes at the gene expression level (Fig. 3A).

Similar changes were noted when GO analysis was based on functional gene classes of “cellular compartment” (Fig. 3B). Nuclear transcripts and endoplasmic reticulum genes, as well as mitochondrial transcripts, showed profound dysregulation between failing and nonfailing myocardium, but not during various pacing-induced HF stages (Fig. 3B).

![KEGG pathway analysis](http://physiolgenomics.physiology.org/)

Fig. 1. KEGG pathway analysis of significantly up- and downregulated genes in tachycardia-pacing induced heart failure (HF). Nine KEGG pathways are plotted as percentage of up- and downregulated transcripts that were statistically significant between nonfailing and HF myocardium. Transcripts up- and downregulated by ventricular tachypacing are represented by white and black columns, respectively. Studies (a) and (b) compare nonfailing animals (D0) to dogs that were paced for three days only (study a) or to later time points [W1+W2+HF, study (b)]. Data for study (c) was based on an independent microarray dataset (Gene Expression Omnibus accession no. GSE5247) that is publicly available, comparing nonfailing to failing ventricular myocardium. It becomes evident that energy-deriving processes including oxidative phosphorylation and TCA cycle were greatly downregulated as early as D3, while various cell signaling pathways and extracellular matrix components were upregulated. When data from the present study were compared with an independent microarray dataset (Gene Expression Omnibus accession no. GSE5247), we found a high concordance regarding disease-specific gene expression patterns between both studies, suggesting a common disease-specific genomic fingerprint.
Correspondence between transcriptional remodeling and key functional parameters associated with HF development. It remains an open question as to whether or not transcriptional and functional remodeling are associated or correlated with the progression of left ventricular dysfunction. To address this question, we measured hemodynamic and electrophysiological variables [end-diastolic pressure, dP/dt(max), APD, and CV] over time during pacing-induced HF (Fig. 4A). The relationships between the five time points were derived from joint analysis of the 1,185 differentially expressed genes and the

![Gene Ontology (GO) analysis](image)

Fig. 3. Gene Ontology (GO) analysis comparing nonfailing (D0) to various pacing stages (D3, W1, W2, and HF). The number of transcripts exhibiting altered expression between different stages in various GO biological processes (A) and cellular components (B) are plotted. A: stage-specific GO classification based on “biological processes” revealed that the gene expression changes characteristic of pacing-induced HF occurred as early as D3. Corresponding to the marked downregulation of the KEGG pathways of oxidative phosphorylation and TCA cycle, GO analysis identified a significant downregulation of transcripts encoding for “cellular metabolic processes” and “catabolic processes.” Likewise, functional gene classes of “response to stress,” “cell communication,” and “cell death” showed marked dysregulation between failing and nonfailing hearts, but not when comparing only paced animals. B: similar changes were noted when GO analysis was based on functional gene classes of “cellular compartment.”
functional data using PCA. In the PCA gene expression-based patterns of the five stages, all four disease stages are closely clustered while the D0 stage is distinct in the PC1-PC2 plot, (Fig. 4B3); in the PC2-PC3 plot, the pattern of relations among the four HF stages is associated with PC3 while PC2 separates normal from other HF stages (Fig. 4B4). These projection patterns are remarkably similar to that determined from PCA of the time course of hemodynamic and electrophysiological variables (Fig. 4, B1 and B2). Moreover, both patterns show that the control D0 stage is distinct from any pacing stages irrespective of degree of LV dysfunction.

APD-centered gene association network involved in HF development. To characterize functional relations between genes in HF progression, we constructed what we refer to as phenotype-centered gene association networks. Phenotype-centered association networks characterize associations between genes whose expression correlates with the time evolution of phenotypic changes. APD prolongation is the hallmark cellular electrophysiological feature of HF (29, 30). APD measurements in the canine HF model show that the APD is significantly prolonged as early as the D3 pacing stage and exhibits no further change after the W1 stage (Fig. 4A) (2). An APD-centered gene association network was constructed to explore associations between genes whose expression shares a similar temporal pattern with APD changes in pacing-induced HF progression. Pairwise associations of genes in this network are plotted in the gene-gene matrix in Fig. 5A (additional phenotype-centered networks and their relations are shown in Suppl. Fig. S1). Analysis of network topology provides us an opportunity to explore the functional implications of the network modules.

The cluster of positively correlated genes in module 1 is central to the topology of the APD network (Fig. 5A); expression changes of all the genes in this module are inversely correlated with APD changes in pacing-induced HF. The overwhelming majority of the genes in module 1 (25 out of 31) involve major energy generation pathways–oxidative phosphorylation, ATP biosynthesis, fatty acid β-oxidation, and citrate cycle–and mitochondrial protein translation (Fig. 5B). The genes encoding components of the protein complexes in the electron transport chain (complex I, III, and IV), ATP synthase complex, and mitochondrial ribosomal complex are linked based on the concordant gene expression patterns.

Network modules 2 and 4 consist of a cluster of genes positively correlated with APD prolongation in HF progression. Modules 2 and 4 include genes involved in signal transduction as well as transcriptional and translational regulation. About half of the genes in module 3 are involved in the cytoskeleton and cell-cell adhesion: myosin light chain alkali smooth muscle isoform (MLC3SM), nectin-like protein 2
Sarcoplasmic reticulum membrane, central to Ca^{2+} handling, can be found in the supplement (Suppl. Table S1). The list of the genes of this APD-centered gene association network representing homeostasis, is represented in the APD-centered network to explore their functional relations (Fig. 6, D and E). Most importantly, about half of the genes associated with SERCA2 (18 out of 37) were again directly linked to oxidative phosphorylation, ATP synthesis, fatty acid β-oxidation, TCA, and mitochondrial ribosomal complex. This suggests a coordinate dysregulation shared by SERCA2 and energy pathways/protein translation machinery during pacing tachycardia-induced HF.

It is well known that altered functional expression of SERCA2 plays an important role in the abnormal Ca^{2+} homeostasis associated with HF; however, the mechanisms underlying SERCA2A dysregulation are not fully understood. We extracted SERCA2 and its associated genes from the APD-centered network to explore their functional relations (Fig. 6, D and E). Most importantly, about half of the genes associated with SERCA2 (18 out of 37) were again directly linked to oxidative phosphorylation, ATP synthesis, fatty acid β-oxidation, TCA, and mitochondrial ribosomal complex. This suggests a coordinate dysregulation shared by SERCA2 and energy pathways/protein translation machinery during pacing tachycardia-induced HF.

**DISCUSSION**

HF is a progressive clinical syndrome characterized by myocardial remodeling in response to stress signals. Detailed characterization of the molecular processes associated with the progression of HF is essential to the development of new therapeutic strategies. Taking advantage of a well-controlled animal model of canine tachycardia-induced HF that mimics key features of human HF, including cardiac dilatation, impairment of contractile function, APD prolongation, and sudden cardiac death (22), we performed gene expression profiling using transmural LV tissue from nonfailing animals and dogs that were tachycardia-paced for different time periods. The genomic HF profile observed in this model is characterized by predominant downregulation of metabolic processes, while stress-response and profibrotic pathways are upregulated (Fig. 1). Thus, the genomic alterations are consistent with our previous findings (15) as well as other independent studies (17, 23).

The key question that we examined herein is how the cardiac transcriptome responds to an index event and changes over time with progressive ventricular dysfunction. We found that the characteristic genomic changes in this model were evident after as little as 3 days of pacing and paralleled the functional changes of contractile dysfunction and electrical remodeling. This is exemplified by KEGG and GO analyses, demonstrating that changes in gene expression for all major classes characteristic of the genomic signature of HF occurred already at D3 (Figs. 1 and 3). The most prominent transcriptional changes affect metabolic processes, such as oxidative phosphorylation and TCA cycle. Given the increased \(O_2\) consumption and energy demand due to fast pacing rates, the pronounced downregulation of transcripts encoding for mitochondrial and TCA enzymes seems paradoxical, as these changes can be envisaged to exacerbate the increased energy demand caused by ventricular tachypacing. Yet, reductions in specific mitochondrial respiratory enzyme activities represent a central pathophysiological feature in pacing-induced HF (21). Several lines of evidence suggest that the bioenergetic deficit might be a computational model of the canine ventricular myocyte action potential and intracellular Ca^{2+} handling (32). The simulation revealed that a 50% decrease of SERCA2 protein at HF end-stage was associated with 100 ms increase in APD (Fig. 6C). These results suggest that SERCA2 expression on both the mRNA and protein levels decreased during the course of rapid pacing-induced HF progression, contributing to prolongation of APD.

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common downstream event in the pathways leading to the HF phenotype. Ventricular tachypacing has been shown to increase proinflammatory cytokines, including TNF-α and IL-1β, which leads to the generation of reactive oxygen species (ROS) in mitochondria and early impairment in mitochondrial function (25, 34). In addition to free radical-induced damage in myocardium of paced animals, as evidenced by markedly increased levels of left ventricular tissue aldehyde levels (21), a decreased GSH/GSSG ratio and increased level of oxidation products of mitochondrial DNA (19), ROS could act as molecular intermediates in the translation of electrical/mechanical stimuli to a cellular response. These effects could be mediated by activation of different ROS-sensitive signal transduction pathways, including MAPK, JAK-STAT, and TGF-β signaling pathways (13, 16), all of which were found to have an increased transcriptional activity in the present study (Fig. 1). A contributory role of a systemic activation of cell signaling pathways in mitochondrial dysfunction is also suggested by the fact that mitochondrial dysfunction in pacing-induced HF is not only limited to the heart but is also evident in skeletal muscle (21). The activation of these signaling pathways early in the course of the disease provides a rationale for early pharmacological interventions aimed at blocking these cell signaling cascades. It is tempting to speculate that the im-

Fig. 6. SERCA2 in the APD-centered gene association network. A: change in SERCA2 mRNA over the time course of HF progression, measured by microarray. SERCA2 was identified in the APD network due to its temporal association with the change in APD over the time course of pacing. The difference in RNA level between D0 and end stage was statistically significant (FDR < 0.5%, SAM analysis). B: change in SERCA2 protein (average of SERCA2 protein from transmural sections of left ventricle) over the time course, with a representative Western blot below the bar plot. Wilcoxon test indicates that SERCA2 protein level was significantly different between D0 and end stage (P = 0.012). C: functional contribution of reduced SERCA2 to APD prolongation. The normal and HF canine myocyte models were used to simulate the changes APD in response to SERCA2 reduction. The blue line represents ADP at end-stage HF with a 50% reduction of SERCA2 protein. D: genes associated with SERCA2 in the APD-centered network. The orange nodes represent genes positively correlated with SERCA2, and green nodes represent genes negatively correlated with SERCA2. E: schematic of multiple pathways involved in SERCA2 subnetwork. The genes in the SERCA2 subnetwork are related to energy generation, mitochondrial protein translation, Ca²⁺ homeostasis, ER protein folding and transport, and hypertrophic signaling.
proved clinical outcomes, observed after early treatment with beta-blockers or ACE inhibitors might not only be related to modulation of neurohumoral blockade but also to significant antioxidant and antiapoptotic effects of these drugs (19).

HF is characterized by progressive structural and electrical remodeling processes. The early genomic changes in tachypacing-induced HF paralleled changes in electrophysiological remodeling, with action potential prolongation reaching a plateau during the first week. The temporal correlation between gene expression profiles and electrophysiological measurements suggest a mechanistic interdependence (Fig. 4). By developing an APD-centered gene association network, we have identified key pathways associated with transcriptional remodeling in the development of tachypacing induced HF, including an orchestrated regulation of major energy generation pathways, transcriptional and translational regulation, Ca\(^{2+}\) homeostasis, protein folding/transport, and hypertrophic signaling. Of note, the largest group of genes in this network encoded for processes involved in cellular energy generation, thus linking altered cellular electrophysiology to metabolic activity (module 1, Fig. 5B). It can be envisaged that the drop in high energy phosphates observed in pacing-induced HF (26) has important consequences on ion homeostasis, as intracellular Na\(^{+}\) and Ca\(^{2+}\) concentrations are maintained at low levels by energy-intensive processes, involving the sarcosomal Na\(^{+}\)-K\(^{+}\) pump and the sarcoplasmic reticulum Ca\(^{2+}\) pump (SERCA2). To our knowledge, this is the first report linking changes in action potential duration and SERCA2 mRNA transcript levels to metabolic activity in left ventricular dysfunction. The APD-centered network can provide a unique opportunity to conduct case studies on individual genes to explore their associations with functional partners. As a preliminary functional validation, we studied the temporal pattern of SERCA2 protein expression and APD prolongation.

Sequential comparisons between the pacing tachycardia-induced HF stages suggest that major transcriptional events may occur early after initiation of ventricular tachypacing. The development of clinically overt HF was associated with only limited further changes in the myocardial transcriptome. Considering the profound phenotypic alterations of the failing heart, this finding seems surprising, however, it is in agreement with studies showing that the transition from compensated to decompensated HF during chronic ventricular tachypacing in dogs is associated with only few transcriptional changes (6, 23). Our study extends this observation, demonstrating that ventricular tachypacing-associated transcriptional changes occur very early after initiation of pacing, setting the stage for maladaptive remodeling processes and the subsequent development of HF. Therefore, early intervention may hold great promise to slow disease progression in HF. Of interest, first studies aimed at activating peroxisome proliferator-activated receptor-α by fenofibrate to reverse downregulation of enzymes of free fatty acid oxidation have shown promising results (9, 20). In this respect, global gene expression profiling holds great promise to identify additional key pathways in the genesis of HF to develop new therapeutic strategies.

**Study Limitations**

In the present study, we noted a striking correlation of transcriptional changes with the decline in pump function and prolongation of the action potential in a pacing model of HF. Importantly, correlations do not prove cause and effect. Therefore, our study cannot prove a direct link between the transcriptional changes observed and APD prolongation or the decline in pump function.

As our study protocol did not include animals prior to day 3, we cannot differentiate between transcriptional changes caused by tachycardia vs. HF. For instance, we cannot exclude that pacing itself contributed to the initial action potential prolongation observed in our present study, especially as APD prolongation and remodeling of ionic currents has been shown to occur within minutes after instituting ventricular pacing even at slower rates (11). Future studies are needed focusing on the transcriptional changes prior to the development of APD prolongation and compromise in LV function. These studies will be critical to differentiate transcriptional changes associated with altered electrical activation of the ventricles and dysynchronous mechanical contraction independent of HF.

The rapid pacing model is a widely used animal model of nonischemic HF. However, it must be recognized that this model will not fully represent the complex clinical spectrum of congestive heart failure. Even though pacing-induced HF shares major electrophysiological (APD prolongation, high incidence of sudden cardiac death, atrial arrhythmias), morphological (biventricular dilatation), and functional (depressed contractility) hallmarks of human HF, it might not mimic all features of human HF, as the changes in myocardial structure occurring with tachypacing are dissimilar to clinical forms of HF due to chronic ischemia or hypertensive disease. Thus, extrapolation of the findings from this HF model to clinical forms of HF should be done with caution.

In summary, our study identifies several key pathways, including energy generation and metabolism, Ca\(^{2+}\) homeostasis, and cell signaling, associated with myocardial remodeling during the progression of tachypacing-induced HF. Most importantly, the changes in contractility, electrophysiology and transcriptional activity show temporal correspondence in this well-controlled large animal model of HF. This study provides unique insights into transcriptome-wide and dynamic molecular processes underlying HF progression.

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