Construction of microRNA and transcription factor regulatory network based on gene expression data in cardiomyopathy

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Abstract

Background: Cardiomyopathy is a progressive myocardial disorder. Here, we attempted to reveal the possible mechanism of cardiomyopathy at the transcription level with the roles of microRNAs (miRNAs) and transcription factors (TFs) taken into account.

Method: We firstly identified differentially expressed genes (DEGs) between cardiomyopathy patients and controls with data from the gene expression omnibus (GEO) database. DEGs were associated with the canonical pathways, molecular and cellular functions, physiological system development and function in the Ingenuity Knowledge Base by using the Ingenuity Pathway Analysis (IPA) software. TFs and miRNAs that DEGs significantly enriched were identified and a double-factor regulatory network was constructed.

Results: A total of 1,680 DEGs were identified. The DEGs were enriched for various pathways, with glucocorticoid receptor signaling as the most significant. A double-factor regulatory network was constructed, including seven TFs and two miRNAs. A subnetwork under the regulation of MEF2C and SRF was also constructed to illustrate their regulatory effects on cardiac functions.

Conclusion: Our results may provide new understanding of cardiomyopathy and may facilitate further therapeutic studies.

Keywords: Cardiomyopathy, Transcription factors, miRNAs, Gene expression

Background

Cardiomyopathy is a progressive myocardial disorder, usually leading to cardiovascular death or heart failure-related disability [1]. It develops at any age, in either sex, and in any population [2,3]. The etiology of cardiomyopathy is highly complex and improving its treatments has become a research hotspot.

Using bioinformatics combined with gene expression data to identify potential therapeutic targets has shown great application prospects. The majority of the previous studies mainly focused on the analyses of differentially expressed genes (DEGs), without considering microRNAs (miRNAs) and transcription factors (TFs) that regulate the expression of DEGs. miRNAs are small non-coding RNAs that control various biological processes through affecting the stability and translation of target mRNAs. Previous studies have proposed several miRNAs as being involved in the pathogenesis of cardiomyopathy, such as miR-1 [4] and miR-21 [5]. TFs can regulate gene expression through binding to the cis-elements in target genes’ promoter regions. TFs, such as MEF2, have been reported to be associated with cardiomyopathy [6]. Considering the important regulatory roles of miRNAs and TFs in the pathogenesis of cardiomyopathy, identification of miRNAs and TFs that enriched with target DEGs and construction of a double-factor regulatory network may provide new understanding of the molecular mechanism of cardiomyopathy.

In the current study, based on gene expression data from the Gene Expression Omnibus (GEO) database, we acquired DEGs, miRNAs and TFs that enriched with target DEGs and constructed a double-factor regulatory network.
Our results may reveal the possible mechanism of cardiomyopathy at the transcription level.

**Methods**

**Ethics Statement**

This study was approved by the institutional review board of the Xi’an Children’s Hospital (20140518). Written informed consent was obtained from all patients for the publication of this report and any accompanying images.

**Microarray data**

The gene expression profile GSE5406 from the GEO database was used. This dataset includes transcription

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*Figure 1* Ingenuity Pathway Analysis results. The significant canonical pathways in which differently expressed genes (DEGs) were enriched are shown.
profiles of 210 left ventricular myocardial tissue samples, 194 of which were from patients with advanced cardiomyopathy and 16 of which were from healthy donors. All patients had New York Heart Association class 3 to 4 symptoms and left ventricular systolic dysfunction, with ejection fraction of 14 ± 8% (mean ± SD). Patients suffered from heart failure due to ischemic (n = 86) or idiopathic dilated (n = 108) cardiomyopathy. Control samples had normal left ventricular function with ejection fraction of 56 ± 7% (P = 0.0001 versus patients). None of the subjects received mechanical support with left ventricular assist devices. Myocardial tissue samples were obtained from patients undergoing heart transplantation and from controls deemed unsuitable for transplantation. Whole hearts were removed after preservation in cold cardioplegia at the time of transplantation or donor harvest. Then, segments of noninfarcted left ventricular free wall were snap-frozen in liquid nitrogen. For each sample, RNA was isolated by using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The dataset was generated by using the (HG-U133A) Affymetrix Human Genome U133A Array (Affymetrix, Santa Clara, CA, USA).

Identification of differentially expressed genes (DEGs)
Raw data from all arrays were normalized using Robust Multi-array Analysis (RMA) [7] in the R software (version 3.0.0). The resulting expression values were used to identify DEGs with the limma package (3.12.1) in R. DEGs were detected by using t-tests and multiple test corrections were carried out with the Benjamini-Hochberg method [8]. The threshold for significance was set as P < 0.01.

Enrichment analysis
To explore the functions and pathways of DEGs, DEGs were associated with the canonical pathways, molecular and cellular functions, physiological system development and function in the Ingenuity Knowledge Base by using the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, http://www.ingenuity.com).

Table 1 Ingenuity Pathway Analysis: functions related to differentially expressed genes

| Name                                | P-value                   | Number of molecules |
|-------------------------------------|---------------------------|---------------------|
| **Molecular and Cellular Functions** |                           |                     |
| Cell Death and Survival             | 2.53 × 10^{-24} to 1.36 × 10^{-3} | 405                 |
| Cellular Growth and Proliferation   | 3.37 × 10^{-24} to 1.36 × 10^{-3} | 414                 |
| Cellular Movement                   | 5.41 × 10^{-13} to 1.36 × 10^{-3} | 236                 |
| Gene Expression                     | 8.14 × 10^{-11} to 1.20 × 10^{-3} | 247                 |
| Cellular Development                | 1.13 × 10^{-10} to 1.36 × 10^{-3} | 328                 |
| **Physiological System Development and Function** |                           |                     |
| Cardiovascular System Development and Function | 4.17 × 10^{-12} to 1.20 × 10^{-3} | 125                 |
| Organismal Development              | 3.96 × 10^{-11} to 1.22 × 10^{-3} | 92                  |
| Tissue Development                  | 3.68 × 10^{-9} to 1.22 × 10^{-3} | 165                 |
| Tumor Morphology                    | 3.84 × 10^{-7} to 1.20 × 10^{-3} | 117                 |
| Connective Tissue Development and Function | 8.52 × 10^{-8} to 1.24 × 10^{-3} | 40                  |
The results of miRNA and TF enrichment analysis are listed in Table 2. A network was constructed to illustrate the regulatory relationships (Figure 2). Considering the important role of SRF and MEF2C in the progression of cardiomyopathy, a subnetwork under their regulation was constructed and the biological functions they may affect were also indicated (Figure 3).

**Discussion**

In the current study, based on GSE5406 from the GEO database, 1,680 DEGs were identified between advanced cardiomyopathy patients and healthy controls. DEGs were mapped into the Ingenuity Knowledge Base and several canonical pathways were screened out, with glucocorticoid receptor signaling as the most significant. Glucocorticoid receptors are involved in cardiovascular homeostasis [12] and glucocorticoid could protect rodent hearts from ischemia/reperfusion injury [13]. Our results confirmed the essential roles of this pathway in the pathogenesis of cardiomyopathy. Molecular and cellular functions analysis revealed

| Name             | P-value        |
|------------------|----------------|
| MicroRNA         |                |
| miR-30c-5p (and other miRNAs w/seed GUAAACA) | $3.12 \times 10^{-4}$ |
| miR-125b-5p (and other miRNAs w/seed CCCUGAG) | $2.15 \times 10^{-3}$ |
| Transcription factors |        |
| MEF2C            | $5.65 \times 10^{-6}$ |
| GATA1            | $6.11 \times 10^{-5}$ |
| SRF              | $1.53 \times 10^{-4}$ |
| NUPR1            | $4.18 \times 10^{-4}$ |
| ATF4             | $7.25 \times 10^{-4}$ |
| RELA             | $9.35 \times 10^{-3}$ |
| GLI2             | $1.42 \times 10^{-2}$ |

![Figure 2](image_url) The microRNA (miRNA) and transcription factor (TF) regulatory network. TFs are shown with ellipse and miRNAs are shown with semicircle. Up-regulated genes are shown in red and down-regulated genes are shown in green.
that ‘Cell Death and Survival’ \( (P = 2.53 \times 10^{-24} \text{ to } 1.36 \times 10^{-23}) \) was the top molecular function affected by DEGs, suggesting that dysregulation of the cell function may contribute to the progressive heart failure induced by cardiomyopathy.

TFs or miRNAs that DEGs significantly enriched were identified and a double-factor regulatory network was constructed, including seven TFs and two miRNAs (Figure 2). All these miRNAs and TFs have previously been implicated in cardiomyogenesis or cardiac function. Considering their important regulatory roles in controlling various biological processes, these miRNAs and TFs may provide new avenues for the therapeutic strategies of cardiomyopathy. In addition, since all these miRNAs and TFs were detected in patients suffered from New York Heart Association class 3 to 4 symptoms, they may also be considered as prognosis markers in clinical practice. A subnetwork under the regulation of SRF and MEF2C was also constructed and the functions they may affect were also indicated (Figure 3) considering the considerable evidence in support of their involvement in cardiomyopathy [14-17]. As shown in Figure 3, both SRF and MEF2C may inhibit the expression of MYH6 and CNN1. MYH6 is a famous cardiac muscle myosin gene and mutations of this gene are implicated in cardiomyopathy in various studies [18]. Inhibition of CNN1 expression may disrupt its suppression of cardiomyopathy through the ePKC pathway [19]. In addition, both SRF and MEF2C may regulate the expression of NPPA, which was overexpressed in patients in our results. This gene is involved in many cardiac dysfunctions (Figure 3), including myocardial infarction, heart failure, and high ventricular pressure [20,21].
Conclusion
In summary, with DEGs identified by gene chip data generated by high-throughput technologies from the GEO database, we constructed a cardiomyopathy double-factor regulatory network and revealed the possible mechanism on regulation level. A subnetwork under the regulation of SRF and MEF2C was also constructed to illustrate their possible regulatory roles. Our results may facilitate further therapeutic studies of cardiomyopathy.

Abbreviations
DEG: differentially expressed genes; GEO: Gene Expression Omnibus; IPA: Ingenuity Pathway Analysis; miRNAs: microRNAs; RMA: Robust Multi-array Analysis; TF: transcription factor; UCSC: University of California Santa Cruz.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
LW conceived of the study and drafted the manuscript. JH, HK and MS performed the statistical analysis. JW, QJ and HY revised the manuscript. All authors read and approved the final manuscript.

Acknowledgement
This study is supported by Provincial Natural Science Basic Research Foundation of Shaanxi (2014JM4152) and the Science and Technology Project of Xi’an Municipal Health Bureau (Number 20130208).

Received: 31 July 2014 Accepted: 14 October 2014
Published online: 24 October 2014

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Cite this article as: Wang et al.: Construction of microRNA and transcription factor regulatory network based on gene expression data in cardiomyopathy. European Journal of Medical Research 2014 19:57.