Left ventricular global transcriptional profiling in human end-stage dilated cardiomyopathy

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ABSTRACT

We employed ABI high-density oligonucleotide microarrays containing 31,700 sixty-mer probes (representing 27,868 annotated human genes) to determine differential gene expression in idiopathic dilated cardiomyopathy (DCM). We identified 626 up-regulated and 636 down-regulated genes in DCM compared to controls. Most significant changes occurred in the tricarboxylic acid cycle, angiogenesis, and apoptotic signaling pathways, among which 32 apoptosis- and 13 MAPK activity-related genes were altered. Inorganic cation transporter, catalytic activities, energy metabolism and electron transport-related processes were among the most critically influenced pathways. Among the up-regulated genes were HTRA1 (6.9-fold), PDCD8(AIFM1) (5.2) and PRDX2 (4.4) and the down-regulated genes were NR4A2 (4.8), MX1 (4.3), LGALS9 (4), IFNA13 (4), UNC5D (3.6) and HDAC2 (3) ($p<0.05$), all of which have no clearly defined cardiac-related function yet. Gene ontology and enrichment analysis also revealed significant alterations in mitochondrial oxidative phosphorylation, metabolism and Alzheimer's disease pathways. Concordance was also confirmed for a significant number of genes and pathways in an independent validation microarray dataset. Furthermore, verification by real-time RT-PCR showed a high degree of consistency with the microarray results. Our data demonstrate an association of DCM with alterations in various cellular events and multiple yet undeciphered genes that may contribute to heart muscle disease pathways.

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Background

Idiopathic dilated cardiomyopathy (DCM) is the thinning of one or both ventricle(s) from an unknown cause, with the resultant impaired cardiac contractility often leading to overt congestive heart failure or cardiac arrhythmias. While no clear cause is evident in the majority of cases, DCM is probably an end product of myocardial damage triggered by a variety of toxic, metabolic or infectious agents [1]. Besides, some forms of familial DCM, in particular, also appear to be triggered by mutations in genes encoding cytoskeletal, contractile or other myocardial proteins [2–5]. The ensuing progression of heart failure is associated with left ventricular remodeling, which manifests as a gradual increase in left ventricular end-diastolic and end-systolic volumes, wall thinning and alteration in the shape of the chambers to a more spherical and less elongated form [6]. Several molecular and cellular alterations have been identified that contribute to cardiac muscle contractility and relaxation abnormalities in this process.

These include, among others, the cyclic AMP (cAMP)-dependent pathways, calcium (Ca^{2+}) homeostasis, neurohumoral activation and myofibrillar function [7]. Essentially, cAMP-dependent pathways are desensitized due to alterations in β-adrenoceptors (β-AR), β-AR kinases and guanine nucleotide binding proteins (G-proteins) [8]. Calcium ion (Ca^{2+}) homeostasis is impaired, characterized by a reduced sarcoplasmic reticulum Ca^{2+} reuptake rate, elevated Ca^{2+} release channel threshold and an increase in sodium ion (Na^+)/Ca^{2+} exchanger expression [9,10]. Myofibrillar function may also be influenced by a decrease in Mg^{2+}-ATPase activity and in troponin I phosphorylation, as well as changes in troponin T isoform expression [9,11–13]. Accumulating data also suggests a link between alterations and/or deficiencies in cytoskeletal proteins and the progression of cardiomyopathy to heart failure. Moreover, the remodeling process appears to be regulated by a number of pathways including cytokines and growth factors [14].

Despite great efforts to understand the mechanism involved in the progress of DCM to overt heart failure, the underlying triggering factors for the disease remain to be elucidated. Accumulating evidence from gene profiling and other studies implicates diverse pathways, including among others, the vascular renin–angiotensin system [15], G_i-coupled receptors [16], TGFβ-activin-A/Smad signaling pathway

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[17], SH2-containing cytoplasmic tyrosine phosphatase (Shp) [18] and apoptotic signaling [15,17–20], to name a few. While classical opinion might argue that several of these alterations occur independently of the underlying etiology of the disease, it has also become apparent that the greater part of the familiar myocardial changes is probably triggered by chronic neurohumoral activation and abnormal mechanical load [21], which greatly promote the progression of heart failure as part of a vicious circle. However, the molecular basis for this link remains unclear. Several studies have been performed using different microarray-based and other techniques to evaluate alterations in gene expression in DCM [22–26], and recently intraplatform consistency in terms of sample sources as well as a high level of interplatform concordance with respect to genes identified as differentially expressed have been demonstrated [27,28]. Hence, deciphering the pattern of alterations in gene expression in DCM using the microarray system provides a valuable basis for elucidating some of the mechanisms involved in this vicious circle. In particular, the ABI high-density oligonucleotide microarray platform allows analysis of a greater number of genes than most platforms, as it includes annotated genes from both public and Celera databases. ABI platform is also a chemiluminescent based array by which signal is enhanced to fentomol sensitivity which may help to detect rare mRNAs. It has also been shown that ABI 1700 platform has substantially higher sensitivity, detecting four times as many changes in an identical experimental design and results are well correlated ($R^2 > 0.7$) with qRT-PCR compared to other microarray platforms [29–31]. In this study, we therefore sought to establish left ventricular differential gene expression in DCM employing the ABI 1700 platform, in order to be able to detect a relatively rare class of mRNAs and obtain further insight into the mechanism of heart muscle disease pathways.

Materials and methods

Study patients

For the gene expression and subsequent experiments, 300 mg of tissue were harvested from left ventricles of five DCM hearts excised from patients (3 male and 2 female; 42.3 ± 6.3 years) with end-stage heart failure undergoing cardiac transplantation at our institution. All samples were procured from identical myocardial loci to ensure optimal uniformity. The patients had New York Heart Association class 3–4 symptoms, and received anti-heart failure treatment and/or inotropic support. None of the patients was on a left ventricle assist device or any other mechanical support. Four healthy hearts procured from organ donors (three male and 1 female; 34.1 ± 4.7 years) who died of traffic accidents with no history of cardiac disease served as controls. The mean age of the controls was not significantly different from that of the patients ($p = 0.37$). These hearts had originally been intended for transplantation, but failed to get suitable matching recipients. At the time of harvesting, whole hearts were explanted after preservation in cold cardioplegia, followed by immediate dissection into small portions, snap-frozen in liquid nitrogen, and maintained at -80 °C until use. Minimum time possible (usually < 3 h) was allowed between harvesting donor hearts and freezing the samples in liquid nitrogen. Fully informed consent was obtained from all patients or family members before participating in the study. This study was performed in accordance with the Declaration of Helsinki as adopted and promulgated by the US National Institutes of Health as well as rules and regulations laid down by our Institutional Ethics Committee.

Expression array analysis

Total RNA was isolated from similar left ventricular biopsies using Applied Biosystems (ABI) Totally RNA Isolation Kit (ABI-Ambion,

Foster City, CA, USA), quantified with the NanoDrop® ND-1000 Spectrophotometer (Nanodrop Inc., Wilmington, DE, USA) and further analyzed by RNA 6000 Nano Assay using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Digoxigenin-UTP labeled cRNA was generated and amplified from 1 μg of total RNA using Applied Biosystems (ABI) Chemiluminescent RT-IVT Labeling Kit v 1.0. The array hybridization was performed for 16 h at 55 °C and detection, image acquisition and analysis were performed using ABI Chemiluminescence Detection Kit on ABI 1700 Chemiluminescent Microarray Analyzer (ABI, Foster City, CA, USA).

Real-time RT-PCR

In order to validate our microarray results, confirmatory quantitative real-time RT-PCR (qRT-PCR) was performed using the ABI 7500 Sequence Detection System (ABI, Foster City, CA, USA). For this purpose, 50 ng total RNA procured from the same microarray study samples were transcribed into cDNA using Sensiscript Kit (QIAGEN Inc., Valencia, CA, USA) under the following conditions: 25 °C for 10 min, 42 °C for 2 h, and 70 °C for 15 min in a total volume of 20 μl. Six differentially expressed genes (CRYM, NR4A2, PDK4, RASD1, TNNI3K, and AIFM1) were randomly selected and primers designed using Primer3 software. After primer optimization, the PCR assays were performed in 6 μl of the cDNA using the QIAGEN Quantitet SyBR Green Kit, employing GAPDH as the endogenous control gene. All reactions were conducted in triplicates and the data was analyzed using the delta delta C_T method [32].

Data analysis

Hybridization images were analyzed using the ABI 1700 Chemiluminescent Microarray Analyzer software v 1.1, with the detection threshold set at signal to noise (S/N) ratio > 3 (a value that indicates 99.9% confidence level for the signal being above the background level, "present" probes) and quality flag < 5000. The open source Bioconductor packages, ab1700, limma, multtest and affy (Fred Hutchinson Cancer Research Center, Seattle, WA, USA) and Partek Genomics Suite (Partek Inc.) were employed to normalize the data via quantile normalization and to determine significant differences in gene expression levels between DCM patients and normal controls [33]. When comparing DCM patients and normal controls to identify the differentially expressed genes, we used a combination of three criteria. We considered genes that are "present" in at least half of the samples in either group. Given the nature of the data, and statistical tests selected, adjusting for multiple testing errors is critical. We used Benjamini-Hochberg [34] step-up procedure to control the false discovery rate (FDR). As an alternative approach, we employed the two-class SAM procedure to estimate the FDR [35]. Significantly modulated genes were defined as those with absolute fold change (FC) > 1.8 and controlling FDR at 5%. A validation data set was generated from an independent study by Barth et al. [22] using Affymetrix HG-U133A array, and the raw data was analyzed by using dChip [36] and open source R/Bioconductor packages. The dChip outlier detection algorithm was used to identify outlier arrays (all arrays passed), and probes "present" in at least 50% of the samples in either group were filtered. The data was normalized by the GC Robust Multi-array Average (GC-RMA) algorithm [37,38]. Unpaired *t*-tests were performed to determine significant differences in gene expression levels between patients and normal controls, Multi Experiment Viewer (MeV4.0) [39] was used to perform two-dimensional hierarchical clustering employing Euclidean distance as well as Pearson correlation with average linkage clustering. Functional annotation and biological term enrichment analysis were performed using DAVID Bioinformatics Resources [40], Expression Analysis Systematic Explorer (EASE) [41], Protein ANalysis Through Evolutionary Relationships (PANTHER™) classification systems [42], and

Ingenuity Pathways Analysis (IPA) 6.3 (Ingenuity Systems, Mountain View, CA). Gene Set Enrichment Analysis/MSigDB was used to determine whether an *a priori* defined set of genes showed statistically significant, concordant differences between the 2 groups (DCM vs normal). Statistical analyses were performed with the MATLAB software packages (Mathworks, Natick, MA, USA), R and Bioconductor and PARTEK Genomics Suite (Partek Inc., St. Lois, MO, USA).

Results

Global gene expression analysis

The mRNA expression was analyzed using the ABI human whole genome array version 2. The ABI Human Genome Survey Microarray contains 31,700 sixty-mer oligonucleotide probes representing 27,868 individual human genes. Approximately 19,000 of these probes were detectable based on the above criteria. We have found 1309 probes, of which 655 probes (626 genes) were up-regulated and 654 probes (636 genes) were down-regulated, whose expression varied at least 1.8-fold and were statistically significant at a false discovery rate of <5% between DCM patients and normal controls (Supplementary Table 1). The hierarchical clustering in both dimensions (samples and genes) clearly distinguished individuals as either DCM or controls (Fig. 1). The 50 most significantly altered genes (>3-fold change) are listed in Tables 1A and 1B.

Gene ontology analysis

The gene ontology and functional analysis of DCM specific up/down-regulated genes were performed using the Ingenuity knowledge base (Fig. 2A). The biological functions assigned to the data set are ranked by significance ($-\log P$ value). As demonstrated in Fig. 2A, highly significant functions include lipid metabolism, cell death, amino acid metabolism, small molecule biochemistry, molecular transport, cellular growth and proliferation, nucleic acid metabolism, tissue development, and cellular development. We further identified altered biological processes, molecular functions and pathways among the differentially expressed genes using PANTHER™ classification systems [42]. The numbers of genes identified in each of the three categories were calculated and compared using the binomial test to determine if there were more genes than expected in the differentially regulated list [43]. Based on this analysis, genes related to electron transport ($p = 1.9 \times 10^{-16}$), oxidative phosphorylation ($p = 4.7 \times 10^{-16}$), protein metabolism and modification ($p = 3.4 \times 10^{-9}$), carbohydrate metabolism ($p = 1.3 \times 10^{-8}$), fatty acid metabolism ($p = 1.2 \times 10^{-7}$), cell proliferation and differentiation ($p = 1.7 \times 10^{-3}$) belonged to the most significantly enriched among the up-regulated genes. Genes related to signal transduction ($p = 6.1 \times 10^{-7}$), cell communication ($p = 5.6 \times 10^{-5}$), mesoderm development ($p = 4.2 \times 10^{-3}$), mRNA transcription ($p = 1.4 \times 10^{-4}$), cell surface receptor-mediated signaling ($p = 1.5 \times 10^{-3}$), developmental processes ($p = 1.3 \times 10^{-2}$) and cell adhesion ($p = 8.7 \times 10^{-3}$) were the most significantly enriched among the down-regulated genes (data not shown). Biological themes associated with the differentially expressed genes were also identified by using three gene ontology categories of biological processes, molecular functions and cellular components. The most significantly overrepresented GO categories (EASE score<0.01) among the up-regulated genes were related to catalytic activity, electron transport, mitochondrial metabolism and energy pathways, whereas among the down-regulated genes were those associated with cell adhesion, signal

transduction, binding and transcription, which was consistent with the categories identified by PANTHER.

We also investigated biological pathways significantly represented among the differentially expressed genes. The most significantly overrepresented pathways included the TCA cycle, asparagine and aspartate biosyntheses, apoptosis signaling, Parkinson's disease, cell cycle, and salvage pyrimidine ribonucleotide pathways enriched among the up-regulated genes, and TGF-β signaling, p53, apoptosis signaling, Ras, integrin signaling and Alzheimer's disease-presenilin pathways among the down-regulated genes (Table 2). The IPA analysis of DCM specific genes (up/down-regulated) also revealed that citrate cycle, mitochondrial dysfunction, oxidative phosphorylation and TGF-β signaling are among the most significantly altered canonical pathways (Fig. 2B). The Gene Set Enrichment Analysis/MSigDB further complements the ontology analysis with significant enrichment of gene sets or pathways related to cytoplasm, mitochondrial genes, Alzheimer disease, metabolism and oxidative phosphorylation.

Gene interaction network analysis

To obtain a deeper insight into the interactions of the dysregulated genes among the various pathways, the DCM specific genes were mapped to the gene networks using the Ingenuity knowledge base. These genes were mapped primarily to top networks (Fig. 3A and B) related to, among others, cell death, cellular growth and proliferation, cardiovascular and nervous system development and function, post-translational modification, protein folding, cell cycle, tissue development, lipid metabolism and small molecule biochemistry.

Among the differentially expressed genes, 32 were apoptosis-related and 13 were associated with mitogen-activated protein kinase (MAPK) activities. Also noteworthy was the large number of up-regulated genes pertaining to oxidoreductase activity, synthases, ribosomal proteins, nucleic acid binding, mitochondrial function and metabolism on the one hand, and the down-regulated genes involved in homeobox transcription, signal transduction, receptor signaling, growth, extracellular matrix or DNA-binding on the other. Furthermore, the most highly (>4-fold change) elevated genes included pyruvate dehydrogenase kinase, isozyme 4 (PDK4), malonyl-CoA decarboxylase (MLYCD), the programmed cell death (AIFM1 also known as apoptosis-inducing factor or mitochondrial programmed cell-death protein 8), ubiquitin B (UBB), human troponin I subtype 3 (TNNI3) interacting kinase (TNNI3K), mitochondrial branched chain aminotransferase 2 (BCAT2), crystalline-mu (CRYM), and peroxiredoxin 2 (PRDX2), while the most significantly down-regulated genes included the pyruvate dehydrogenase kinase, isoenzyme 4 (NR4A2), dexamethasome-induced RAS encoding subtype 1 gene (RASD1), B-cell receptor-inducible gene BIC(MIRHG2), myxovirus 1 (MX1), interferon A13 (IFNA13), unc-5 homolog D (UNC5D), histone deacetylase 2 (HDAC2) and potassium voltage-gated channel, member 2 (KCNQ2) genes, just to name a few.

Independent validation set analysis

As a validation of our results, we analyzed an independently performed microarray dataset for DCM from Barth et al. [22] using Affymetrix short oligo array using the analysis procedure defined in the "Materials and methods" section on the new dataset. The data is composed of 12 samples for non-failing heart ($n = 5$) and DCM heart patients ($n = 7$). We found 1223 genes differentially expressed between DCM and normal controls. The validation

Fig. 1. Heatmap of genes that were significantly modulated due to DCM. Hierarchical clustering clearly separated individuals as either DCM patients or normal controls. Highly expressed genes are indicated in red, intermediate in black, and weakly expressed in green. Only 100 of the most significantly altered genes are shown for readability.
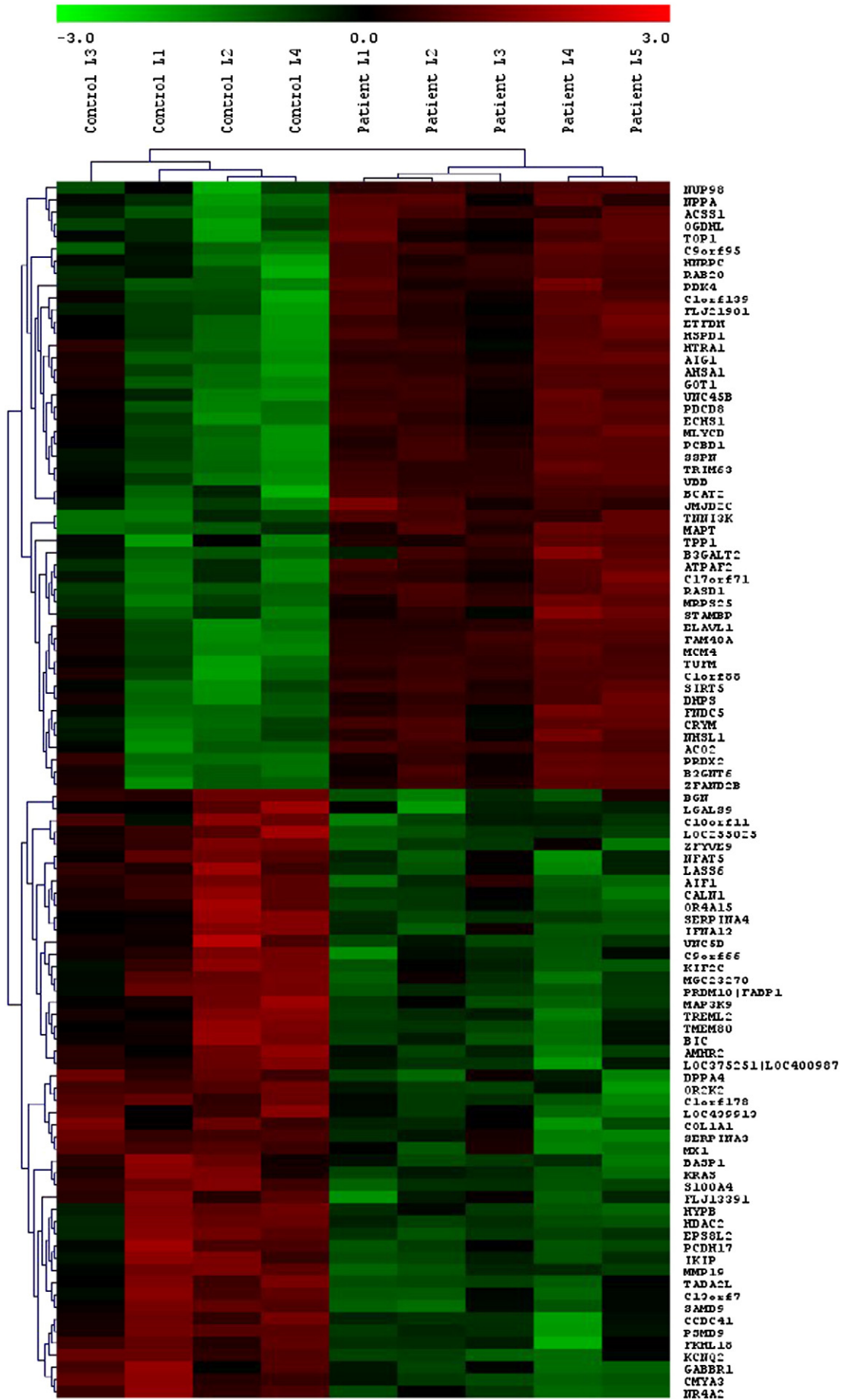
Table 1A
Top 50 genes that were most significantly increased in expression in DCM.

Probe ID	UniGene ID	Symbol	Gene_name	FC[a]
184159	Hs.75640	NPPA	natriuretic peptide precursor A	24.27
101060	Hs.8364	PDK4	pyruvate dehydrogenase kinase, isozyme 4	16.44
127385	Hs.461571	MLYCD	malonyl-CoA decarboxylase	9.23
212992	Hs.356190	UBB	ubiquitin B	7.21
115431	Hs.25829	RASD1	RAS, dexamethasone-induced 1	7.18
199204	Hs.567501	AIG1	androgen-induced 1	7.12
119696	Hs.3192	PCBD1	pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1)	7.03
168813	Hs.17860	OGDHL	oxoglutarate dehydrogenase-like	6.94
111748	Hs.501280	HTRA1	HtrA serine peptidase 1	6.87
117167	Hs.204041	AHSA1	AHA1, activator of heat shock 90 kDa protein ATPase homolog 1 (yeast)	6.73
118421	Hs.480085	TNNI3K	TNNI3 interacting kinase	6.68
146066	Hs.494186	C9orf95	chromosome 9 open reading frame 95	5.85
211439	Hs.500756	GOT1	glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)	5.83
162178	Hs.512670	BCAT2	branched chain aminotransferase 2, mitochondrial	5.71
209321	Hs.518834	B3GALT2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	5.54
122333	Hs.529276	FLJ21901(FASTKD1)		5.46
112942	Hs.479491	C1orf139(GPR177)	chromosome 1 open reading frame 139	5.46
161710	Hs.924	CRYM	crystallin, mu	5.31
205457	Hs.424932	AIFM1	programmed cell death 8 (apoptosis-inducing factor)	5.25
146299	Hs.567431	SIRT5	sirtuin (silent mating type information regulation 2 homolog) 5 (*S. cerevisiae*)	5.24
214987	Hs.12084	TUFM	Tu translation elongation factor, mitochondrial	5.06
214864	Hs.184492	ELAVL1	ELAV (embryonic lethal, abnormal vision, *Drosophila*)-like 1 (Hu antigen R)	4.98
200831	Hs.499916	FAM48A	family with sequence similarity 48, member A	4.93
158867	Hs.523454	TPP1	tripeptidyl peptidase I	4.92
155357	Hs.183428	SSPN	sarcospan (Kras oncogene-associated gene)	4.91
202365	Hs.524234	FNDC5	fibronectin type III domain containing 5	4.87
115589	Hs.460184	MCM4	MCM4 minichromosome maintenance deficient 4 (*S. cerevisiae*)	4.79
210061	Hs.486596	NHSL1	NHS-like 1	4.79
195766	Hs.155729	ETFDH	electron-transferring-flavoprotein dehydrogenase	4.77
182995	Hs.379636	UNC45B	unc-45 homolog B (*C. elegans*)	4.76
124513	Hs.172510	C1orf88	chromosome 1 open reading frame 88	4.74
217302	Hs.524750	NUP98	nucleoporin 98 kDa	4.56
202618	Hs.8526	B3GNT6	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6	4.51
158513	Hs.432121	PRDX2	peroxiredoxin 2	4.43
102257	Hs.508848	HNRPC(HNRNPC)	heterogeneous nuclear ribonucleoprotein C (C1/C2)	4.21
190596	Hs.529353	ACSS1	acyl-CoA synthetase short-chain family member 1	4.17
213069	Hs.279709	TRIM63	tripartite motif-containing 63	4.16
107417	Hs.13434	ATPAF2	ATP synthase mitochondrial F1 complex assembly factor 2	4.05
115807	Hs.76394	ECHS1	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	4.05
100079	Hs.113684|Hs.567290	HSPD1	heat shock 60 kDa protein 1 (chaperonin)	4.04
218816	Hs.101174|Hs.569810	MAPT	microtubule-associated protein tau	4.00
203523	Hs.79064	DHPS	deoxyhypusine synthase	3.98
134459	Hs.508720	RAB20	RAB20, member RAS oncogene family	3.95
179728	Hs.472737	TOP1	topoisomerase (DNA) I	3.88
101676	Hs.7296	C17orf71	chromosome 17 open reading frame 71	3.88
169529	Hs.555973	MRPS25	mitochondrial ribosomal protein S25	3.79
169750	Hs.183070	STAMBP	STAM binding protein	3.73
144908	Hs.474982	ACO2	aconitase 2, mitochondrial	3.71
195306	Hs.157106	JMJD2C	jumonji domain containing 2C	3.65
162286	Hs.534540	ZFAND2B	zinc finger, AN1-type domain 2B	3.65

[a] FC was calculated between the mean values of control and DCM.

dataset showed a significant number of genes ($p < 10^{-5}$) in common with our analysis results. Significance of overlaps was calculated using hypergeometric distributional assumption [44] and *P* values were adjusted using Bonferroni correction for multiple comparisons [45]. In addition, unsupervised clustering was performed using our signature gene list to cluster Barth et al. [22] data. We found that using our signature gene list was sufficient to separate individuals in Barth et al.'s study as either DCM patients or normal controls (Supplemental Fig. 1).

Furthermore, almost 70% of all GO categories from our analysis were retained in the validation dataset ($p \sim 0$), and a significant number of overrepresented GO categories was in common. Of note, metabolism, catalytic activity, ribosome, energy derivation by oxidation, protein metabolism, muscle development, and biosynthesis come up as significantly enriched GO categories in both the validation and our analyses. The gene set enrichment analysis (GSEA) showed highly significant enrichment of sets related to cytoplasm, neuronal stem cell, Alzheimer's disease and protein metabolism. The similarities between our results and the independent validation results

argues against random chance accounting for the observed enrichment of these gene sets.

Validation of selected differentially expressed genes

We next used quantitative real-time PCR (qRT-PCR) to validate selected differentially expressed genes, CRYM, NR4A2, PDK4, RASD1, TNNI3K and AIFM1. QRT-PCR analysis revealed a highly significant correlation ($r = 0.97$, *P* value < 0.01) between the microarray and the qRT-PCR data (Fig. 4), thus demonstrating the reliability of our gene expression measurements. These sets of genes and their interaction networks are shown (Supplementary Fig. 1A–E).

Discussion

The present study investigates alterations in gene expression associated with heart muscle disease using DCM as a study model. While the sample sizes employed in this study are comparatively small, it should be noted that all tissues were procured from identical

Table 1B
Top 50 gene that were most significantly decreased in expression in DCM.

Probe ID	UniGene ID	Gene symbol	Gene_name	FC[a]
123450	Hs.165258	NR4A2	nuclear receptor subfamily 4, group A, member 2	−4.83
221732	Hs.161851	KCNQ2	potassium voltage-gated channel, KQT-like subfamily, member 2	−4.78
179577	Hs.535591|	LOC375251		−4.72
652781	Hs.388313	MIRHG2		−4.67
155850	Hs.81256	S100A4	S100 calcium binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog)	−4.39
215752	Hs.65641	SAMD9	sterile alpha motif domain containing 9	−4.29
209986	Hs.517307	MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	−4.25
162446	Hs.275086|Hs.380135	PRDM10|FABP1	PR domain containing 10|fatty acid binding protein 1, liver	−4.23
217544	Hs.352220	MGC23270		−4.23
207085	Hs.445496	MAP3K9	mitogen-activated protein kinase kinase kinase 9	−4.11
171927	Hs.154057	MMP19	matrix metallopeptidase 19	−4.11
159046	Hs.81337	LGALS9	lectin, galactoside-binding, soluble, 9 (galectin 9)	−4.10
200670	Hs.500066	TADA2L	transcriptional adaptor 2 (ADA2 homolog, yeast)-like	−4.03
132165	Hs.533471	IFNA13	interferon, alpha 13	−4.02
224571	Hs.516971	FKHL18(FOXS1)	forkhead-like 18 (*Drosophila*)	−4.02
145309	Hs.532345	ZFYVE9	zinc finger, FYVE domain containing 9	−4.02
206143	Hs.437877	AMHR2	anti-Mullerian hormone receptor, type II	−3.96
223807	Hs.252543	IKIP		−3.90
691815	Hs.196484	C1orf178	chromosome 1 open reading frame 178	−3.86
223796	Hs.190877	C9orf66	chromosome 9 open reading frame 66	−3.80
102968		KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	−3.69
158586	Hs.317659	DPPA4	developmental pluripotency associated 4	−3.66
141970	Hs.238889	UNC5D	unc-5 homolog D (*C. elegans*)	−3.64
126259	Hs.567576	RNF219	chromosome 13 open reading frame 7	−3.60
114684	Hs.506829	LASS6	LAG1 longevity assurance homolog 6 (*S. cerevisiae*)	−3.58
107450	Hs.448664	TMEM80	transmembrane protein 80	−3.57
129347	Hs.381312	OR2K2	olfactory receptor, family 2, subfamily K, member 2	−3.51
177348	Hs.159628	SERPINA4	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	−3.45
141595	Hs.164797	TREML2	triggering receptor expressed on myeloid cells-like 2	−3.39
127023	Hs.554529	OR4A15	olfactory receptor, family 4, subfamily A, member 15	−3.39
145949	Hs.371987	NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	−3.37
171525	Hs.821	BGN	biglycan	−3.32
169674	Hs.131151	PSMD9	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	−3.32
102572	Hs.279209	CCDC41	coiled-coil domain containing 41	−3.28
224005	Hs.73680|Hs.470488	CMYA3(XIRP2)	cardiomyopathy associated 3	−3.27
111499	Hs.434720|Hs.55016	EPS8L2	EPS8-like 2	−3.24
198318	Hs.201641	BASP1	brain abundant, membrane attached signal protein 1	−3.21
110723	Hs.549368	LOC439913		−3.19
134951	Hs.106511	PCDH17	protocadherin 17	−3.18
212531	Hs.69360	KIF2C	kinesin family member 2C	−3.10
194003	Hs.172928	COL1A1	collagen, type I, alpha 1	−3.08
171591	Hs.552755	LOC255025		−3.08
165806	Hs.534293	SERPINA3	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	−3.05
195643	Hs.167017	GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	−3.02
167343	Hs.576884|Hs.118161	C10orf11	chromosome 10 open reading frame 11	−3.01
180037	Hs.76364	AIF1	allograft inflammatory factor 1	−3.00
143136	Hs.333274	CALN1	calneuron 1	−2.99
133640	Hs.302346	FLJ13391(FAM176A)		−2.98
230512		HDAC2	histone deacetylase 2	−2.97
135102	Hs.517941	HYPB(SETD2)		−2.97

[a] FC was calculated between the mean values of control and DCM.

myocardial loci, to ensure optimal uniformity in cell content. This procedure should produce comparatively reliable and informative results with the microarray platform employed, in particular, if considered together with similar gene expression studies employing different platforms [46–48]. Most importantly, a validation analysis demonstrated great concordance of our results with other data sets using a different microarray platform. Besides ABI 1700 system has a unique approach in identifying the dysregulated genes since it targets genes from both Celera and Public databases and utilizes chemiluminescently enhanced detection that is likely to determine relatively rare mRNAs. Also, our confirmatory qRT-PCR experiments displayed very good correlation with the microarray results, adding to the validity of the present observations. This is in concordance with some recent studies showing linear relationship for real-time and conventional reverse transcription and therefore validity of robustness of mRNA quantification using either microarrays or quantitative RT-PCR

[49]. Hence the aim of the present work is to identify potentially novel and signature genes for DCM, in order to gain further insight into the mechanism of heart muscle disease pathways.

We identified differentially expressed probes satisfying the set criteria of S/N ratio >3 in >50% of the samples, a 5% FDR and absolute fold change >1.8 in DCM patients compared to controls. These observations are consistent with the study of Guo et al. [28], in which gene lists ranked by fold change and filtered with non-stringent statistically significant tests were more reproducible across platforms than those generated through other analytical procedures. Our study reveals several differentially and highly expressed genes and gene families, the majority of which encode apoptotic, cell proliferation and differentiation, homeostatic and mitochondrial energy metabolizing proteins. In general, genes and pathways involved in apoptosis, growth, communication and cardiomyoctye structure were down-regulated, whereas those involved in energy metabolic processes, cell
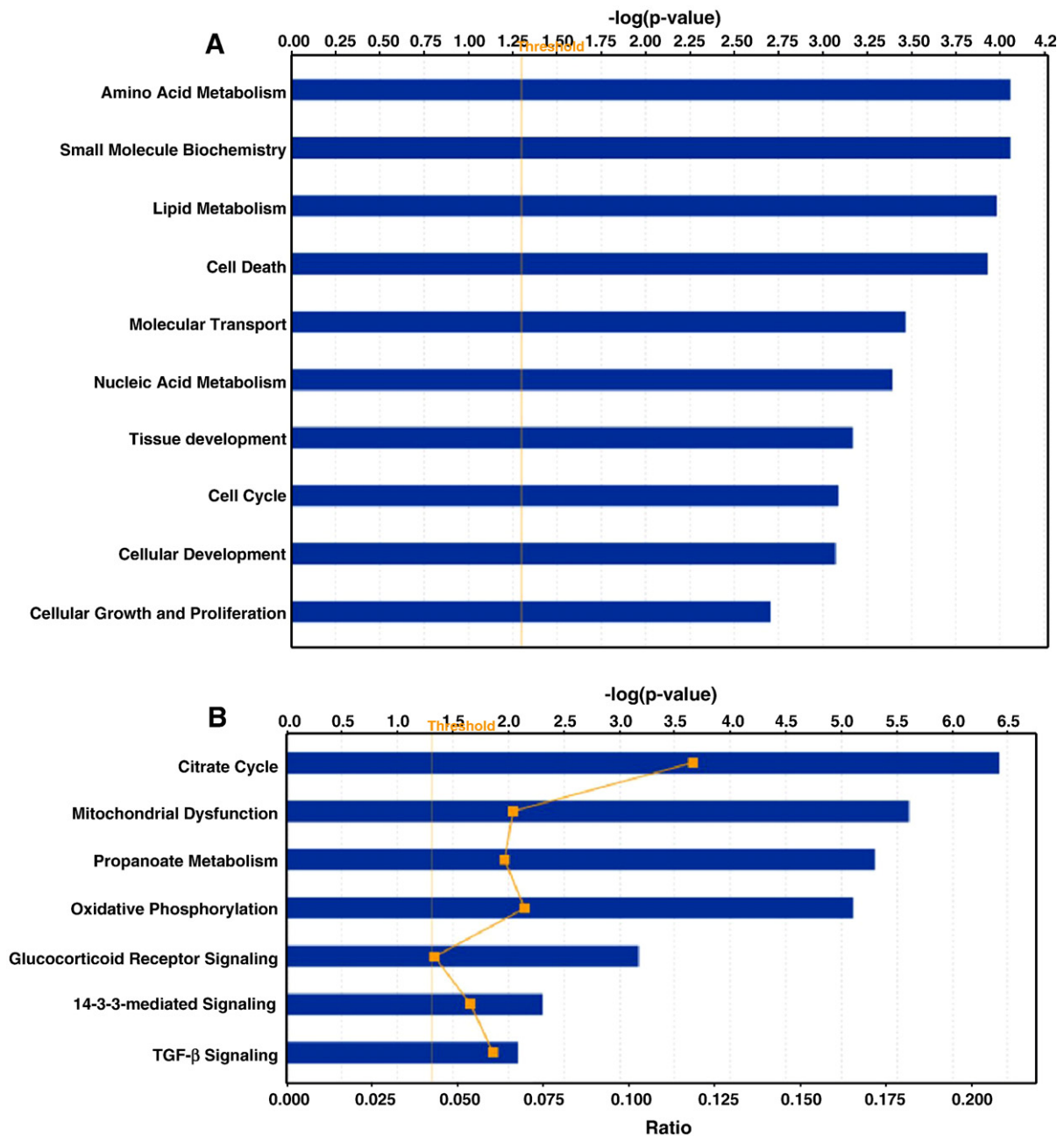
Fig. 2. Functional (A) and canonical pathway (B) analysis of DCM specific genes. X-axis indicates the significance (−log P value) of the functional/pathway association that is dependent on the number of genes in a class as well as biologic relevance. The threshold line represents a P value of 0.05.

survival, cell cycle, ion transport were up-regulated. Thus, our study furnishes supporting evidence for the prevalence of apoptosis in DCM, as indicated by the presence of DNA fragmentation in conjunction with changes in apoptotic signaling components [50,51]. These results are also consistent with other microarray and small-scale studies demonstrating changes in related genes, including cytokines, tumor necrosis factor (TNF)-induced genes, MAPKs, cell survival and stress response genes as well as other components of apoptotic signaling pathways [52–54]. Taken together, these data strongly implicate perturbations in death-related signaling in the pathogenesis of DCM.

Other similarly described changes in DCM thus far involve genes contributing to diverse cellular processes, including transcription [55], sarcomeric and cytoskeletal function [24,56], extracellular matrix remodeling [57], membrane transport [23], ion channels [55] and immune responses [22]. Also consistently implicated in this disease is the alteration in myofilament function in conjunction with depressed myofibrillar ATPase activity [58] and increased myofilament Ca^{2+} sensitivity, which presumably contribute to slowed or incomplete muscle relaxation, and therefore depression of the force-frequency relation [12]. In this regard, it is noteworthy that altered post-translational modification of particularly the phosphorylation state of troponins I and T, and possibly myosin light chain, has been postulated as the most important mechanism of myofilament dysfunction in DCM, with possible contributions of other modifications, such as oxidation and glycation [13]. Therefore, the down-regulation of extracellular matrix genes and the increase in cell structural and myofilament gene expression established in this and various other studies substantiate the potential involvement of cardiomyocyte modification(s) as an integral component of events occurring in DCM.

Apart from modifications of the contractile muscle structure, changes in mitochondrial gene expression were also evident, ranging from the up-regulation of genes involved in energy metabolism, such

Table 2
Overrepresented pathways among up/down-regulated genes.

Pathways	List hits[a]	Expected value[b]	P value[c]
Overrepresented pathways among up-regulated genes			
TCA cycle	7	0.44	4.40E−07
Asparagine and aspartate biosynthesis	4	0.2	5.32E−05
Fructose galactose metabolism	4	0.32	3.37E−04
ATP synthesis	4	0.37	5.74E−04
Methylmalonyl pathway	2	0.1	4.54E−03
Glycolysis	4	0.74	6.88E−03
Vitamin B6 metabolism	2	0.12	6.98E−03
Methylcitrate cycle	2	0.12	6.98E−03
Acetate utilization	2	0.22	2.12E−02
Threonine biosynthesis	1	0.05	4.81E−02
Cell cycle	3	0.81	4.91E−02
Salvage pyrimidine ribonucleotides	2	0.42	6.66E−02
Parkinson disease	6	2.86	6.97E−02
Overrepresented pathways among down-regulated genes			
TGF-beta signaling pathway	11	3.67	1.42E−03
p53 pathway feedback loops 2	5	1.53	1.97E−02
Ornithine degradation	1	0.02	2.43E−02
EGF receptor signaling pathway	8	3.57	2.92E−02
Interleukin signaling pathway	13	7.49	4.12E−02
Angiogenesis	10	5.49	5.25E−02
Integrin signaling pathway	10	5.54	5.51E−02
Alzheimer disease-presenilin pathway	7	3.4	5.72E−02

[a] The number of genes in respective PANTHER classification categories.
[b] The expected value is the number of genes expected in the differentially expressed genes for this PANTHER category, based on the reference list.
[c] P values for each category were calculated from the binomial test statistic.

as the citric acid cycle and ATP synthesis, and those important in maintaining energy metabolic pathways, such as MLYCD or PDK4, to alterations at the level of oxidative phosphorylation (e.g. TNNI3K) and transamination (e.g. BCAT1). Similar changes have also been described by other investigators in genes related to oxidative phosphorylation [59], mitochondrial ADP/ATP transport and adenine nucleotide translocase (SLC25A4) [60,61], for example. In this regard, it should be noted that changes in DCM have also been associated with more economical and efficient energy utilization by the contractile machinery, which may offer myocardial protection [58]. Interestingly, in our study, metabolic pathways were by far the most significantly altered and well-represented among the over-expressed biological processes, implicitly placing them at the centre of events occurring in this disease. Therefore, there appears to be a heightened level of metabolic activity, which may be ascribable to adaptive or compensatory mechanisms in this disease process.

In addition to the above well-characterized pathways and processes, we also discovered changes in genes thought to be physiologically dormant, which may not necessarily fit into the picture of the failing heart. These include the attenuation in functional expression of genes encoding growth factors, such as TGF-β, EGF and FGF, as well as numerous unclassified molecular functions on the one hand, and the up-regulation of those encoding homeobox transcription factors and processes, such as protein, nucleoside, nucleotide, nucleic acid metabolisms and cardiac development, on the other. This scenario points to a functional state in which regulatory processes that naturally occur in early stages of cardiac development are suppressed, while those that may be attributed to sustention of cellular integrity are elevated or possibly stimulated under these disease conditions. Our findings are consistent with similar microarray studies postulating specific role of various transcription factors in heart failure [62,63]. This implies therefore, that the dormancy of these entities in an adult heart serves definable functional purpose(s), which can be mobilized or may alternatively contribute to the shaping the disease pathway(s) to heart failure.

Interestingly, a large number of genes were similarly uncovered with currently undefined cardiac-related function. These include the up-regulated HTRA1, AIFM1, CRYM and PRDX2 genes and the down-regulated NR4A2, LGALS9, IFNA13, MX1, UNC5D, and HDAC2 in DCM. To date, the HTRA1 has been associated with, among others, Alzheimer's disease [64,65], various neoplasms [66,67] and regulation of several signaling pathways [68]. The AIFM1 is a mitochondrial oxidoreductase which has been implicated in neurological disorders, and may influence homeostasis possibly by interacting with apoptosis-related signaling protein [69–72]. The PRDX2 is a peroxidoreductase that catalyzes oxidation-reduction reactions and has been implicated in Down's syndrome [72], Alzheimer's disease [73] and various forms of neoplasms [74]. The protein may be involved in the regulation of apoptosis and response to oxidative stress [75], possibly by influencing oxidoreductase enzyme activities [67,69,76], among others. The CRYM has also been associated with various forms of cancer [77]. Of the down-regulated genes, the LGALS9 has been linked with various neoplasms and positive regulation of I-κB kinase/NF-κB cascade, Ras signalling and protein amino acid phosphorylation [78,79]. It probably inhibits cell growth in a fashion that is regulated by NF-κB [80]. The MIRHG2 [81], UNC5D [82] and HDAC2 [83,84] have similarly been associated with different types of cancer. However, the functional roles particularly with respect to cardiovascular function of their putative protein products remain largely ill-defined.

Thus, it appears that several altered pathways, processes and yet undefined genes described in the present study are also implicated in various non-cardiac disorders. These actions may be related to alterations in apoptotic signaling components, such as MAPKs, p90RSK, NF-κB, caspase-3 and Src, that have in turn been partly implicated in the pathogenesis of heart muscle disease [50,53,85]. The concomitant up-regulation and down-regulation of the apoptotic signaling components points to a dual role for this pathway, possibly contributing to both the progression of the disease to heart failure and to compensatory/adaptive mechanisms in response to the ventricular overload [80,86,87]. Besides, it has also been suggested that in HF, apoptosis may be interrupted and is therefore potentially reversible [20]. This might also explain in part some of these apparently incongruous signalling events under these disease conditions.

The fact that some of the above genes are similarly associated with neurodegenerative disorders, such as Alzheimer's disease, Huntington's disease or Down's syndrome, attracts speculations with respect to the relevance of their existence in the myocardium. Interesting in this regard is also a recent finding of a mouse model of hypertension revealing the induction of Alzheimer's disease pathways [88]. It is therefore appealing to hypothesize common underlying mechanism(s) leading to or triggered by these biological processes with a missing link connecting cardiac disease pathways with these disorders. It is also noteworthy that the formation of amyloid plaques in Alzheimer's disease is associated partly with perturbations in Ca^{2+} metabolism, a pivotal second messenger in the regulation of cardiac contractility. Moreover, mitochondrial dysfunction and particularly oxidative stress are well-established major players in Alzheimer's disease [89,90] and possibly Down's syndrome [91], pointing to the likelihood of an inter-regulation of these disorders at the level of mitochondrial function or second messenger signal transduction. Hence, these observations necessitate more precisely focused studies to enhance our understanding of the missing links coupling such diverse forms of human disease with one another.

A further important question remains as to whether or not the observed alterations in gene expression are exclusive for DCM per se, heart muscle disease as a whole, or could be eventually ascribable to heart failure in general. Although the current study was not directed at addressing this issue, it is noteworthy that investigations involving global gene expression in DCM and heart failure thus far have yielded varying results. While some investigators purport disease-specific alterations in gene expression [25], others view these changes as ultimately describing the events associated with heart failure. It has
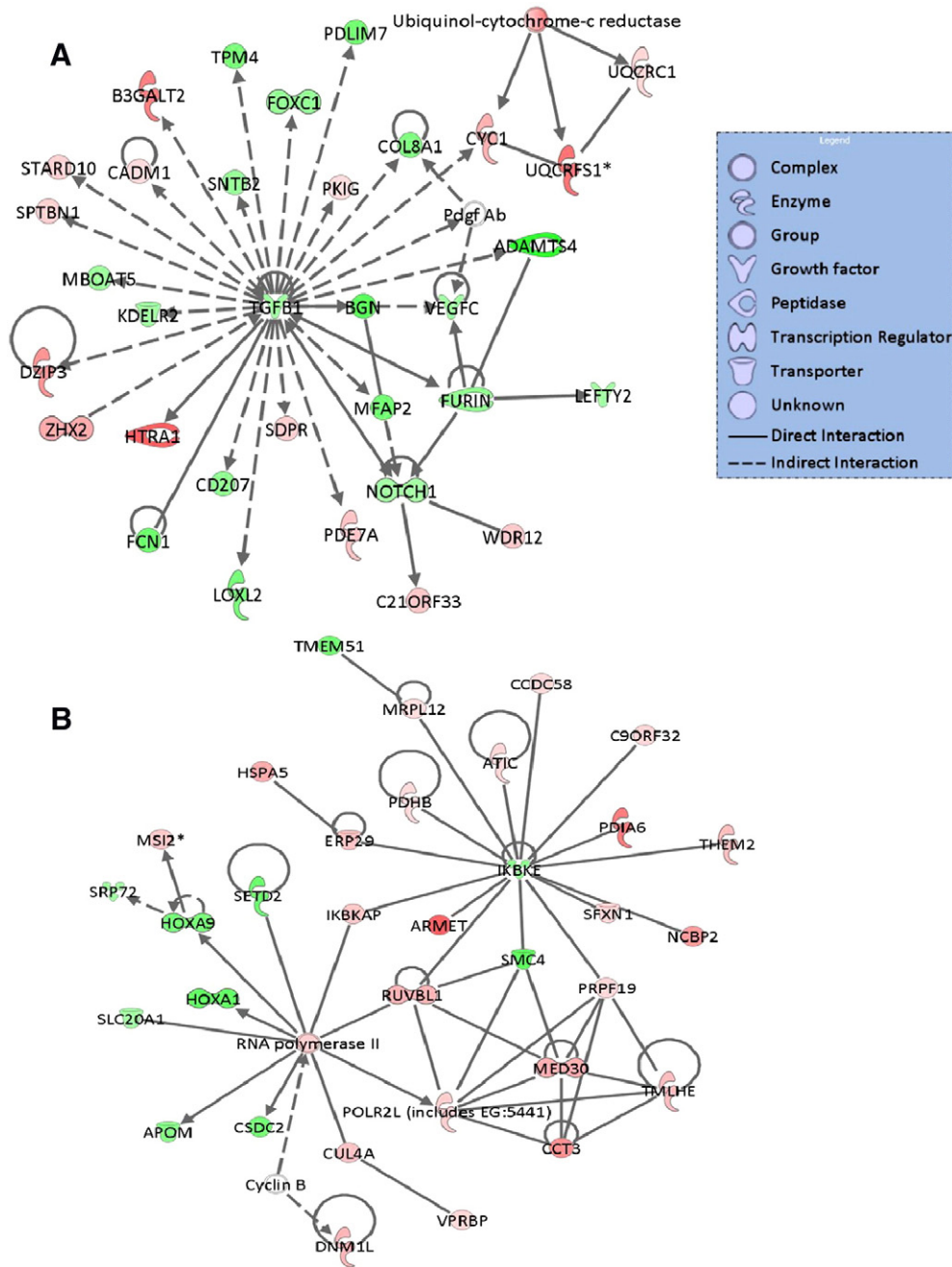
Fig. 3. Functional network analysis of DCM specific genes. Top two scoring gene interaction networks with high relevancy scores (significance: score = 48) for the DCM specific genes. A score of three indicates that there is 1/1000 (score = −log (P value)) chance that the focus genes are assigned to a network randomly. Green indicates down-regulated, red, up-regulated. The color intensity is correlated with fold change. Straight lines are for direct gene to gene interactions, dashed lines are for indirect ones.

also been argued that the alterations in gene expression in explanted DCM hearts are reflective of events pertaining to the progression of heart failure rather than the heart muscle disease per se [25,55,85,92]. However, while these studies commonly discuss the sharing of alterations in gene expression under different disease conditions, they do not necessarily preclude the possibility of yet undefined changes in signaling pathways exclusively related to DCM being discernible from events specifically pertinent to end-stage heart failure, in general. Indeed, differences have been described in gene expression between ischemic heart disease (IHD) and DCM [26,54,85,93]. On the other hand, it is believed that remodeling is a feature of both IHD and DCM, suggesting common mechanisms for their progression to cardiac dysfunction. However, it has also been

asserted that, although heart failure emanating from these two diseases results in similar clinical endpoints, it progresses through different remodeling and molecular pathways [55]. Hence, further studies are necessary to ascertain the events determining various disease pathways to overt heart failure.

In summary, evaluation of global gene expression patterns provides a molecular depiction specific to DCM, yields insights into the pathophysiological aspects of heart muscle disease, and identifies novel genes and pathways whose cardiac-related functions have yet to be deciphered. The present study demonstrates not only concomitant activation of signaling components regulating partly counteractive mechanisms involved in cell death, survival and homeostasis, but also novel gene expression previously unknown to
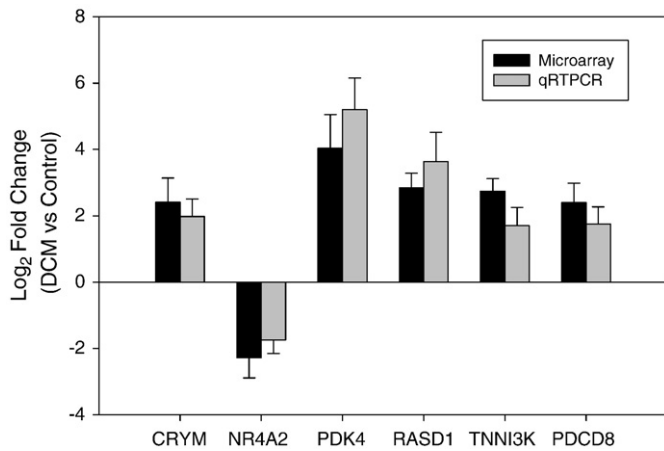
Fig. 4. Confirmation of the microarray gene expression for six randomly selected differentially expressed genes by qRT-PCR. Ratio of expression for each gene in DCM group to normal control (fold change) was $\log_2$ transformed for microarray data and real-time RT-PCR. Dark bars represent microarray hybridizations, and, and grey bars represent values from qRT-PCR. The error bar represents standard deviation (SD) over three experiments.

be related to cardiac function. The resemblance of DCM with disorders, such as cancer or neurodegenerative disorders, in the pattern of differential expression of several genes, molecular functions and pathways points to a link of these diseases at the level of apoptotic signaling, energy metabolism and maintenance of cellular structural integrity.

Acknowledgments

Appendix A

The original microarray data is available in the ArrayExpress database (Accession: E-TABM-480).

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygeno.2009.03.003.

References

[1] T. Arimura, T. Hayashi, A. Kimura, Molecular etiology of idiopathic cardiomyopathy, Acta Myol. 26 (3) (2007) 153–158.
[2] J. Ross Jr, Dilated cardiomyopathy: concepts derived from gene deficient and transgenic animal models, Circ. J. 66 (3) (2002) 219–224.
[3] A.N. Chang, J.D. Potter, Sarcomeric protein mutations in dilated cardiomyopathy, Heart Fail. Rev. 10 (3) (2005) 225–235.
[4] H. Granzier, Y. Wu, L. Siegfried, M. LeWinter, Titin: physiological function and role in cardiomyopathy and failure, Heart Fail. Rev. 10 (3) (2005) 211–223.
[5] F. Ahmad, J.G. Seidman, C.E. Seidman, The genetic basis for cardiac remodeling, Annu. Rev. Genomics Hum. Genet. 6 (2005) 185–216.
[6] M.G. Sutton, N. Sharpe, Left ventricular remodeling after myocardial infarction: pathophysiology and therapy, Circulation 101 (25) (2000) 2981–2988.
[7] M.R. Wolff, S.H. Buck, S.W. Stoker, M.L. Greaser, R.M. Mentzer, Myofibrillar calcium sensitivity of isometric tension is increased in human dilated cardiomyopathies: role of altered beta-adrenergically mediated protein phosphorylation, J. Clin. Invest. 98 (1) (1996) 167–176.
[8] N. Dzimiri, Regulation of beta-adrenoceptor signaling in cardiac function and disease, Pharmacol. Rev. 51 (3) (1999) 465–501.
[9] C. Mittmann, T. Eschenhagen, H. Scholz, Cellular and molecular aspects of contractile dysfunction in heart failure, Cardiovasc. Res. 39 (2) (1998) 267–275.
[10] M. Periasamy, A. Kalyanasundaram, SERCA pump isoforms: their role in calcium transport and disease, Muscle Nerve 35 (4) (2007) 430–442.
[11] R.J. Solaro, H.M. Rarick, Troponin and tropomyosin: proteins that switch on and tune in the activity of cardiac myofilaments, Circ. Res. 83 (5) (1998) 471–480.
[12] B.M. Palmer, Thick filament proteins and performance in human heart failure, Heart Fail. Rev. 10 (3) (2005) 187–197.
[13] J. Layland, R.J. Solaro, A.M. Shah, Regulation of cardiac contractile function by troponin I phosphorylation, Cardiovasc. Res. 66 (1) (2005) 12–21.
[14] P. Sivakumar, S. Gupta, S. Sarkar, S. Sen, Upregulation of lysyl oxidase and MMPs during cardiac remodeling in human dilated cardiomyopathy, Mol. Cell. Biochem. 307 (1–2) (2008) 159–167.
[15] N. Escobales, M.J. Crespo, Early pathophysiological alterations in experimental cardiomyopathy: the Syrian cardiomyopathic hamster, P. R. Health Sci. J. 27 (4) (2008) 307–314.
[16] D.T. McCloskey, S. Turcato, G.Y. Wang, L. Turnbull, B.Q. Zhu, T. Bambino, A.P. Nguyen, D.H. Lovett, R.A. Nissenson, J.S. Karliner, A.J. Baker, Expression of a Gi-coupled receptor in the heart causes impaired Ca2+ handling, myofilament injury, and dilated cardiomyopathy, Am. J. Physiol. Heart Circ. Physiol. 294 (1) (2008) H205–212.
[17] M. Mahmoudabady, M. Mathieu, L. Dewachter, I. Hadad, L. Ray, P. Jespers, S. Brimioulle, R. Naeije, K. McEntee, Activin-A, transforming growth factor-beta, and myostatin signaling pathway in experimental dilated cardiomyopathy, J. Card. Fail. 14 (8) (2008) 703–709.
[18] F. Princen, E. Bard, F. Sheikh, S.S. Zhang, J. Wang, W.M. Zago, D. Wu, R.D. Trelles, B. Bailly-Maitre, C.R. Kahn, Y. Chen, J.C. Reed, G.G. Tong, M. Mercola, J. Chen, G.S. Feng, Deletion of Shp2 tyrosine phosphatase in muscle leads to dilated cardiomyopathy, insulin resistance, and premature death, Mol. Cell. Biol. 29 (2) (2009) 378–388.
[19] M. Zheng, H. Cheng, X. Li, J. Zhang, L. Cui, K. Ouyang, L. Han, T. Zhao, Y. Gu, N.D. Dalton, M.L. Bang, K.L. Peterson, J. Chen, Cardiac-specific ablation of cypher leads to a severe form of dilated cardiomyopathy with premature death, Hum. Mol. Genet. (2008).
[20] E.J. Birks, N. Latif, K. Enesa, T. Folkvang, A. Luong le, P. Sarathchandra, M. Khan, H. Ovaa, C.M. Terracciano, P.J. Barton, M.H. Yacoub, P.C. Evans, Elevated p53 expression is associated with dysregulation of the ubiquitin–proteasome system in dilated cardiomyopathy, Cardiovasc. Res. 79 (3) (2008) 472–480.
[21] P. Tavi, M. Laine, M. Weckstrom, H. Ruskoaho, Cardiac mechanotransduction: from sensing to disease and treatment, Trends Pharmacol. Sci. 22 (5) (2001) 254–260.
[22] A.S. Barth, R. Kuner, A. Buness, M. Ruschhaupt, S. Merk, L. Zwermann, S. Kaab, E. Kreuzer, G. Steinbeck, U. Mansmann, A. Poustka, M. Nabauer, H. Sultmann, Identification of a common gene expression signature in dilated cardiomyopathy across independent microarray studies, J. Am. Coll. Cardiol. 48 (8) (2006) 1610–1617.
[23] C.K. Yung, V.L. Halperin, G.F. Tomaselli, R.L. Winslow, Gene expression profiles in end-stage human idiopathic dilated cardiomyopathy: altered expression of apoptotic and cytoskeletal genes, Genomics 83 (2) (2004) 281–297.
[24] J.D. Barrans, P.D. Allen, D. Stamatiou, V.J. Dzau, C.C. Liew, Global gene expression profiling of end-stage dilated cardiomyopathy using a human cardiovascular-based cDNA microarray, Am. J. Pathol. 160 (6) (2002) 2035–2043.
[25] S. Kaab, A.S. Barth, D. Margerie, M. Dugas, M. Gebauer, L. Zwermann, S. Merk, A. Pfeufer, K. Steinmeyer, M. Bleich, E. Kreuzer, G. Steinbeck, M. Nabauer, Global gene expression in human myocardium–oligonucleotide microarray analysis of regional diversity and transcriptional regulation in heart failure, J. Mol. Med. 82 (5) (2004) 308–316.
[26] M.M. Kittleson, K.M. Minhas, R.A. Irizarry, S.Q. Ye, G. Edness, E. Breton, J.V. Conte, G. Tomaselli, J.G. Garcia, J.M. Hare, Gene expression analysis of ischemic and nonischemic cardiomyopathy: shared and distinct genes in the development of heart failure, Physiol. Genomics 21 (3) (2005) 299–307.
[27] L. Shi, L.H. Reid, W.D. Jones, R. Shippy, J.A. Warrington, S.C. Baker, P.J. Collins, F. de Longueville, E.S. Kawasaki, K.Y. Lee, Y. Luo, Y.A. Sun, J.C. Willey, R.A. Setterquist, G.M. Fischer, W. Tong, Y.P. Dragan, D.J. Dix, F.W. Frueh, F.M. Goodsaid, D. Herman, R.V. Jensen, C.D. Johnson, E.K. Lobenhofer, R.K. Puri, U. Schrf, J. Thierry-Mieg, C. Wang, M. Wilson, P.K. Wolber, L. Zhang, S. Amur, W. Bao, C.C. Barbacioru, A.B. Lucas, V. Bertholet, C. Boysen, B. Bromley, D. Brown, A. Brunner, R. Canales, X.M. Cao, T.A. Cebula, J.J. Chen, J. Cheng, T.M. Chu, E. Chudin, J. Corson, J.C. Corton, L.J. Croner, C. Davies, T.S. Davison, G. Delenstarr, X. Deng, D. Dorris, A.C. Eklund, X.H. Fan, H. Fang, S. Fulmer-Smentek, J.C. Fuscoe, K. Gallagher, W. Ge, L. Guo, X. Guo, J. Hager, P.K. Haje, J. Han, T. Han, H.C. Harbottle, S.C. Harris, E. Hatchwell, C.A. Hauser, S. Hester, H. Hong, P. Hurban, S.A. Jackson, H. Ji, C.R. Knight, W.P. Kuo, J.E. LeClerc, S. Levy, Q.Z. Li, C. Liu, Y. Liu, M.J. Lombardi, Y. Ma, S.R. Magnuson, B. Maqsodi, T. McDaniel, N. Mei, O. Myklebost, B. Ning, N. Novoradovskaya, M.S. Orr, T.W. Osborn, A. Papallo, T.A. Patterson, R.G. Perkins, E.H. Peters, R. Peterson, K.L. Philips, P.S. Pine, L. Pusztai, F. Qian, H. Ren, M. Rosen, B.A. Rosenzweig, R.R. Samaha, M. Schena, G.P. Schroth, S. Shchegrova, D.D. Smith, F. Staedtler, Z. Su, H. Sun, Z. Szallasi, Z. Tezak, D. Thierry-Mieg, K.L. Thompson, I. Tikhonova, Y. Turpaz, B. Vallanat, C. Van, S.J. Walker, S.J. Wang, Y. Wang, R. Wolfinger, A. Wong, J. Wu, C. Xiao, Q. Xie, J. Xu, W. Yang, L. Zhang, S. Zhong, Y. Zong, W. Slikker Jr, The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements, Nat. Biotechnol. 24 (9) (2006) 1151–1161.
[28] L. Guo, E.K. Lobenhofer, C. Wang, R. Shippy, S.C. Harris, L. Zhang, N. Mei, T. Chen, D. Herman, F.M. Goodsaid, P. Hurban, K.L. Phillips, J. Xu, X. Deng, Y.A. Sun, W. Tong, Y.P. Dragan, L. Shi, Rat toxicogenomic study reveals analytical consistency across microarray platforms, Nat. Biotechnol. 24 (9) (2006) 1162–1169.
[29] M. Ali-Seyed, N. Laycock, S. Karanam, W. Xiao, E.T. Blair, C.S. Moreno, Cross-platform expression profiling demonstrates that SV40 small tumor antigen activates Notch, Hedgehog, and Wnt signaling in human cells, BMC Cancer 6 (2006) 54.
[30] S.J. Walker, Y. Wang, K.A. Grant, F. Chan, G.M. Hellmann, Long versus short oligonucleotide microarrays for the study of gene expression in nonhuman primates, J. Neurosci. Methods 152 (1–2) (2006) 179–189.

[31] Y. Wang, C. Barbacioru, F. Hyland, W. Xiao, K.L. Hunkapiller, J. Blake, F. Chan, C. Gonzalez, L. Zhang, R.R. Samaha, Large scale real-time PCR validation on gene expression measurements from two commercial long-oligonucleotide micro-arrays, BMC Genomics 7 (2006) 59.

[32] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method, Methods 25 (4) (2001) 402–408.

[33] R.C. Gentleman, V.J. Carey, D.M. Bates, B. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, J. Gentry, K. Hornik, T. Hothorn, W. Huber, S. Iacus, R. Irizarry, F. Leisch, C. Li, M. Maechler, A.J. Rossini, G. Sawitzki, C. Smith, G. Smyth, L. Tierney, J.Y. Yang, J. Zhang, Bioconductor: open software development for computational biology and bioinformatics, Genome Biol. 5 (10) (2004) R80.

[34] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. R. Stat. Soc. Ser. B 57 (1995) 289–300.

[35] V.G. Tusher, R. Tibshirani, G. Chu, Significance analysis of microarrays applied to the ionizing radiation response, Proc. Natl. Acad. Sci. U. S. A. 98 (9) (2001) 5116–5121.

[36] C. Li, W. Hung Wong, Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application, Genome Biol. 2 (8) (2001) RESEARCH0032.

[37] Z. Wu, R.A. Irizarry, Stochastic models inspired by hybridization theory for short oligonucleotide arrays, J. Comput. Biol. 12 (6) (2005) 882–893.

[38] Z. Wu, R.A. Irizarry, Preprocessing of oligonucleotide array data, Nat. Biotechnol. 22 (6) (2004) 656–658 author reply 658.

[39] A.I. Saeed, V. Sharov, J. White, J. Li, W. Liang, N. Bhagabati, J. Braisted, M. Klapa, T. Currier, M. Thiagarajan, A. Sturn, M. Snuffin, A. Rezantsev, D. Popov, A. Ryltsov, E. Kostukovich, I. Borisovsky, Z. Liu, A. Vinsavich, V. Trush, J. Quackenbush, TM4: a free, open-source system for microarray data management and analysis, Biotechniques 34 (2) (2003) 374–378.

[40] B.T. Sherman, D.W. Huang, Q. Tan, Y. Guo, S. Bour, D. Liu, R. Stephens, M.W. Baseler, H.C. Lane, R.A. Lempicki, DAVID Knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis, BMC Bioinformatics 8 (1) (2007) 426.

[41] D.A. Hosack, G. Dennis Jr., B.T. Sherman, H.C. Lane, R.A. Lempicki, Identifying biological themes within lists of genes with EASE, Genome Biol. 4 (10) (2003) R70.

[42] P.D. Thomas, A. Kejariwal, M.J. Campbell, H. Mi, K. Diemer, N. Guo, I. Ladunga, B. Ulitsky-Lazareva, A. Muruganujan, S. Rabkin, J.A. Vandergriff, O. Doremieux, PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification, Nucleic. Acids Res. 31 (1) (2003) 334–341.

[43] P.D. Thomas, A. Kejariwal, N. Guo, H. Mi, M.J. Campbell, A. Muruganujan, B. Lazareva-Ulitsky, Applications for protein sequence-function evolution data: mRNA/protein expression analysis and coding SNP scoring tools, Nucleic. Acids Res. 34 (2006) W645–650 (Web Server issue).

[44] N.B. Ivanova, J.T. Dimos, C. Schaniel, J.A. Hackney, K.A. Moore, I.R. Lemischka, A stem cell molecular signature, Science 298 (5593) (2002) 601–604.

[45] S.S.P. Dudoit, J.C. Boldrick, Multiple hypothesis testing in microarray experiments, Statist. Sci. 18 (1) (2003) 71–103.

[46] P. Pedotti, P.A. t Hoen, E. Vreugdenhil, G.J. Schenk, R.H. Vossen, Y. Ariyurek, M. de Hollander, R. Kuiper, G.J. van Ommen, J.T. den Dunnen, J.M. Boer, R.X. de Menezes, Can subtle changes in gene expression be consistently detected with different microarray platforms? BMC Genomics 9 (2008) 124.

[47] L.Y. Chuang, C.H. Ke, H.W. Chang, C.H. Yang, A two-stage feature selection method for gene expression data, Omics (2009).

[48] A.B. Barrett, J.H. Phan, M.D. Wang, Combining multiple microarray studies using bootstrap meta-analysis, Conf. Proc. IEEE. Eng. Med. Biol. Soc. 1 (2008) 5660–5663.

[49] P. Francois, C. Garzoni, M. Bento, J. Schrenzel, Comparison of amplification methods for transcriptomic analyses of low abundance prokaryotic RNA sources, J. Microbiol. Methods 68 (2) (2007) 385–391.

[50] N. Dzimiri, B. Afrane, C.C. Canver, Preferential existence of death-inducing proteins in the human cardiomyopathic left ventricle, J. Surg. Res. (2007).

[51] N. Dzimiri, P. Muiya, E. Andes, B. Afrane, Differential regulation of myocyte death/survival signalling in human dilated cardiomyopathy, FASEB J. 20 (2006) 164.

[52] C. Steenbergen, C.A. Afshari, J.G. Petranka, J. Collins, K. Martin, L. Bennett, A. Haugen, P. Bushel, E. Murphy, Alterations in apoptotic signaling in human idiopathic cardiomyopathic hearts in failure, Am. J. Physiol. Heart Circ. Physiol. 284 (1) (2003) H268–276.

[53] Y. Takeishi, Q. Huang, J. Abe, W. Che, J.D. Lee, H. Kawakatsu, B.D. Hoit, B.C. Berk, R.A. Walsh, Activation of mitogen-activated protein kinases and p90 ribosomal S6 kinase in failing human hearts with dilated cardiomyopathy, Cardiovasc. Res. 53 (1) (2002) 131–137.

[54] G. Plenz, Z.F. Song, S. Reichenberg, T.D. Tjan, H. Robenek, M.C. Deng, Left-ventricular expression of interleukin-6 messenger-RNA higher in idiopathic dilated than in ischemic cardiomyopathy, Thorac. Cardiovasc. Surg. 46 (4) (1998) 213–216.

[55] J.J. Hwang, P.D. Allen, G.C. Tseng, C.W. Lam, L. Fananapazir, V.J. Dzau, C.C. Liew, Microarray gene expression profiles in dilated and hypertrophic cardiomyopathic end-stage heart failure, Physiol. Genomics 10 (1) (2002) 31–44.

[56] R. Grzeskowiak, H. Witt, M. Drungowski, R. Thermann, S. Hennig, A. Perrot, K.J. Osterziel, D. Klingbiel, S. Scheid, R. Spang, H. Lehrach, P. Ruiz, Expression profiling of human idiopathic dilated cardiomyopathy, Cardiovasc. Res. 59 (2) (2003) 400–411.

[57] J. Xu, N.L. Gong, I. Bodi, B.J. Aronow, P.H. Backx, J.D. Molkentin, Myocyte enhancer factors 2A and 2C induce dilated cardiomyopathy in transgenic mice, J. Biol. Chem. 281 (14) (2006) 9152–9162.

[58] M.M. LeWinter, Functional consequences of sarcomeric protein abnormalities in failing myocardium, Heart Fail. Rev. 10 (3) (2005) 249–257.

[59] R. Lopes, P.F. Solter, D.D. Sisson, M.A. Oyama, R. Prosek, Characterization of canine mitochondrial protein expression in natural and induced forms of idiopathic dilated cardiomyopathy, Am. J. Vet. Res. 67 (6) (2006) 963–970.

[60] M.A. Portman, The adenine nucleotide translocator: regulation and function during myocardial development and hypertrophy, Clin. Exp. Pharmacol. Physiol. 29 (4) (2002) 334–338.

[61] A. Dorner, S. Giessen, R. Gaub, H. Grosse Siestrup, P.L. Schwimmbeck, R. Hetzer, W. Poller, H.P. Schultheiss, An isoform shift in the cardiac adenine nucleotide translocase expression alters the kinetic properties of the carrier in dilated cardiomyopathy, Eur. J. Heart Fail. 8 (1) (2006) 81–89.

[62] K.B. Margulies, S. Matiwala, C. Cornejo, H. Olsen, W.A. Craven, D. Bednarik, Mixed messages: transcription patterns in failing and recovering human myocardium, Circ. Res. 96 (5) (2005) 592–599.

[63] S. Hannenhalli, M.E. Putt, J.M. Gilmore, J. Wang, M.S. Parmacek, J.A. Epstein, E.E. Morrisey, K.B. Margulies, T.P. Cappola, Transcriptional genomics associates FOX transcription factors with human heart failure, Circulation 114 (12) (2006) 1269–1276.

[64] S. Grau, A. Baldi, R. Bussani, X. Tian, R. Stefanescu, M. Przybylski, P. Richards, S.A. Jones, V. Shridhar, T. Clausen, M. Ehrmann, Implications of the serine protease HtrA1 in amyloid precursor protein processing, Proc. Natl. Acad. Sci. U. S. A. 102 (17) (2005) 6021–6026.

[65] Z. Yang, N.J. Camp, H. Sun, Z. Tong, D. Gibbs, D.J. Cameron, H. Chen, Y. Zhao, E. Pearson, X. Li, J. Chien, A. Dewan, J. Harmon, P.S. Bernstein, V. Shridhar, N.A. Zabriskie, J. Hoh, K. Howes, K. Zhang, A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration, Science 314 (5801) (2006) 992–993.

[66] F. Carta, P.P. Demuro, C. Zanini, A. Santona, D. Castiglia, S. D'Atri, P.A. Ascierto, M. Napolitano, A. Cossu, B. Tadolini, F. Turrini, A. Manca, M.C. Sini, G. Palmieri, A.C. Rozzo, Analysis of candidate genes through a proteomics-based approach in primary cell lines from malignant melanomas and their metastases, Melanoma Res. 15 (4) (2005) 235–244.

[67] J. Furuta, Y. Nobeyama, Y. Umebayashi, F. Otsuka, K. Kikuchi, T. Ushijima, Silencing of Peroxiredoxin 2 and aberrant methylation of 33 CpG islands in putative promoter regions in human malignant melanomas, Cancer Res. 66 (12) (2006) 6080–6086.

[68] C. Oka, R. Tsujimoto, M. Kajikawa, K. Koshiba-Takeuchi, J. Ina, M. Yano, A. Tsuchiya, Y. Ueta, A. Soma, H. Kanda, M. Matsumoto, M. Kawaichi, HtrA1 serine protease inhibits signaling mediated by Tgfbeta family proteins, Development 131 (5) (2004) 1041–1053.

[69] N. Vahsen, C. Cande, J.J. Briere, P. Benit, N. Joza, N. Larochette, P.G. Mastrobe-rardino, M.O. Pequignot, N. Casares, V. Lazar, O. Feraud, N. Debili, S. Wissing, S. Engelhardt, F. Madeo, M. Piacentini, J.M. Penninger, H. Schagger, P. Rustin, G. Kroemer, AIF deficiency compromises oxidative phosphorylation, EMBO J. 23 (23) (2004) 4679–4689.

[70] A. Urbano, U. Lakshmanan, P.H. Choo, J.C. Kwan, P.Y. Ng, K. Guo, S. Dhakshina-moorthy, A. Porter, AIF suppresses chemical stress-induced apoptosis and maintains the transformed state of tumor cells, EMBO J. 24 (15) (2005) 2815–2826.

[71] C. Delettre, V.J. Yuste, R.S. Moubarak, M. Bras, N. Robert, S.A. Susin, Identification and characterization of AIFsh2, a mitochondrial apoptosis-inducing factor (AIF) isoform with NADH oxidase activity, J. Biol. Chem. 281 (27) (2006) 18507–18518.

[72] M.F. Sanchez-Font, J. Sebastia, C. Sanfeliu, R. Cristofol, G. Marfany, R. Gonzalez-Duarte, Peroxiredoxin 2 (PRDX2), an antioxidant enzyme, is under-expressed in Down syndrome fetal brains, Cell. Mol. Life Sci. 60 (7) (2003) 1513–1523.

[73] J. Yao, M. Taylor, F. Davey, Y. Ren, J. Aiton, P. Coote, F. Fang, J.X. Chen, S.D. Yan, F.J. Gunn-Moore, Interaction of amyloid binding alcohol dehydrogenase/Abeta mediates up-regulation of peroxiredoxin II in the brains of Alzheimer's disease patients and a transgenic Alzheimer's disease mouse model, Mol. Cell. Neurosci. 35 (2) (2007) 377–382.

[74] E. Huang, S.H. Cheng, H. Dressman, J. Pittman, M.H. Tsou, C.F. Horng, A. Bild, E.S. Iversen, M. Liao, C.M. Chen, M. West, J.R. Nevins, A.T. Huang, Gene expression predictors of breast cancer outcomes, Lancet 361 (9369) (2003) 1590–1596.

[75] J.A. Klein, C.M. Longo-Guess, M.P. Rossmann, K.L. Seburn, R.E. Hurd, W.N. Frankel, R.T. Bronson, S.L. Ackerman, The harlequin mouse mutation downregulates apoptosis-inducing factor, Nature 419 (6905) (2002) 367–374.

[76] K. Chikamori, J.E. Hill, D.R. Grabowski, E. Zarkhin, A.G. Grozav, S.A. Vaziri, J. Wang, A.V. Gudkov, L.R. Rybicki, R.M. Bukowski, A. Yen, M. Tanimoto, M.K. Ganapathi, R. Ganapathi, Downregulation of topoisomerase IIbeta in myeloid leukemia cell lines leads to activation of apoptosis following all-trans retinoic acid-induced differentiation/growth arrest, Leukemia 20 (10) (2006) 1809–1818.

[77] S. Mousses, L. Bubendorf, U. Wagner, G. Hostetter, J. Kononen, R. Cornelison, N. Goldberger, A.G. Elkahloun, N. Willi, P. Koivisto, W. Ferhle, M. Raffeld, G. Sauter, O.P. Kallioniemi, Clinical validation of candidate genes associated with prostate cancer progression in the CWR22 model system using tissue microarrays, Cancer Res. 62 (5) (2002) 1256–1260.

[78] M.J. Abedin, Y. Kashio, M. Seki, K. Nakamura, M. Hirashima, Potential roles of galectins in myeloid differentiation into three different lineages, J. Leukoc. Biol. 73 (5) (2003) 650–656.

[79] A. Kasamatsu, K. Uzawa, D. Nakashima, H. Koike, M. Shiiba, H. Bukawa, H. Yokoe, H. Tanzawa, Galectin-9 as a regulator of cellular adhesion in human oral squamous cell carcinoma cell lines, Int. J. Mol. Med. 16 (2) (2005) 269–273.

[80] T. Okudaira, M. Hirashima, C. Ishikawa, S. Makishi, M. Tomita, T. Matsuda, H. Kawakami, N. Taira, K. Ohshiro, M. Masuda, N. Takasu, N. Mori, A modified version of galectin-9 suppresses cell growth and induces apoptosis of human T-cell leukemia virus type I-infected T-cell lines, Int. J. Cancer 120 (10) (2007) 2251–2261.

[81] P.S. Eis, W. Tam, L. Sun, A. Chadburn, Z. Li, M.F. Gomez, E. Lund, J.E. Dahlberg,

Accumulation of miR-155 and BIC RNA in human B cell lymphomas, Proc. Natl. Acad. Sci. U. S. A. 102 (10) (2005) 3627–3632.

[82] K. Koed, C. Wiuf, L.L. Christensen, F.P. Wikman, K. Zieger, K. Moller, H. von der Maase, T.F. Orntoft, High-density single nucleotide polymorphism array defines novel stage and location-dependent allelic imbalances in human bladder tumors, Cancer Res. 65 (1) (2005) 34–45.

[83] S. Zimmermann, F. Kiefer, M. Prudenziati, C. Spiller, J. Hansen, T. Floss, W. Wurst, S. Minucci, M. Gottlicher, Reduced body size and decreased intestinal tumor rates in HDAC2-mutant mice, Cancer Res. 67 (19) (2007) 9047–9054.

[84] T. Tateno, H. Izumiyama, M. Doi, T. Yoshimoto, M. Shichiri, N. Inoshita, K. Oyama, S. Yamada, Y. Hirata, Differential gene expression in ACTH-secreting and non-functioning pituitary tumors, Eur. J. Endocrinol. 157 (6) (2007) 717–724.

[85] M. Steenman, Y.W. Chen, M. Le Cunff, G. Lamirault, A. Varro, E. Hoffman, J.J. Leger, Transcriptomal analysis of failing and nonfailing human hearts, Physiol. Genomics 12 (2) (2003) 97–112.

[86] H. Lou, I. Danelisen, P.K. Singal, Involvement of mitogen-activated protein kinases in adriamycin-induced cardiomyopathy, Am. J. Physiol. Heart Circ. Physiol. 288 (4) (2005) H1925–1930.

[87] O.F. Bueno, J.D. Molkentin, Involvement of extracellular signal-regulated kinases 1/2 in cardiac hypertrophy and cell death, Circ. Res. 91 (9) (2002) 776–781.

[88] J.E. Larkin, B.C. Frank, R.M. Gaspard, I. Duka, H. Gavras, J. Quackenbush, Cardiac transcriptional response to acute and chronic angiotensin II treatments, Physiol. Genomics 18 (2) (2004) 152–166.

[89] B. Su, X. Wang, A. Nunomura, P.I. Moreira, H.G. Lee, G. Perry, M.A. Smith, X. Zhu, Oxidative stress signaling in Alzheimer's disease, Curr. Alzheimer Res. 5 (6) (2008) 525–532.

[90] H. Fukui, C.T. Moraes, The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis? Trends Neurosci. 31 (5) (2008) 251–256.

[91] S. Arbuzova, T. Hutchin, H. Cuckle, Mitochondrial dysfunction and Down's syndrome, Bioessays. 24 (8) (2002) 681–684.

[92] F.L. Tan, C.S. Moravec, J. Li, C. Apperson-Hansen, P.M. McCarthy, J.B. Young, M. Bond, The gene expression fingerprint of human heart failure, Proc. Natl. Acad. Sci. U. S. A. 99 (17) (2002) 11387–11392.

[93] S. Ikeda, S.W. Kong, J. Lu, E. Bisping, H. Zhang, P.D. Allen, T.R. Golub, B. Pieske, W.T. Pu, Altered microRNA expression in human heart disease, Physiol. Genomics (2007).

Glossary

ANT(SLC25A4): adenine nucleotide translocase
β-AR: β-adrenoceptor
BCAT2: mitochondrial branched chain aminotransferase 2
MIRHG2: B-cell receptor-inducible gene
BMP: bone morphogenic protein
CRYM: crystalline-mu
EGF: epidermal growth factor
FDR: false discovery rate
FGF: fibroblast growth factor
HCM: hypertrophic cardiomyopathy
HDAC2: histone deacetylase 2
HTRA1: human high-temperature requirement factor A1
IFNA13: interferon A13
KCNQ2: potassium voltage-gated channel, member 2
LGALS9: lectin, galactose binding, soluble 9
MAPK: mitogen-activated protein kinase
MLYCD: malonyl-CoA decarboxylase
MX1: myxovirus subtype 1
NF-κB: nuclear factor κB
NR4A2: pyruvate dehydrogenase kinase, isoenzyme 4
p90RSK: p90 Ribosomal S6 Kinase
AIFM1: mitochondrial programmed cell-death protein 8
PDK4: pyruvate dehydrogenase kinase, isozyme 4
PRDX2: peroxiredoxin 2
RASD1: dexamethasome-induced RAS encoding subtype 1 gene
SERCA: sarco-endoplasmic reticulum Ca^{2+}-ATPase
TCA: tricarboxylic acid
TGFR-β: transforming growth factor-β receptor
TNF: tumor necrosis factor
TNNI3: human troponin I subtype 3
TNNI3K: TNNI3 interacting kinase
UBB: ubiquitin B
UNC5D: unc-5 homolog D