Microarray analysis of active cardiac remodeling genes in a familial hypertrophic cardiomyopathy mouse model rescued by a phospholamban knockout

Sudarsan Rajan,1 James R. Pena,2 Anil G. Jegga,3 Bruce J. Aronow,3 Beata M. Wolska,2 and David F. Wieczorek
1Department of Molecular Genetics, Biochemistry, & Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio; 2Department of Medicine, Section of Cardiology and Department of Physiology and Biophysics, Center for Cardiovascular Research, University of Illinois, Chicago, Chicago, Illinois; and 3Division of Biomedical Informatics, Children’s Hospital Medical Center, Cincinnati, Ohio

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Rajan S, Pena JR, Jegga AG, Aronow BJ, Wolska BM, Wieczorek DF. Microarray analysis of active cardiac remodeling genes in a familial hypertrophic cardiomyopathy mouse model rescued by phospholamban knockout. Physiol Genomics 45: 764–773, 2013. First published June 25, 2013; doi:10.1152/physiolgenomics.00023.2013.—Familial hypertrophic cardiomyopathy (FHC) is a disease characterized by ventricular hypertrophy, fibrosis, and aberrant systolic and/or diastolic function. Our laboratories have previously developed two mouse models that affect cardiac performance. One mouse model encodes an FHC-associated mutation in α-tropomyosin: Glu → Gly at amino acid 180, designated as Tm180. These mice display a phenotype that is characteristic of FHC, including severe cardiac hypertrophy with fibrosis and impaired physiological performance. The other model was a gene knockout of phospholamban (PLN KO), a regulator of calcium uptake in the sarcoplasmic reticulum of cardiomyocytes; these hearts exhibit hypercontractility with no pathological abnormalities. Previous work in our laboratories shows that when mice were genetically crossed between the PLN KO and Tm180, the progeny (PLN KO/Tm180) display a rescued hypertrophic phenotype with improved morphology and cardiac function. To understand the changes in gene expression that occur in these models undergoing cardiac remodeling (Tm180, PLN KO, PLN KO/Tm180, and nontransgenic control mice), we conducted microarray analyses of left ventricular tissue at 4 and 12 mo of age. Expression profiling reveals that 1,187 genes changed expression in direct response to the three genetic models. With these 1,187 genes, 11 clusters emerged showing normalization of transcript expression in the PLN KO/Tm180 hearts. In addition, 62 transcripts are highly involved in suppression of the hypertrophic phenotype. Confirmation of the microarray analysis was conducted by quantitative RT-PCR. These results provide insight into genes that alter expression during cardiac remodeling and are active during modulation of the cardiomyopathic phenotype.

Familial hypertrophic cardiomyopathy (FHC) is associated with mutations in contractile proteins of the cardiac sarcomere, Z-disc proteins, and Ca2+-handling proteins (5). α-tropomyosin (Tm), a thin filament protein of the sarcomere, has been found to encode 11 mutations that lead to FHC (23). Previous work in our laboratories established a transgenic mouse model expressing mutant FHC Tm180 protein (1, 7, 16, 17). This mouse develops severe concentric cardiac hypertrophy with significant ventricular fibrosis and atrial enlargement that is detected by 2 wk. These disease-associated changes progressively increase with significant physiological alterations in cardiac function, including diastolic dysfunction in addition to myofilaments that demonstrate an increased activation of the thin filament through enhanced calcium sensitivity of steady-state force.

In addition to the FHC Tm180 model, a gene knockout (KO) model of phospholamban (PLN) was also developed (10). PLN is a sarcoplasmic reticulum (SR) protein that negatively regulates Ca2+ reuptake by the SERCA2a pump in its unphosphorylated state, and thus knocking out PLN is expected to reduce Ca2+ availability to the sarcomere. These PLN KO mice do not exhibit any gross morphological or pathological abnormalities in the heart. In fact, physiological alterations in cardiac performance show there is enhanced myocardial contractility, coupled with increased relaxation. These physiological changes are due to an increased resequestration of calcium into the SR.

Several studies demonstrate that the FHC phenotype can be modulated through alterations in calcium handling by the myofilaments and calcium buffering (2, 4, 8, 15, 22, 24). Recently, we examined whether modification of SERCA2a activity and Ca2+ handling would rescue the hypertrophic phenotype in the Tm180 mice (4, 7, 8, 13). To test the hypothesis that increasing the rate of calcium resequestration into the SR through PLN ablation would attenuate the FHC pathological and physiological phenotype, we crossed the FHC Tm180 mice with PLN KO mice (7). Results show there is a dramatic improvement in the FHC phenotype in the PLN KO/Tm180 progeny. The hearts of these PLN KO/Tm180 rescued mice demonstrate normalization of hypertrophic gene markers, normalization of cardiac function as assessed by in situ pressure-volume analysis and echocardiography, and decreased fibrosis and adverse cardiac remodeling associated with hypertrophic cardiomyopathy (7).

The rescue of the Tm180 phenotype when mated with PLN KO mice offers a unique opportunity to examine the molecular

Address for reprint requests and other correspondence: D. F. Wieczorek, 231 Albert Sabin Way, Cincinnati, OH 45267-0524 (e-mail: David.Wieczorek@uc.edu).
changes in cardiac gene expression that occur with the attenuated cardiac hypertrophy. We tested the hypothesis that model-specific gene expression changes can provide insight into cardiac disease and remodeling. To examine the gene expression profiles of the two mouse models (Tm180 and PLN KO) and the genetically crossed mice (PLN KO/Tm180), we conducted extensive microarray analyses using nontransgenic (NTG), Tm180, PLN KO, and PLN KO/Tm180 left ventricular tissue mRNAs. Overall, there were 1,187 genes (from a total of 28,869 genes that were probed) that had significant changes in expression at a 1.3-fold level in response to the Tm180 and/or PLN KO genotypes (P < 0.01). Quantitative RT-PCR analyses validated the results of the microarray analyses. The differentially regulated genes (1,187 genes) were then subject to enrichment analysis using Topclust (9), a web server application, and 11 different clusters were generated based on the phenotypic changes that occurred among the four genotypes. Of the 11 clusters, we chose three (clusters 3, 4, and 5) where there was a marked downregulation of gene expression in PLN KO/Tm180 mice from the Tm180 mice, and then we created a connectivity network of linked biological pathways involved in the rescue of the hypertrophic phenotype. An additional more stringent analysis identified the most significant 62 genes involved in suppression of the hypertrophic phenotype. Many of these genes are associated with cardiac hypertrophy, the vasculature, and/or inflammation. As such, these results reveal an intricate pathway of co-regulated genes that are involved in the rescue of cardiac hypertrophy in the PLN KO/Tm180 mouse hearts.

MATERIALS AND METHODS

PLN KO, Tm180, and PLN KO/Tm180 mouse. A comprehensive description of the generation and analyses of the PLN KO, Tm180, and PLN KO/Tm180 mice has been documented in our previous studies (7, 10, 16). The Institutional Animal Care and Use Committee approved the handling and maintenance of animals. Ventricular tissue RNA from individual 4 and 12 mo old mice was isolated, and total RNA was prepared using the Trizol method (Invitrogen) followed by purification using the RNeasy Cleanup kit (Qiagen). These ages were chosen to study the effect of established disease and adverse cardiac remodeling on gene expression over time. (Changes in early gene expression associated with developing cardiomyopathy may be missed with the current experimental design.) The quality of the total RNA was analyzed with an Agilent Bioanalyzer 2100 (Hewlett Packard) using the RNA 6000 Nano Assay to ensure that samples prepared for microarray hybridization meet the Affymetrix guidelines. Microarray hybridization and data analysis. Gene expression profiling was performed on 24 individual mouse ventricular tissue total RNA samples that were collected from three mice each from each genotypic group (NTG, α-Tm180, PLN KO, and PLN KO/Tm180) at 4 and 12 mo of age. Standard Affymetrix Whole Transcript Sense Target Labeling Assay (Affymetrix) ST labeling kits were used to convert 100–300 ng of total RNA into biotin-labeled, complementary RNA (cRNA) that were each hybridized to Affymetrix GeneChip Mouse Gene 1.0 ST whole genome arrays, which enables profiling of 28,869 genes via ~26 probes derived from sequences spread across the full length of the gene. With the FHC α-Tm180 mice, we have not observed sex-biased differences in the FHC phenotype. Images were scanned using a GeneArray scanner (Agilent Technologies, Palo Alto, CA) and GeneChip cel files were subsequently processed by RMAExpress 0.1 (http://rmaexpress.bmbolstad.com) using the default options and the Affymetrix version 31 CDF chip definition file. RNA data were loaded into GeneSpring 7.3.1 software (Agilent Technologies, Santa Clara, CA), and differentially expressed genes were identified compared with the baseline for each gene relative to that of its median level expression across the NTG samples. Raw and robust multichip average (RMA) experimental data were deposited in the National Center for Biotechnology Information’s Gene Expression Omnibus (GEO) repository and are accessible through GEO Series accession number GSE42892 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42892). Gene expression in the mutant (Tm180, PLN KO, and PLN KO/Tm180) mouse hearts was examined relative to the average NTG heart. To do this, we used MAExpress, which normalizes gene expression in three steps: a background adjustment, quartile normalization, and finally summarization. Log-scaled gene expression levels for each probe set of each sample were transformed to ratios relative to the mean of expression level values in the NTG heart. Thus, a ratio of 1 represents an equivalent level of expression in mutant and NTG hearts. To be sensitive to expression changes in a less than majority fraction of cell types in the ventricle, we filtered for genes that exhibited at least a 1.3× change or greater between two specific genotypic groups; with P ≤ 0.01 (ANOVA Student’s t-test as implemented in GeneSpring). Furthermore, we also performed additional analysis using a fold change of at least 1.4 using similar criteria. From the list of genes obtained, a Student’s t-test with a P value cut-off of 0.01 was performed to identify genes that were significantly regulated between groups being compared. These results were pooled and subject to hierarchical clustering using a Pearson correlation. Topcluster (9) and Cytoscape (21) were used to visualize the biological pathways network analysis.

RESULTS

To examine the gene expression profiles of the FHC Tm180 and the cardiac hypercontractile PLN KO mice, along with the genetically crossed PLN KO/Tm180 mice, we conducted an extensive microarray analyses. Total RNAs from left ventricular tissue of 4 and 12 mo old hearts were isolated for use in gene expression matrices. The advantage of comparing gene expression profiles in the PLN KO and Tm180 models at two distinct time intervals is the potential to identify model-specific molecular events and how those changes in gene expression may vary over time and disease progression. Furthermore, by examining the PLN KO/Tm180 hearts, changes in gene expression that may potentially modulate or rescue the FHC phenotype can be identified. Thus, we tested the hypothesis that model-specific gene expression changes can provide insight into cardiac disease and remodeling. Results reveal there is significance divergence in gene expression among the four models, with a total of 1,187 differentially expressed genes being in the hearts of the NTG and three mouse models.
the PLN KO genotype elicits a dominant effect on many genes
matrix and ion regulation. As such, in has many genes associated with the cytoskeletal/extracellular
Cluster 5
myofibrils, cytoskeletal proteins, and hypertrophy. with inflammation, and
Cluster 4
contains numerous genes that are associated with membranes and fatty
Clusters 6, 7, 8, and 9 (55 genes) show patterns of expression where genes in the
Tm180 hearts have lower levels of expression than the levels in the PLN KO/Tm180 hearts. Many of the genes in these clusters are associated with the metabolism, membranes, amino acid metabolic processes, and amino acid biosynthesis. Thus, these genes demonstrate dominant expression of the PLN KO genotype over the Tm180 hearts by increased expression. This decreased expression of genes associated with metabolic pathways in the hypertrophic Tm180 hearts is in agreement with our previous work (18).

Gene clusters 2 (103 genes), 8 (112 genes), and 11 (45 genes) display minimal changes in gene expression from the Tm180 to the PLN KO/Tm180 genotype (Fig. 2, green lines). These clusters show gene expression levels that are below NTG levels in the hearts of all three models. Many of the genes in clusters 2 and 8 are membrane-associated proteins and involved with lipid and fatty acid metabolism. Cluster 11 shows a pattern of genes expressed at NTG control levels with decreased expression only at 12 mo in the three models. Many of the genes in these clusters are also associated with membranes and fatty acid metabolism.

Table 1. Primers used for real-time quantitative PCR

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>GenBank Accession No.</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>NM_008084</td>
<td>forward</td>
<td>TGACCCAGATCGATGCTGATC</td>
</tr>
<tr>
<td>α-MHC</td>
<td>NM_010856</td>
<td>forward</td>
<td>GACGACAGACATTGGGGTQAG</td>
</tr>
<tr>
<td>β-MHC</td>
<td>NM_080728</td>
<td>forward</td>
<td>ACAATCTCCTGGGCCACCTTC</td>
</tr>
<tr>
<td>OSF-2</td>
<td>NM_015784</td>
<td>reverse</td>
<td>GTCAATCTGAGGGGTGTGTTC</td>
</tr>
<tr>
<td>α-Sk. actin</td>
<td>NM_009606</td>
<td>forward</td>
<td>GCTCTGCAGGCAATTTGAG</td>
</tr>
<tr>
<td>ANF</td>
<td>NM_008725</td>
<td>reverse</td>
<td>GGCCAGTAGAGACATCATTT</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>NM_009367</td>
<td>forward</td>
<td>TCGACATGAGACATTGGGG</td>
</tr>
<tr>
<td>Edn3</td>
<td>NM_007903</td>
<td>forward</td>
<td>GCCCTGATCTTTTGTAGATGG</td>
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<tr>
<td>Thbo4</td>
<td>NM_011582</td>
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<td>AGCGCTGAAAGACSCATC</td>
</tr>
<tr>
<td>IL6</td>
<td>NM_031168</td>
<td>reverse</td>
<td>TTGGCTCAGTCCAGGAAACCC</td>
</tr>
<tr>
<td>Gsta1</td>
<td>NM_008181.3</td>
<td>forward</td>
<td>ACGCTGATGTCATACGAGAAAC</td>
</tr>
<tr>
<td>C3</td>
<td>NM_009778</td>
<td>reverse</td>
<td>GCCATTGGGCCATATTGGAG</td>
</tr>
</tbody>
</table>

MHC, myosin heavy chain; OSF, osteoblast-specific factor; Sk. actin, skeletal actin; ANF, atrial natriuretic factor; TGF, transforming growth factor; Edn, endothelin; Thbs, thrombospondin; IL, interleukin; Gsta, glutathione s-transferase; C, complement component.

(1) These are genes that display the greatest changes in cardiac expression from all four genotypes at both ages (4 and 12 mo). Expression was increased or decreased by at least 1.3-fold (P < 0.01, in at least three experiments). Hierarchical clustering (Pearson correlation) was applied to the expression profile of the 1,187 genes. These data are represented by a “gene tree” in which genes of most similar expression patterns are closest to one another within a node of a tree (Fig. 1).

We grouped the 1,187 genes according to their responses in expression with respect to their genotype and changes that occur with age. Figure 2 shows 11 distinct cluster patterns. Gene clusters 1 (107 genes), 3 (78 genes), 4 (140 genes), and 5 (148 genes) show patterns where highly expressed Tm180 genes have attenuated expression due to the effects of the PLN KO background (Fig. 2, blue lines). This expression pattern is evident by the decrease in transcript levels for clusters 1, 3, 4, and 5 in the PLN KO/Tm180 hearts compared with the Tm180 alone. The genes affiliated with cluster 3 (120 mo) and 5 (4 and 12 mo) display the most significant decreases in expression between the Tm180 and PLN KO/Tm180 hearts, respectively. In all four of the clusters, the lowest level of gene expression occurs in the NTG genotype, thereby showing increased mRNA levels for the other three genotypes. Many of these “hypertrophic” responsive genes in cluster 1 are associated with the extracellular matrix and structural components of the cell. Cluster 3 contains numerous genes that are associated with inflammation, and cluster 4 has genes associated with myofibrils, cytoskeletal proteins, and hypertrophy. Cluster 5 has many genes associated with the cytoskeletal/extracellular matrix and ion regulation. As such, in clusters 1, 3, 4, and 5, the PLN KO genotype elicits a dominant effect on many genes expressed in the Tm180 hearts by decreasing expression from the levels observed in Tm180 hearts alone. It is not surprising that with the rescue of the hypertrophic phenotype in the PLN KO/Tm180 mice, there is decreased expression of genes associated with the extracellular matrix/cytoskeleton, inflammation, and myofibrils from Tm180 levels. The PLN KO/Tm180 mice exhibit decreased fibrosis, inflammation, and cardiac hypertrophy, which are all prominent features of the Tm180 hearts (7, 16, 17).

Gene clusters 6 (123 genes), 7 (152 genes), 9 (124 genes), and 10 (55 genes) show patterns of expression where genes in the Tm180 hearts have lower levels of expression than the levels in the PLN KO/Tm180 hearts (Fig. 2, red lines). In addition, genes in these clusters are more highly expressed in the PLN KO/Tm180 hearts than in the Tm180 or PLN KO/Tm180 hearts. Many of the genes in these clusters are associated with the metabolism, membranes, amino acid metabolic processes, and amino acid biosynthesis. Thus, these genes demonstrate dominant expression of the PLN KO genotype over the Tm180 hearts by increased expression. This decreased expression of genes associated with metabolic pathways in the hypertrophic Tm180 hearts is in agreement with our previous work (18).

Gene clusters 2 (103 genes), 8 (112 genes), and 11 (45 genes) display minimal changes in gene expression from the Tm180 to the PLN KO/Tm180 genotype (Fig. 2, green lines). These clusters show gene expression levels that are below NTG levels in the hearts of all three models. Many of the genes in clusters 2 and 8 are membrane-associated proteins and involved with lipid and fatty acid metabolism. Cluster 11 shows a pattern of genes expressed at NTG control levels with decreased expression only at 12 mo in the three models. Many of these genes are also associated with membranes and fatty acid metabolism.

1 The online version of this article contains supplemental material.
Fig. 1. Hierarchical cluster analysis of the 1,187 hypertrophic-responsive genes. Heat-map representation of transcriptome analyses that displayed the greatest changes in expression in the hearts from all 4 genotypes at both ages (4 and 12 mo). Expression was increased or decreased by at least 1.3-fold ($P < 0.01$) in at least 3 experiments. Colors show the range of expression from blue (decreased expression) to yellow (increased expression).
To identify the gene transcripts undergoing the most significant changes in expression, we conducted an additional analysis of the 1.3-fold gene expression data by focusing on a more significant 1.4-fold change in gene expression levels. Results show that 464 transcripts differ in expression at the 1.4-fold level between NTG vs. PLN KO, Tm180, or PLN KO/Tm180 genotypes at 4 and 12 mo (Supplemental Table S2). At 4 mo, there are 220 altered mRNAs expressed between NTG hearts vs. PLN KO, 388 mRNAs vs. the Tm180, and 379 transcripts vs. PLN KO/Tm180 genotypes; 271 of the gene transcripts are the same between Tm180 and PLN KO/Tm180 genotypes. At 12 mo of age, 117 mRNAs differ between NTG and PLN KO genotypes, 321 mRNAs vs. Tm180, and 259 mRNAs vs. PLN KO/Tm180 genotypes. Between Tm180 and PLN KO/Tm180 groups, there are 62 mRNAs that differ at the 1.4-fold level of expression at 12 mo of age; these transcripts represent genes that are potentially involved in the suppression of the hypertrophic cardiac phenotype. Hierarchical clustering was applied to the expression profile of these 62 genes and is represented by a gene tree in Fig. 3. In this figure, it is apparent from the colors...
indicating different expression levels that $\frac{2}{3}$ of the genes are more highly expressed in the Tm180 vs. the PLN KO/Tm180 hearts.

In examining the biological function of the 62 genes that are most highly expressed between the Tm180 and PLN KO/Tm180 genotypes at 12 mo of age, six are often associated with heart failure and cardiac hypertrophy: complement component 3 (C3), PLN, interleukin 6 (IL6), histone deacetylase 5 (HDAC5), alpha-2-HS-glycoprotein (AHSG), and aquaporin (AQP1). Three of these genes (C3, IL6, AHSG) increase their expression in the Tm180 hearts, six are often associated with heart failure and cardiac hypertrophy: complement component 3 (C3), PLN, interleukin 6 (IL6), histone deacetylase 5 (HDAC5), alpha-2-HS-glycoprotein (AHSG), and aquaporin (AQP1). Three of these genes (C3, IL6, AHSG) increase their expression in the
Tm180 hearts, perhaps due to the inflammatory response often associated with cardiac hypertrophy. Three of the genes (PLN, HDAC5, AQP1) decrease their expression in the Tm180 hearts perhaps due to decreases in the movement of cellular components, specifically calcium into the SR and water movement. These alterations in gene expression correlate with the phenotypic differences observed between the Tm180 and PLN KO/ Tm180 hearts (7, 16, 17).

To verify the results of regulated gene expression detected in the microarray analysis, we conducted real-time quantitative RT-PCR analysis on a select number of transcripts associated with cardiac hypertrophy using ventricular RNAs isolated from the left ventricles of NTG, PLN KO, Tm180, and PLN KO/ Tm180 mice. The following RNAs were examined: atrial natriuretic factor (ANF), α- and β-myosin heavy chain (MHC), skeletal actin, osteoblast-specific factor (OSF-2, also called periostin), and transforming growth factor-β2 (TGF-β2). Results show that for these transcripts, there are generally higher levels in the Tm180 hearts, with lower levels in the PLN KO/ Tm180 rescued mice (Table 2). Overall, that there is a strong concordance between the values obtained for the various transcripts and the two methods of quantification serves to verify the results between the gene matrix and RT-PCR analyses.

We also conducted quantitative RT-PCR on additional transcripts that were identified in the microarray analysis but are not typically associated with cardiomyocytes. These RNAs were endothelin 3 (Edn3), thrombospondin 4 (Thbs4), IL6, glutathione s-transferase 1 (Gsta1), and C3. Results (Table 2) show concordance among quantified levels in the four genotypic groups, and the trend of gene regulations was relatively constant between the microarray and quantitative RT-PCR assays and further validates the results of our analyses.

**DISCUSSION**

As mentioned previously, the Tm180 mice develop a severe cardiomyopathy that entails cardiomyocyte hypertrophy, fibrosis, thrombosis, atrial and ventricular enlargement, diastolic dysfunction, and abnormalities in myofilament sensitivity to calcium (1, 7, 16, 17). By mating the Tm180 mice with the PLN KO mice, we rescued the resulting progeny (PLN KO/ Tm180 mice) from the disease parameters manifest in the hypertrophic cardiomyopathy phenotype (7). This rescue occurs by modulating SR calcium cycling, an approach previously used in other models of cardiomyopathy (3, 12, 19).

The results from this study show that there are 1,187 genes that are differentially expressed at a 1.3-fold level \((P < 0.01)\) between the four genotypic groups: NTG, PLN KO, Tm180, and PLN KO/Tm180 at 4 and 12 mo of age (Fig. 1 and Supplemental Table S1). These genes can be grouped according to their levels of expression into 11 clusters. Generally, the expression of each clustered gene group (i.e., NTG, PLN KO, Tm180, and PLN KO/Tm180) is similar between the 4 and 12 mo time period.

With a 1.4-fold change in gene expression, results show in the three mouse models there are 464 genes that significantly differ from each other (Supplemental Table S2). These genes are distributed across the different clusters and encompass a wide range of functions, including cardiac ion transport, signalling, and metabolism. The results from this study further support the hypothesis that modulating SR calcium cycling is a critical factor in the development and progression of severe cardiomyopathies.
change their expression from NTG levels. At 12 mo of age, there are 62 genes that differ at the 1.4-fold level of expression between the hypertrophic Tm180 and the rescued PLN KO/Tm180 hearts (Fig. 3 and Supplemental Table S2). As mentioned, six of these genes (C3, PLN, IL6, HDAC5, AHSG, and AQP1) are frequently associated with heart failure and cardiac hypertrophy. However, up to 34 of the genes (>50%) have at least one publication indicating their role in cardiac hypertrophy and/or heart failure. It may be somewhat surprising that more of these genes that change expression at the 1.4-fold level are not involved in these cardiac hypertrophy/heart failure functions, considering the complete reversal of the cardiomyopathy that occurs between the Tm180 and PLN KO/Tm180 mice. However, one must consider that in the adverse cardiac remodeling of the PLN KO/Tm180 mouse hearts, there are multiple cell types that alter their expression, including cardiomyocytes, cardiac fibroblasts, smooth muscle cells, and inflammatory response cells. Results show that a substantial number of these 62 genes are involved with vein and arterial contraction and the vasculature; these genes include EDN3, NR4A1, CHI3L1, HDAC5, LAMA5, IL6, TMEM100, APOD, C3, and AQP1. It should be noted that some of these genes are involved in multiple processes and are not restricted to one particular biological function. Also, it should not be surprising that cardiac hypertrophy and adverse cardiac remodeling would involve genes associated with the vasculature/smooth muscle because of the significance of the blood vessels in the heart during cardiac disease, prevention, and remodeling. Another major process involved with cardiac disease and adverse remodeling is that of inflammation. Genes associated with the inflammatory response include: CHI3L1, ITGB6, HDAC5, LBP, IL6, S100A8, AHSG, CXCL1, CXCL2, CXCL14, APOD, TNFAIP6, C3, C7, C4G, PENK, TNFAIP6, C4B, CLEC4D, and PRG4. This list of 20 genes (~1/3 of 62) represents the largest class of highly responsive genes that alter their expression in response to the adverse cardiac remodeling process between the Tm180 and PLN KO/Tm180 hearts. This high number of genes represents the importance of the inflammatory response that occurs during severe cardiac disease.

Furthermore, the nonhypertrophic genes that were also validated by our real-time RT-PCR analysis presents a clear pattern of molecular signatures for the rescue of hypertrophic phenotype. Edn3, essential in neurocrest-derived cell lineages, also plays a role as a vasoactive peptide. Il6 is a cytokine that functions in inflammation and maturation of B cells. Gsta1 functions in the detoxification of electrophilic compounds, including carcinogens and toxins. C3 plays a central role in the activation of the complement system involved in innate and adaptive immune responses. Thbs4 is an adhesive glycoprotein that is expressed in the heart and plays a role in vessel remodeling and repair. The gene expression profile suggests that the rescue of the hypertrophic phenotype involves a coordinated regulation of genes involved in remodeling, inflammation, and vessel function.
involved in cell-cell and cell-matrix interactions. It can also stimulate early erythroid progenitor proliferation. The role of thrombospondins during cardiac remodeling has also been identified by several other studies (6, 11, 20).

The results show that changes in gene expression that occur with hypertrophic processes (Tm180 hearts), hyperdynamic cardiac function (PLN KO hearts), and adverse cardiac remodeled hearts (PLN KO/Tm180) are varied and complex. This is best exemplified by the results shown in Fig. 2, where each genotypic group displays unique sets of gene transcript patterns. In most of the gene clusters, the genes associated with the PLN KO/Tm180 profile appear to have expression levels that are intermediate between the PLN KO and TM180 levels. The strong influence of the PLN KO profile in the PLN KO/Tm180 mice is not surprising since these mice have improved cardiac function and are more efficient in handling calcium resquestration. The data indicate that these intermediate levels of expression are sufficient to rescue the cardiomyopathic phenotype associated with the Tm180 mice. However, there are also transcript-associated changes seen in Tm180 profile that are dominant over the PLN KO/Tm180 genotype (cluster 4). The dominance of these genes does not appear to be crucial in establishing the “rescued” phenotype and are more recalcitrant in their “plasticity” to change their expression patterns. Whether it is the property of some genes to be more resistant or adaptable to changing their levels of expression is a subject of future investigations.

One reason for choosing the 4 and 12 mo time intervals for the microarray time points was to determine whether there would be significant changes in gene expression with aging of the mice of the various phenotypes. Results show there are some changes in gene expression over this time interval within any given genotype, but not for all of the gene clusters. This is best exemplified by a comparison of the 4 and 12-mo times for clusters 3 and 5 when all four genotypes exhibit significant increases in gene expression (Fig. 2). Other clusters of genes (i.e., 4 and 8) do not show significant changes in gene expression between these two time periods. Thus, it appears that the gene expression profile is not predictable and can be dramatically changed from 4 to 12 mo.

To examine the identity, interaction, and complementation between the gene clusters, we chose three clusters (sets 3, 4, and 5) associated with the rescue of the hypertrophic phenotype for analysis. As seen in Fig. 4, these chosen sets share many properties and biological features. Many of the genes that are key activators of signaling pathways (red hexagons) are shared between the cluster sets, as are their other functional relationships (gene ontology and mouse phenotype terms represented as green and brown boxes, respectively). Additional gene interactions (yellow boxes), microRNA involvement (purple boxes), and transcription factor activators (pink boxes) are also highlighted.

The ability to develop and rescue animal model systems that mimic human pathological conditions is one goal of research utilizing transgenic and KO mice. Several strategies have been employed to rescue hypertrophic cardiomyopathic mice by regulating the cardiomyocyte’s response to calcium and its handling. Previous work demonstrating this involved mating the Tm180 mice with a transgenic mouse expressing a chimeric α/β-Tm protein that induces a desensitization of the sarcomere to calcium (16, 17). The resulting double transgenic mice were phenotypically rescued, showing a normal heart size and morphology, significantly improved cardiac function, and normal myofilament calcium sensitivity. Also, studies demonstrate that hypertrophy and cardiac dysfunction can be improved by ablation of PLN in various animal models (3, 7, 12, 19). In addition, gene transfer of SERCA2a into the Tm180 neonates also improves cardiac hypertrophy and hemodynamic performance (13). Cardiac relaxation abnormalities can be corrected when Tm180 mice are genetically crossed to transgenic mice expressing parvalbumin, a calcium buffer (2). By performing a microarray analysis on the PLN KO/Tm180 mice, we hypothesized that genes involved in rescue of the hypertrophic phenotype (PLN KO/Tm180 mice) could be distinguished from those associated with cardiac hypertrophy (Tm180 mice) and hyperdynamic performance of the heart (PLN KO mice). Most of these genes that were identified in the PLN KO/Tm180 hearts revert their expression levels toward NTG levels, instead of the levels observed in the Tm180 hearts alone. Future studies will address whether targeting specific genes and/or pathways for over-/underexpression will also serve to rescue the hypertrophic phenotype in cardiac disease.

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Current address for S. Rajan: Heart Institute, Cincinnati Children’s Hospital Medical Center, 240 Albert Sabin Way, Cincinnati, OH 45229-3039.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


