Pathology – Research and Practice 213 (2017) 548–557

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Gene expression profiling reveals genes and transcription factors associated with dilated and ischemic cardiomyopathies

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ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 9 October 2016
Accepted 22 December 2016

Keywords:
Differentially expressed gene
Dilated cardiomyopathy
Functional interaction
Ischemic cardiomyopathy
Transcription factor

ABSTRACT

Aims: Dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM) can cause heart failure, and this study aims to identify genes and transcription factors (TFs) associated with DCM and ICM.
Methods: Gene expression dataset GSE42955 was generated from GEO database, and it contained 12 DCM, 12 ICM, and 5 control samples. Differentially expressed genes (DEGs) were identified between DCM (or ICM) and controls. Gene functions were investigated, and their associations were analyzed using Enrichmentmap plugin in Cytoscape. Protein-protein interactions (PPIs) between DEGs were determined, and DEGs with high degree were defined as key DEGs. Potential TFs of key DEGs were predicted using iRegulon plugin. Common DEGs were found, and their functional interactions were investigated using GeneMANIA.
Results: A total of 362 and 300 DEGs were respectively identified for DCM and ICM in comparison with controls, and these DEGs mainly participated in similar functions about extracellular region, membrane, immune process, and defense response. PPI networks were respectively constructed for DCM and ICM, and 26 key DEGs (e.g. CXCL10, IL6, TLR3, and VCAM1) were found, which might be targeted by 35 TFs (e.g. IRF1). Besides, 47 common up-regulated DEGs were found, which participated in 14 pathways like Apoptosis, Collagen formation, as well as 127 common down-regulated DEGs that involved in 20 pathways like Adaptive immune system, Interferon γ signaling (e.g. IRF1, VCAM1), and Toll-like receptor signaling pathway (e.g. CXCL10, IL6, TLR3).
Conclusion: DCM and ICM may share similar mechanism, and TFs (e.g. IRF1) play crucial roles in their development via regulating gene expression.

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1. Introduction

Ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM) frequently cause heart failure [1,2]. DCM is characterized by systolic dysfunction and left ventricular (LV) dilatation in most patients, whereas ICM is characterized by LV enlargement and LV ejection fraction depression. Although the prevalence rates of DCM and ICM increase in recent years, their mechanism is not fully understood.

Previous studies investigated the molecular mechanism of ICM and DCM during the past decades, and cardiomyopathy-related genes were found. Interleukin-1β modulates inflammatory response during ICM progression, and its blockade improves left ventricular systolic/diastolic function in severe ICM in mice [3]. RNA-sequencing and quantitative real-time PCR have revealed that transcription factors (TFs) like SP100, CITED2, CEBPD, and BCL3 are down-regulated in myocardial tissue of ICM when compared with healthy control [4]. Besides, 17 HIF1A target genes like IGFBP3 and EGLN3 are up-regulated in ICM patients [4]. The endocannabinoid-CB2 receptor axis plays a cardioprotective role at the early stage of ICM [5]. Hearts of CB2-deficient mice cannot induce transformation between myosin heavy chain isoforms during repetitive ischemia and reperfusion (I/R) injury, resulting in cell apoptosis, cardiomyocytes loss, and left ventricular dysfunction [5]. Metabolic and proteomic data have revealed that ASK1/p38- and DJ-1/PTEN/AKT-mediated cell signaling pathways are associated with ICM [6].

In DCM hearts, mutations in titin, desmoplakin, plakophilin-2, ryanodine receptor 2, myosin-binding protein-C 3, desmocollin-

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http://dx.doi.org/10.1016/j.prp.2016.12.017
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2, sodium channel voltage-gated type V α, and desmoglein-2 are frequently observed [7]. Whole-exome sequencing has suggested that DCM is associated with a 10-nucleotide deletion in BAG3 gene, which causes a shift in the corresponding open reading frame, a loss of 135 amino acids, and a diminished protein level of BAG3 [8]. Moreover, microarray profiling has showed that most of the cardiac ion channel genes are abnormally expressed in DCM, e.g. up-regulated SCN2B, down-regulated KCNJ5, down-regulated KCNJ8, down-regulated CLIC2, and down-regulated CACNB2 [9]. Nevertheless, Molina-Navarro et al. only focus on the cardiac ion channel genes in the microarray analysis, and the molecular mechanism and global regulatory mechanism of cardiomyopathy still remains unclear. Also, there are few studies investigating the common genes and mechanisms involved in ICM and DCM.

In order to further identify genes and TFs associated with DCM and ICM, we comprehensively re-analyzed the high-throughput whole-genome gene expression profiling up-loaded to public database in previous study [9] by using bioinformatics tools and databases about functions, pathways, interactions and TFs (Fig. 1). Besides, the common genes involved in both ICM and DCM were identified, and these genes might contribute to the development of cardiomyopathy.

2. Methods

2.1. Gene expression profiling

Global gene expression data about DCM and ICM (No.GSE42955) were generated from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42955) that deposited public expression data. The platform of GSE42955 was Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]. A total of 29 transmural samples were involved in GSE42955, including 12 DCM samples, 12 ICM samples, and 5 control samples. Only gender information was shown in GEO, and all of the 29 samples were provided by men. According to the description in previous study [9], these samples were taken from the same place (near the apex) of the left ventricle in human hearts of patients undergoing cardiac transplantation and non-diseased donor hearts, after generating approval from ethics committee and all participants. Before microarray detection, Qiagen RNeasy Fibrous Tissue Mini kit (Qiagen Iberia SL, Spain) was utilized to extract RNA, whose concentration and quality were assessed using NanoDrop 2000 spectrophotometer and 2100 Bio-analyzer (Agilent Technologies, Spain SL), respectively.

2.2. Preprocessing the microarray data

Microarray data were firstly preprocessed using the affy software package [10] downloaded from Bioconductor (http://www.bioconductor.org/). In this process, background signal was corrected, data were normalized, and expression values were calculated. Thereafter, each probe identifier (ID) was changed into gene symbol using the hugene10sttranscriptcluster.db package [11] in the microarray platform, and expression values were summarized.

2.3. Differential expression analysis

To identify the differentially expressed genes (DEGs) between DCM and normal healthy hearts (namely, controls), as well as the DEGs between ICM and controls, limma package [12] in Bioconductor was utilized, which provided unpaired *t*-test method to calculate the p-value for expression difference. Only the DEGs with p-value < 0.05 and fold change (FC) ≥ 1.414 (namely, $|\log_2 FC| \geq 0.5$) were defined as significant DEGs.

2.4. Clustering analysis of DEGs

To determine whether DCM, ICM, and control samples could be distinguished by DEGs, clustering analysis was performed using pvclust package [13]. Generally, clusters with approximately unbiased p-value (AU) >90% are strongly supported by data.

2.5. Functions of DEGs

To investigate the bio-functions of DEGs, annotation, visualization and integrated discovery (DAVID) [14], an online tool, was utilized to conduct enrichment analysis. Thus, pathway terms and function terms participated by DEGs were identified based on the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.jp/) database and Gene Ontology (GO, http://www.geneontology.org/) database, respectively. Only the terms with DEG count ≥ 2 and p-value < 0.05 were defined as significant terms.

2.6. Associations between the enriched terms

To research the associations between these enriched terms, Enrichmentmap plugin [15] in Cytoscape 2.8 software [16] was utilized with the following parameters: p-value cutoff = 0.005; false discovery rate (FDR) cutoff = 0.1; similarity coefficient cutoff = 0.5. Based on the overlapped DEGs, the associations between two terms were calculated, and terms with strong associations were utilized to construct the corresponding association networks.

2.7. Interactions between DEGs

Generally, the proteins encoded by genes play their roles through interacting with each other, and Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) v10 database [17] stores protein–protein interactions (PPIs) like neighborhood, co-expression, and gene fusion. Therefore, the PPIs between DEGs were determined using STRING v10 with a criterion of medium confidence >0.4. Then, PPI networks of DEGs were constructed for DCM and ICM, and node degrees were calculated. DEGs with high degree were defined as key DEGs that might play crucial roles in network and cardiomyopathies, as degree stood for the number of nodes linked to a certain node.

2.8. Potential TFs of key DEGs

The TFs that might target key DEGs were predicted using iRegulon plugin [18] in Cytoscape 2.8 software [16]. Reportedly, iRegulon can enrich TF motifs based on their direct targets using the position weight matrix method [18]. In this study, the criteria for motif enrichment analysis were set as identity between orthologous genes ≥ 0.05 and FDR on motif similarity ≤ 0.001, and the TF motifs with normalized enrichment score (NES) > 7 were utilized to further identify the corresponding TFs. Thereafter, TF-target pairs were obtained based on the TRANSFAC and jasper databases included in iRegulon plugin. Furthermore, the TF family information was generated from the human database in AnimalTFDB 2.0 (http://bioinfo.life.hust.edu.cn/AnimalTFDB/) [19].

2.9. Common DEGs

DEG-set of DCM was compared with DEG-set of ICM using VennPlex software (http://www.irp.nia.nih.gov/bioinformatics/vennplex.html) [20], and the overlapped DEGs were identified based on $\log_2 FC$ value, including common up-regulated DEGs, common down-regulated DEGs, and DEGs with contrary expression changes (namely, contra-regulated genes).

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