Original Paper

The Effect of Mecp2 on Heart Failure

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Key Words

Mecp2 • Heart failure • Protein–protein interactions • Functional enrichment

Abstract

Background/Aims: Heart failure is the end result of various kinds of cardiovascular diseases. It has a high rate of morbidity and mortality. This article aims to determine the effect of MeCP2, a key epigenetic regulator, on heart failure. **Methods:** The genes associated with heart failure were selected and analyzed using Gene Ontology (GO) term analysis and protein–protein interaction (PPI) network analysis. Significantly up- or downregulated genes in a heart failure animal model were identified, and the genes that had the same or opposite alteration trends as MeCP2 were also recognized. Eighteen hub genes were picked based on topological parameters, and then aberrantly expressed genes with MeCP2 overexpression or knockout were analyzed by GO term, KEGG pathway and PPI analyses. **Results:** MeCP2 was downregulated in the heart failure animal model. Through comparison and alignment, 10 dysregulated genes were selected from the 18 hub genes (JAK1, SETD1B, HRC, TTN, LY22, TPM3, MYH11, MYH6, ALOX5AP, DECR1). These genes were mainly enriched in cytoskeletal regulation mediated by Rho GTPase and inflammation mediated by chemokine and cytokine signaling pathways. **Conclusions:** These dysregulated genes provide a better understanding of the underlying mechanisms of the effect of MeCP2 on heart failure and might be used as targets and prognostic markers of heart failure.

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Introduction

MeCP2, a nuclear protein, is one member of a family of methyl–CpG-binding proteins. It consists of multiple structural domains, including a methyl–CpG-binding domain (MBD) and a transcriptional repression domain (TRD). The MBD binds to methylated DNA, and the TRD recruits HDAC-containing complexes via the mSin3A corepressor to repress transcription [1, 2] (Fig. 1). Recently, it was found that MeCP2 has some unmethylated and active regulatory regions [3]. MeCP2 was first identified as the cause of Rett syndrome (RTT) because of its X-linked gene mutation [4]. MeCP2 function in specific brain regions has been studied extensively, and many studies have confirmed that MeCP2 knockdown or overexpression results in the inverse dysregulation of thousands of genes [5–8]. Some researchers have

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found that MeCP2 mRNA transcripts are highly expressed in lung, skeletal muscle and heart and moderately expressed in brain [9-12], whereas the protein is abundant in brain, lung and spleen; moderately abundant in kidney and heart; and hardly detectable in liver, stomach and small intestine [12]. MeCP2 is involved in the pathologies of many tissues and cells. It has received increasing attention as a nucleus-residing transcriptional regulator in various tissues and cells.

Epigenetic gene regulation plays a crucial role in cardiac fibrosis [13]. MeCP2, as an important epigenetic regulator, is considered to be a critical modulator. MeCP2 is increased in cardiac fibrosis tissue, and siRNA-MeCP2 reduces neonatal rat cardiac fibroblast cell viability by inhibiting cell proliferation [14]. In human and mouse heart failure, MeCP2 is repressed, and prevention of MeCP2 repression in mice after chronic cardiac pressure overload aggravates cardiac hypertrophy and mitochondrial and contractile dysfunction. In contrast, ablation of MeCP2 expression in cardiac myocytes *in vivo* facilitates functional recovery from cardiac failure by mechanical unloading of the heart [15]. It has also been reported that MeCP2-overexpressing human endothelial cells have impaired migration capacity and angiogenic activity, whereas MeCP2-silenced endothelial cells have improved endothelial migration and tube formation capacity [16]. All these data indicate the importance of MeCP2 as a regulator in cardiovascular diseases.

Based on the important role of MeCP2 in cardiovascular diseases, this article aims to further identify genes related to regulation of MeCP2 in heart failure. The original dataset (GSE52796) was downloaded from the Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>). The data were extracted and handled with homogenization. Subsequently, the differentially expressed genes (DEGs) were screened, followed by enrichment analyses for Gene Ontology (GO) and pathway enrichment analysis. Protein-protein interaction (PPI) network analyses were done, and the candidate genes from the PPI network analyses were further analyzed.

Materials and Methods

Initial datasets

The initial gene expression profiles of GSE52786 were downloaded from the GEO database with the GPL6887 annotation platform. The dataset contained 49 samples, including wild-type (WT), MeCP2-overexpressing (TG), and MeCP2-knockout (KO) mice; healthy mice (Sham) that underwent transverse aortic constriction (TAC); and mice that underwent removal of the aortic stenosis (reversible TAC, rTAC) [15].

Process and tools of data analysis

Two tools were used to screen DEGs for further alignment. One was GEO2R, with an indicator-adjusted P value <0.05. The other was online Morpheus (<https://software.broadinstitute.org/morpheus/>), using the indicator P value <0.05 and $|\log FC| > 1$. We performed enrichment analyses for GO terms using three tools for comparison. One was Cytoscape software. The plugin BiNGO was used to perform GO categories in Cytoscape. The predominant functional themes of a given gene set of a map were outputted. The second tool was online DAVID (<https://david.ncifcrf.gov/>). The third was online GoMiner (<https://discover.nci.nih.gov/gominer/index.jsp>). PPI information was evaluated by using the online database STRING (<http://www.string-db.org/>). The DEGs were pasted into STRING, validated interactions were selected, and then the PPI network data were imported to Cytoscape. The plugin MCODE was used to screen the modules of PPI network in Cytoscape.

Category and group of data

The data were classified into two categories, the healthy group and the TAC group. The up- and downregulated genes were compared between the healthy and TAC groups. Through GO term and PPI analyses, the dysregulated genes under TAC were recognized. As another article reported [15], MeCP2 was downregulated under TAC and restored under rTAC. Next, we screened and aligned the genes with the same

regulation trend as MeCP2 and the genes with the reverse regulation trends from MeCP2 under TAC and rTAC. That is, if a gene was downregulated under TAC and upregulated under rTAC, it was aligned as the same trend as MeCP2; otherwise, it was identified as the opposite trend. We identified the pathways that were enriched among these genes. Last, through the analysis of MeCP2 overexpression and knockdown, we found key genes and pathways regulated by MeCP2.

Results

Genes dysregulated under TAC

We analyzed the up- and downregulated genes under TAC, finding 111 upregulated genes and 195 downregulated genes. GO term analysis through an online tool (<http://pantherdb.org/>) showed that up- and downregulated genes were mainly enriched in biological regulation, cellular process, metabolic process and developmental process under the biological process category; in transporter activity, binding, catalytic activity and receptor activity under the molecular function category; and in cell junction, cell part, membrane and organelle under the cellular component category (Fig. 2). Panther pathways are shown in Fig. 3. The top density enrichment of all pathways was inflammation mediated by chemokine and cytokine signaling. These findings demonstrate that inflammatory reactions play an important role in heart failure. Some classical signal pathways were involved in TAC, including apoptosis signaling, PI3K signaling, Wnt signaling and the p53 pathway. PPI networks are shown in Fig. 4. The top hub genes were Myh6 (myosin heavy polypeptide 6 cardiac muscle), Myh11 (myosin heavy polypeptide 11 smooth muscle), Tpm3 (tropomyosin 3, gamma),

Fig. 1. Functional regions of MeCP2.

Fig. 2. Gene ontology analysis of differentially expressed genes under TAC. A. Upregulated genes by Panther molecular function category. B. Upregulated genes by Panther biological process category. C. Upregulated genes by Panther cellular component category. D. Downregulated genes by Panther molecular function category. E. Downregulated genes by Panther biological process category. F. Downregulated genes by Panther cellular component category.

Fig. 3. Pathway analysis of up- and downregulated genes under TAC. A. Upregulated genes by panther Pathway category. B. Downregulated genes by Panther pathway category.

Fig. 4. Protein-protein interaction network of up- and downregulated genes under TAC. Nodes are colored by regulation trend: upregulated (green) and downregulated (red). Edges are colored based on the number of connections: few connections (gray) and highly connected (blue). Node size is proportional to the p value: the lower the value, the larger the node.

Table 1. KEGG pathway analysis of genes with the alteration of MeCP2

Alteration trend	Pathway	count	P value	genes
The same trend	mmu01200:Carbon metabolism	23	2.4×10^{-7}	DLST SHMT1 SHMT2 ACADM ACO2 ACADS ALDOB OGDHL PFKM DLAT GPT ACAT1 IDH3A GOT2 PCX ACSS1 IDH3G H6PD SDHC ENO3 PCCB GPT2 PCCA
	mmu04022:cGMP-PKG signaling pathway	21	0.001	SLC8A1 CREB3 MRV1 ADCY6 ATP1A1 ITPR2 VDAC1 PP1F EDNRA RGS2 MYLK NFATC1 ATP2A2 PDE2A ADCY9 GATA4 ADRA1A CACNA1C NFATC3 PIK3R1 INSR
	mmu04020:Calcium signaling pathway	18	0.01	PHKA2 SLC8A1 PHKG1 GRM1 ITPR2 VDAC1 PP1F EDNRA ATP2A2 ADCY9 CACNA1G PLCD3 ADRA1A CACNA1H PRKACA CACNA1C CAMK2A MYLK
	mmu04152:AMPK signaling pathway	17	0.001	SREBF1 PPP2R1B CPT1B CREB3 PRKAG1 FOXO1 FOXO3 PFKM RPTOR TSC1 TSC2 RAB14 ADRA1A SCD4 INSR LIPE PIK3R1
	mmu05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	9	0.03	SGCG DSG2 DAG1 ITGA3 CTNNA1 CACNA1C TCF7L2 CTNNA3 SGCA
The opposite trend	mmu04068:FoxO signaling pathway	16	2.5×10^{-4}	PIK3CG SGK3 IGF1 PTEN G6PC3 CCNB1 NRAS TNFSF10 CCND1 KRAS CHUK MAPK12 CCND2 SOS1 ARAF GADD45B
	mmu04015:Rap1 signaling pathway	20	7.7×10^{-4}	ACTB PIK3CG ADCY7 GNAI2 IGF1 LPAR1 PRKCB CTNNB1 NRAS VEGFC PFN1 PFN2 KRAS MAPK12 RAPGEF6 GNAS RAPGEF4 RAP1B CALM2 CSF1R
	mmu04010:MAPK signaling pathway	20	0.005	MEF2C PTPRR PPM1A PPM1B STK3 PRKCB NRAS FOS TNFRSF1A RPS6KA3 ATF4 DUSP3 KRAS MAPK12 SOS1 RAP1B GADD45B CHUK HSPA8 DUSP6
	mmu04115:p53 signaling pathway	9	0.01	CCNB1 CCND1 CCND2 IGF1 APAF1 CDK4 GADD45B PTEN
	mmu04210:Apoptosis	7	0.03	PIK3CG TNFRSF1A TNFSF10 BCL2 APAF1 CAPN2 CHUK
	mmu04151:PI3K-Akt signaling pathway	22	0.03	PIK3CG SGK3 PPP2R5C IGF1 LPAR1 CDK4 PTEN G6PC3 NRAS VEGFC ATF4 KRAS CCND1 CCND2 SOS1 BCL2 GNG10 PIK3AP1 CHUK EIF4E2 PPP2R3C CSF1R

Lyz2 (lysozyme 2), Evi2a (ecotropic viral integration site 2a), Fbn1 (fibrillin 1), Junb (Jun B proto-oncogene), Mark2 (MAP/microtubule affinity regulating kinase 2), Decr1 (2, 4-dienoyl CoA reductase 1, mitochondrial), Alox5ap (arachidonate 5-lipoxygenase activating protein), Dcer1 (2, 4-dienoyl CoA reductase 1, mitochondrial), Egr1 (early growth response 1), Ttn (titin), Hrc (histidine rich calcium binding protein), Setd1b (SET domain containing 1B), Fos (FB) osteosarcoma oncogene), Jak1 (Janus kinase 1), Foxo3 (forkhead box O3), and Ppara (peroxisome proliferator activated receptor alpha). These genes were mainly involved in angiogenesis, CCKR signaling and inflammation mediated by chemokine and cytokine signaling. The top 2 significant clusters analyzed by MCODE module analysis are shown in Fig. 5.

Fig. 5. Top 2 clusters from PPI networks under TAC.

Genes that had the same and different regulation trends compared with MeCP2 under TAC and rTAC

In healthy mice, we compared the DEGs under TAC and rTAC and performed further alignment. MeCP2 was downregulated under TAC and upregulated under rTAC. According to the criterion of a p value<0.01, by selecting and aligning the DEGs from TAC and rTAC data, we screened 1055 genes that had the same expression-variation tendency as MeCP2 and 785 genes that had the opposite expression-variation tendency of MeCP2. GO-term analysis that that the DEGs' functions were mainly enriched in metabolic process, cardiovascular development, transcription and protein translation. Metabolic process includes glucose metabolic process, fatty acid catabolic process, amino acid metabolic process and energy metabolism. Cardiovascular development includes heart development, regulation of blood pressure, regulation of cardiac conduction, regulation of cardiac muscle cell contraction and regulation of epithelial cell proliferation. KEGG pathway analysis found that these genes were mainly enriched in metabolic pathway, signal transduction and apoptosis (Table 1).

Genes abnormally expressed with overexpressed MeCP2 under TAC

We screened 125 genes and performed GO term analysis of genes that were dysregulated when MeCP2 was overexpressed in mice under TAC. The GO-term BP analysis result was

mutations. Later, with the determination of the MeCP2 structure, it has become accepted that the effect of MeCP2 is likely to be modulated by the tissue- and cell-specific distribution of methylated and unmethylated CpG units, which clearly constitute an important control mechanism for gene expression [18]. MeCP2 is a multifunctional protein that mediates many downstream targets [19]. MeCP2 not only connects DNA methylation with histone deacetylation but also mediates two global epigenetic modifications, DNA methylation and histone methylation [20].

Cardiovascular events are a major cause of morbidity and mortality worldwide. Their occurrence and development are related to epigenetic regulation. MeCP2, as an epigenetic signature, plays a critical role in regulating cardiovascular diseases. Previous studies described the effect of MeCP2 on neuronal cells [21-23]. It was reported that MeCP2 induces a series of methylation events culminating in myofibroblast transdifferentiation and liver fibrogenesis [24]. It was found that MeCP2 overexpression results in downregulation of a crucial regulator for many endothelial cell functions, SIRT1, and contributes to the impaired angiogenic functions of endothelial cells [16]. Elevated MeCP2 expression has been reported to result in cardiac and skeletal abnormalities during development [25]. It was also reported that MeCP2 is upregulated in infarcted hearts and that inhibition of the MeCP2-upstream microRNA miR-132 decreases myocardial contractility, reparative angiogenesis and interstitial fibrosis in infarcted hearts [26]. We analyzed the data from simulated rat heart failure caused by TAC and rTAC, which alter the expression of MeCP2, and screened many dysregulated genes under MeCP2 overexpression and knockout on a background of TAC. Our findings suggest that MeCP2 plays a key role in cardiovascular activities, including angiogenesis, heart development, blood pressure, myocardial contractility, cardiac conduction and myocardial hypertrophy and fibrosis.

Heart failure is a complicated clinical syndrome involving a multitude of genes and pathways. Our data show that MeCP2 plays an important role in the occurrence and development of heart failure and ventricular remodeling. We also found that MeCP2 is involved in the regulation of metabolic processes, including lipid metabolism, amino acid metabolism, nucleotide metabolism, glucose metabolism and energy metabolism. MeCP2 deficiency results in impaired mitochondrial function [27]. It was shown that MeCP2 regulates lipid homeostasis, and MeCP2 deletion in mice results in a host of severe metabolic defects caused by lipid accumulation, including insulin resistance, fatty liver, perturbed energy utilization, and adipose inflammation by macrophage infiltration [28]. It was also reported that a master regulator of metabolic homeostasis, insulin, is regulated by MeCP2 [29]. MeCP2 was also found to regulate genes involved in tumor processes. It was suggested that MeCP2 could upregulate c-Met expression by silencing miR-137 expression and thus contribute to the colorectal adenoma-carcinoma sequence and tumor progression [30]. Another study reported that MeCP2 promotes gastric cancer progression [31]. In this article, we found that MeCP2 participates in hormone modulation, for example, modulation of growth hormone (GH), thyroid hormone and prolactin. It was reported that MeCP2 can modify the ghrelin/GH/IGF-1 axis in RTT [32].

In summary, MeCP2 participates in the somatic regulation of physiological and pathological activities. It plays a pivotal role not just in brain but in nearly all organs and tissues. It also plays a decisive role in heart failure, which is the end result of various cardiovascular diseases. We screened for genes aberrantly expressed in a simulated heart failure rat model and identified the genes that were abnormally changed with MeCP2 overexpression or knockout by using bioinformatic analysis. Further study of these genes may help to reveal the underlying molecular mechanisms of MeCP2 action in heart failure, leading to better therapies.

Our study has some limitations. The genes were selected from one GEO dataset and lack our own experimental verification. The data were from rats because of the difficulty of operating in humans, so our findings should be confirmed with human data.

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Disclosure Statement

The authors declare to have no competing interests.

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