Genetics and Molecular Research

Transcriptional analysis of atrial and ventricular muscles from rats

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ABSTRACT. Previous studies have used microarray technology to explore gene expression differences between the atrium and the ventricle. However, selection criteria for the differentially expressed genes (DEGs) based only on either the fold change or the P value in these studies. Here, we aim to further identify the DEGs by setting a P value threshold of <0.05 and a fold change of >2, which may yield more specific gene expression differences between the atrium and the ventricle. Gene expression profiling of the atrial appendages and the ventricular free walls in 13 normal male Sprague Dawley rats were obtained from the Gene Expression Omnibus data base (accession No.: GSE5266). DEGs between the atrial and the ventricular samples were screened using the microarray significance analysis. The underlying functions of DEGs were predicted by gene ontology and pathway enrichment analyses. In addition, we also constructed protein interactions networks, and analyzed the function modules of the interacting proteins by MCODE. A total of 757DEGs between the atria and the ventricles

were found. The genes highly expressed in the ventricular myocytes were associated with muscle contraction (e.g., *Myl1*, *Myl2*, *Myl3*, and *Myh7*) and energy production (e.g., *Acadm* and *Acsl6*), while the genes preferentially expressed in the atrial myocytes were involved in the integration of neurohumoral signals (e.g., *Cldn1*). These conclusions were confirmed by pathway enrichment and function module analyses. Our present study provides an overview of the transcript level differences between the atrium and the ventricle, which may be useful for determination of potential biomarkers.

Key words: Brain natriuretic peptide; Cardiovascular disease; Gene expression profile; Functional association analysis; Diagnosis

INTRODUCTION

In mammals, the ventricles primarily generate contractile forces to pump the blood, while the atria, other than its contractile function, serve as a source and target for neurohumoral signals. In order to carry out these functions, the atrial and ventricular myocytes differ in morphology, ultrastructure, and gene expression (Legato, 1973; Forbes et al., 1990). For example, atrial cardiocytes synthesize and secrete more atrial natriuretic factor (Cantin et al., 1987) and phenylethanolamine-*N*-methyltransferase (Tillinger et al., 2006) as compared with ventricular cardiocytes. However, the calcium channel CaV1.2 (Larsen et al., 2002), the inwardly rectifying K^+-channel subunit Kir2.1 (Dhamoon et al., 2004), as well as the KCNE2 protein (Zhang et al., 2012) are more abundantly expressed in the ventricles than in the atria.

In addition, recent studies have also examined differences in gene expression between the atrium and the ventricle via microarray technology, which allows simultaneous determination of the expression levels of thousands of genes (Zhao et al., 2002; Tsubakihara et al., 2004; Barth et al., 2005; McGrath and de Bold., 2009). However, selection criteria for the differentially expressed genes (DEGs) are traditionally only based on either the fold change (FC) alone [>1.8 (McGrath and de Bold, 2009) or 2 (Tsubakihara et al., 2004)], or the adjusted P value alone [false discovery rate (FDR) <0.03] (Barth et al., 2005). In this study, we aimed to further identify the DEGs between the atrium and the ventricle based on an FDR cut-off point of <0.05 and a FC of >2 ($|\log_2 FC|>1$). This method may yield more genes that are specific for the atrium and the ventricle. In addition, we also performed functional analysis using various bioinformatic methods.

MATERIAL AND METHODS

Data sets

Gene expression profiling of the atrial appendages and the ventricular free walls in 13 male Sprague Dawley rats were downloaded from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) under the accession No. GSE5266 (McGrath and de Bold, 2009). The GeneChip platform used was the Rat Genome 230 2.0 Array from Affymetrix (Santa Clara, CA, USA). Tissues were grouped and divided into 4 pools to generate 4 biological replicates for each muscle type. All studies were approved by the University of Ottawa Animal Care Committee.

Data processing and statistical analysis

The raw microarray data were pre-processed using the robust multiarray average analysis function included in the R/Bioconductor software, which performs normalization, background correction, and data summarization. DEGs between the atrial and the ventricular muscle samples were screened using the significance analysis of microarray method (Zhang, 2007), and the raw P values were adjusted according to FDR via the Benjamini & Hochberg procedure (Shaffer, 1995). Genes were considered to be differentially expressed when FDR < 0.05 and expression FC > 2 ($|\log_2 FC|>1$).

Functional classification and gene annotation

Screened genes from each group were classified into different functional categories through clusters of orthologous groups (COGs) (Tatusov et al., 2000; Tatusov et al., 2001; Tatusov et al., 2003). The COGs of proteins were generated by comparing the protein sequences of complete genomes. Each cluster contains proteins or groups of paralogs from at least three lineages. To obtain a better understanding of the biological functions of selected genes, gene ontology (GO) enrichment analyses were conducted using the Inter Pro Scan program. The selected genes were also mapped onto various pathways by the Gene Map Annotator and Pathway Profiler (GenMapp) software (Kanehisa et al., 2010).

Protein-protein interactions and function module analysis

Protein interactions between DEGs were predicted by STRING (http://string-db.org/) (Szklarczyk et al., 2011) with medium confidence, and the interaction network was analyzed by the Cytoscape 2.8.3 software (Shannon et al., 2003). The function modules of the interacting proteins were analyzed by MCODE (Bader and Hogue, 2003), and representation of these functional themes were mapped out by BiNGO (Maere et al., 2005), a plug-in of Cytoscape.

RESULTS

DEGs between atrial and ventricular tissues

Based on the pre-set threshold described above (FDR < 0.05 and $|\log_2 FC|>1$), 757 DEGs were identified between the atrial and ventricular tissues. Among them, 475 genes showed higher expression in the atrial samples, and 282 genes showed higher expression levels in the ventricular tissues. The significantly up- and down-regulated genes in ventricular tissues are listed in **Table S1**.

Gene functional enrichment

As shown in Table 1, DEGs were classified into several different functional categories. It was found that genes involved in signal transductions accounted for the largest proportion of the group, and involved 116 genes (22.14%). Further functional enrichment analysis

showed that overall, DEGs between atrial and ventricular muscles were mainly involved in the regulation of cell proliferation, vasculature development, and identical protein binding (Figure 1 and Table 1). In addition, we also performed pathway analysis for DEGs by using the Gene Map Annotator and Pathway Profiler (GenMapp) software. As a result, we identified 11 significant pathways (Table 2). *Myl2* (myosin, light polypeptide 2, regulatory, cardiac, slow), *Myl3* (myosin, light chain 3, alkali; ventricular, skeletal, slow) and *Myh7* (myosin, heavy polypeptide 7, cardiac muscle, beta) genes were enriched in pathways involving cardiac muscle contraction (rno04260), hypertrophic cardiomyopathy (rno05410), leukocyte transendothelial migration (rno04670), and dilated cardiomyopathy (rno05414). In addition, genes regulating fatty acid metabolism (*Acadm*, acyl-Coenzyme A dehydrogenase, medium chain; *Acsl6*, acyl-CoA synthetase long-chain family member 6) and cell adhesion (*Cldn1*, claudin 1) were also enriched in the ventricular myocytes.

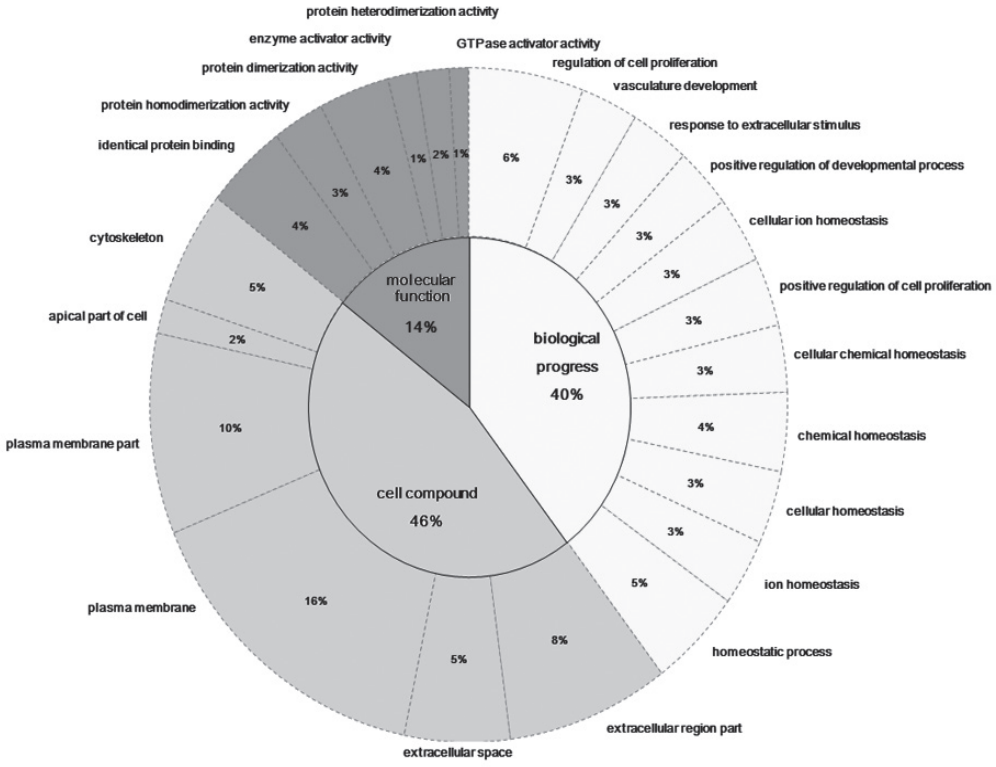

Figure 1. Functional classification of differentially expressed genes between the atria and the ventricles with known gene ontology annotations. White, gray, and black refer to biological progress, cell compound, and molecular function, respectively. Percentages in the figure denote differentially expressed genes plotted according to muscle type, i.e., atrium or ventricle.

Table 1. COG categories of differentially expressed genes between the atria and the ventricles.

Class definition	Number of class	Percent of class (%)
Signal transduction mechanisms	116	22.14
General function prediction only	83	15.84
Transcription	43	8.21
Cytoskeleton	21	4.01
Function unknown	40	7.63
Lipid transport and metabolism	25	4.77
Extracellular structures	16	3.05
Posttranslational modification, protein turnover, chaperones	33	6.30
Carbohydrate transport and metabolism	18	3.44
Energy production and conversion	16	3.05
Inorganic ion transport and metabolism	24	4.58
Amino acid transport and metabolism	17	3.24
Secondary metabolites biosynthesis, transport and catabolism	13	2.48
Cell cycle control, cell division, chromosome partitioning	7	1.34
Nucleotide transport and metabolism	5	0.95
Translation, ribosomal structure and biogenesis	5	0.95
RNA processing and modification	8	1.53
Chromatin structure and dynamics	5	0.95
Intracellular trafficking, secretion, and vesicular transport	16	3.053
Defense mechanisms	5	0.95
Cell motility	2	0.38
Coenzyme transport and metabolism	2	0.38
Cell wall/membrane/envelope biogenesis	2	0.38
Unclassified_Unclassified	1	0.19
Replication, recombination and repair	1	0.19

Table 2. Enriched KEGG pathways for differentially expressed genes between the atria and ventricles.

Term	P value	Genes
rno04260:Cardiac muscle contraction	0.0019	*MYL2, MYL3, ATP1B4, ATP1A1, MYH7, COX6B2, CACNA2D3, CACNA1C, TPM2, LOC687508, CACNA1D*
rno00071:Fatty acid metabolism	0.0020	*ACAA2, ACADSB, ACADM, CPT2, ALDH1B1, ACADS, ALDH2, ACSL6*
rno05410:Hypertrophic cardiomyopathy	0.0032	*PRKAG3, ITGA9, MYL2, MYL3, PRKAG2, MYH7, CACNA2D3, CACNA1C, TPM2, CACNA1D, TGFB2*
rno04512:ECM-receptor interaction	0.0081	*ITGA9, LAMB3, COL4A2, COL4A1, CD44, LAMA5, VTN, SDC2, FN1, LOC681309*
rno04670:Leukocyte transendothelial migration	0.0109	*VCAM1, ITGAL, MYL7, EZR, MYL2, NCF1, CXCR4, CLDN1, CLDN11, VAV2, CXCL12, MYL9*
rno04672:Intestinal immune network for IgA production	0.0125	*CXCR4, RT1-DB1, RT1-DMB, RT1-BA, CXCL12, CCL27, TGFB2*
rno00280:Valine, leucine and isoleucine degradation	0.0138	*ACAA2, ACADSB, ACADM, ALDH1B1, ACADS, AOX1, ALDH2*
rno00340:Histidine metabolism	0.0186	*ASPA, ALDH1B1, ALDH1A3, ALDH2, ALDH3A1*
rno04514:Cell adhesion molecules	0.0268	*ITGAL, PTPRF, RT1-DB1, NEO1, CLDN11, RT1-DMB, RT1-BA, SDC2, ALCAM, VCAM1, ITGA9, CLDN1, VCAN*
rno05414:Dilated cardiomyopathy	0.04010	*ITGA9, MYL2, MYL3, MYH7, CACNA2D3, CACNA1C, TPM2, CACNA1D, TGFB2*
rno04360:Axon guidance	0.0488	*ABLIM1, EPHA4, PAK2, EFNB3, CXCR4, SEMA3G, ABLIM3, RHOD, FES, CXCL12, SLIT3*

Protein-protein interactions and function module analysis

Protein interactions between DEGs were predicted by STRING with medium confidence. An interaction network that included 644 interacting gene pairs was obtained (Figure 2). With the Cytoscape application and its plug-ins, we obtained functional modules of the interaction network, which is presented in Figure 3. In line with the pathway analysis, we also identified modules that were associated with cardiac muscle contraction, fatty acid metabolism, and cell adhesion.

DISCUSSION

According to our microarray data, 757 genes were identified to be differentially expressed in specific regions of the heart; 475 genes were highly expressed in the atrial samples, and 282 genes were more abundantly expressed in the ventricular tissues. Specifically, *Myl1* was highly expressed in the atrial samples, while *Myl2*, *Myl3*, and *Myh7* were highly expressed in the ventricular tissues. These results were in agreement with previous studies. Further functional and PPI network analyses suggested that *Myl1, Myl2, Myl3,* and *Myh7* were important genes involved in muscle contraction.

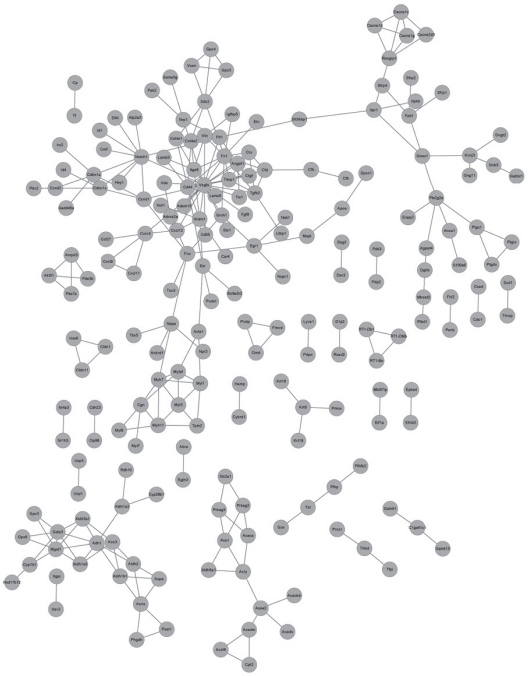

Figure 2. Interaction network of selected gene products. The circular nodes represent gene products, lines refer to interacting relationships between gene products.

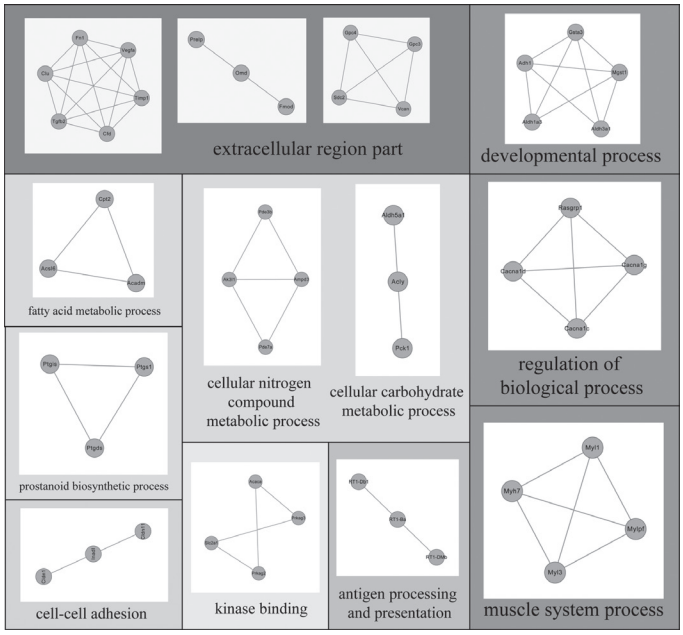

Figure 3. Functional modules of interaction network.

Myosin is a hexameric enzyme, which consists of two pairs of light chains and two heavy chains. *Myl2* makes up the regulatory light chain of myosin, while *Myl3* makes up the essential light chain of myosin. The regulatory light chain binds Ca^{2+} at activating concentrations. In addition, phosphorylation of a serine residue in the N-terminus of *Myl2* is also important for cardiac muscle contractions. In contrast to *Myl2*, *Myl3* contains a unique N-terminal domain that can bind actin, allowing *Myl3* to contribute to force-generating myosin cross-bridges (Timson et al., 1998; Kazmierczak et al., 2009). Several studies have demonstrated that mutations of the *Myl2* and *Myl3* genes are associated with ventricular chamber-type cardiomyopathy (Irie et al., 2011). In addition, myosin light chain-2 has also been shown to be a specific marker for early ventricular hiPSC (human induced pluripotent stem cell)-derived cardiomyocytes, but not atrial hiPSC-derived cardiomyocytes (Bizy et al., 2013). *Myh7*gene encodes the cardiac β-myosin heavy chain, and is the predominant myosin isoform in the ventricular myocardium. Budde et al. (2007) have previously identified the missense mutationc. The 842G>Cin *Myh7*, which affects a highly conserved amino acid in the myosin subfragment-1 (R281T). This mutation prevents salt bridge formation between residues R281 and D325, thereby destabilizing the myosin head (Budde et al., 2007). Richard et al. (2003) reported that changes to the *Myh7* genes accounted for 82% of families with identified mutations, emphasizing its relevance for hypertrophic cardiomyopathy The *Myl1* gene encodes the fast skeletal alkali myosin light polypeptide 1, and is a specific and early marker for development of fast skeletal muscle (Ling et al., 2010; Burguière et al., 2011). However, several studies also have indicated that *Myl1* can be expressed in cardiac muscles including the atria and the left ventricle (Kelly et al., 1995; Franco et al., 1997). In this study, we found that *Myl1* was more abundantly expressed in the atrial samples as compared with the ventricular tissues, which needs to be verified by future studies.

In addition, we also found that genes related to fatty acid metabolism (*Acadm* and *Acsl6*) and cell adhesion (*Cldn1*) were differentially expressed in the ventricles and the atria. Acyl-CoA dehydrogenase is an enzyme that catalyzes the first reactions of long-chain fatty acid β-oxidation in the mitochondria, and thus promotes ATP production in the heart (Le et al., 2000). Inherited deficiency of acyl-CoA dehydrogenase is associated with severe cardiac dysfunction including cardiac hypertrophy, polymorphic ventricular tachycardia, and cardiomyopathy, resulting in sudden death in neonates and children (Exil et al., 2003, 2006; Cox et al., 2009; Marcì and Ajovalasit, 2009). Acyl-CoA synthetase activates long chain fatty acids to form acyl-CoAs, and then directs fatty acids oxidation to generate ATP. Activation of fatty acids is catalyzed by a family of five long-chain acyl-CoA synthetases, namely, ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6 (Ellis et al., 2010). In this study, we found that ACADM and ACSL6 were highly expressed in ventricular tissues, suggesting that energy metabolism occurs more prominently in ventricular cardiomyocytes. CLDN1 is an integral component of epithelial tight junctions, and overexpression of CLDN1 leads to cardiac looping (Simard et al., 2006). Previous studies have demonstrated that another tight junction protein, connexin 43, is critical for migration of cardiac neural crest cells and the formation of peripheral ganglia (Huang et al., 1998). It was found that synaptic proteins were down-regulated in Cx43 null mouse hearts (Iacobas et al., 2005). This finding suggests that tight junction proteins are associated with neuro humoral signals. Based on the proposed functions of the ventricles and the atria, CLDN1 was suggested to be highly expressed in the atria, which was confirmed by our study.

In conclusion, the present study provides insight into the differences in transcript levels between the atrium and the ventricle. We speculate that genes predominately expressed in the ventricular myocytes are associated with muscle contraction and energy production, while genes

preferentially expressed in the atrial myocytes provide integration of neuro humoral signals. However, further studies are still required to confirm the function of these genes.

Conflicts of interest

The authors declare no conflicts of interest.

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Supplementary material

Table S1. COG categories of differentially expressed genes between the atria and the ventricles.

http://www.geneticsmr.com/year2016/vol15-1/pdf/gmr7330_supplementary.pdf