Analysis of Gene Expression During the Development of Congestive Heart Failure After Myocardial Infarction in Rat Models

Zhuo YU,^{1,2*} MD, Hu ZHANG,^{2*} MD, Mingli YU,³ MD, and Qing YE,¹ MD

SUMMARY

Our study aimed to investigate the gene expression at different myocardial infarction (MI) phases and to understand the development mechanisms of congestive heart failure (CHF) after MI. Dataset GSE1957 including 24 samples of rat left ventricles at 1-day post MI or sham operation and 7-day post MI or sham operation was downloaded from Gene Expression Omnibus. The data were normalized with an affyPLM package and differentially expressed genes (DEGs) were identified with a Linear Models for Microarray Data package. Heat maps of the DEGs were constructed using Cluster 3.0. GO (Gene Ontology) enrichment analysis of the DEGs was performed in Database for Annotation, Visualization, and Integrated Discovery. A protein-protein interaction (PPI) network was constructed by Biomolecular Interaction Network Database and visualized by Cytoscape, and a subnetwork was analyzed using plugin ClusterONE in Cytoscape. A total of 5 DEGs at 1-day post-MI, 5 DEGs at 7-day post-MI, and 7 DEGs between the MI and sham groups at 1-day and 7-day post-MI were identified. For the GO category analysis, DEGs at 1-day post-MI were enriched in response to cytokine stimulus. DEGs at 7-day post-MI were enriched in response to inorganic substance and chemical homeostasis. DEGs between 1-day and 7-day post-MI including CDK2 and CDC20 were significantly enriched in mitosis. CDK2, ANXA1, CDC20, and AQP2 were included in the PPI network, and CDK2 was the only DEG included in the subnetwork. In conclusion, the induction of DEGs at 7-day post-MI might participate in the response to a hormone and endogenous stimulus to regulate the development of CHF after MI. (Int Heart J 2015; 56: 444-449)

Key words: Differentially expressed gene, Interaction network, CDK2, AQP2

Myocardial infarction (MI) is commonly known as heart attack. MI is a leading cause of heart failure and one of the major causes of the death and disability, and is a chronic disease and can even go undetected, eventually leading to a sudden death.^{1,2)} Patients with MI sometimes have chest pain while 64% of the cases may not even have any symptoms.³⁾ Therefore, an urgent task is to develop diagnostic and therapeutic methods for MI.

In recent years, studies on genes have provided us with information for disease diagnosis and therapy due to their high sensitivity, specificity, and fast speed. The relation between gene expression and MI has been studied extensively, and the progression of congestive heart failure (CHF) after MI is complex and related to several changes.⁴⁻⁷⁾ For example, the variants of gene ALOX5AP which encodes 5-lipoxygenase activating protein are associated with the pathogenesis of MI by increasing leukotriene production and inflammation.⁴⁾ Andersson, *et al* reported that the expression of 10 neuronal type regulated genes including muscarinic m4, ATP receptor P2rx4, nerve growth factor receptor, and cardiac Kcne1 were all in-

creased in 7-day post-MI CHF hearts, while fewer genes were differentially expressed at 1-day post MI.⁶⁾ However, the underlying functions of the differentially expressed genes (DEGs) and interactions of DEGs were not analyzed.

In order to investigate the mechanisms of CHF progression after MI from a broader perspective, the microarray data GSE1957 of MI rat from Andersson, *et al* was downloaded. Next, DEGs at different stages were identified. Functional enrichment analysis of the DEGs was performed. Finally, a protein-protein interaction (PPI) network and subnetwork were constructed to identify the crucial genes that are related to MI. This study was conducted with the aim of providing more information with which to investigate the development mechanisms of CHF after MI.

METHODS

Microarray data: The gene expression microarray data of GSE1957⁶⁾ were obtained from the National Center of Bio-

From the Departments of ¹ Cardiology, ² Cardiovascular Surgery, the First Affiliated Hospital of Kunming University, and ³ Department of Pharmacy, the Third Hospital of Kunming, Kunming, China.

*These authors contributed equally to this study.

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Address for correspondence: Hu Zhang, MD, Department of Cardiovascular Surgery, the First Affiliated Hospital of Kunming University, Xichang Road, Kunming 650032, China. E-mail: zh_huu@163.com

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technology Information (NCBI) Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>). The platform was GPL153 Atlas Rat 1.2 Array (Cat. #7854-1). A total of 24 chips of left ventricular myocardium from male Wistar rats that underwent MI or sham operation were included in the microarray data, including 6 samples at 1-day post-MI (killed at 1 day postoperation), 6 samples at 1-day post sham operation, 6 samples at 7-day post-MI (killed at 7 day postoperation), and 6 samples at 7-day post sham operation. The raw gene expression microarray data and probe annotation files were downloaded for further analysis.

Data preprocessing and identification of DEGs: The normalization applied to probesets was based upon quantiles of normalize.ExpressionSet.quantiles in afflyPLM package.⁸⁾ Probe sets were mapped to the genes. If there were more than one probe set that corresponded to the same gene, the averaged value was calculated and considered as the expression value of the gene. The probe that had multiple genes was filtered. The LIMMA (Linear Models for Microarray Data) package⁹⁾ was used to identify the DEGs. The cut-off criteria for DEGs were $P < 0.01$ and $|\log_2FC| > 0.5$. Afterwards, the VennDiagram package was used to plot the Venn diagram of DEGs.¹⁰⁾ Cluster analysis of the DEGs was performed by Cluster 3.0, based on Euclidean distance.¹¹⁾

Gene Ontology (GO)-based functional enrichment analysis: GO analysis is a commonly used approach for functional studies of large-scale genomic or transcriptomic data.¹²⁾ DAVID (Database for Annotation, Visualization, and Integrated Discovery; <http://david.abcc.ncifcrf.gov/tools.jsp>) bioinformatics resources consist of an integrated biological knowledge base and analytic tools, including GO functional analytic tool.¹³⁾ In order to investigate the underlying functions of the DEGs, GO-based functional enrichment analyses in biological process

(BP), molecular function (MF), and cellular component (CC) categories were performed using the DAVID online database. A P -value less than 0.05 was regarded as the threshold.

Construction of PPI network and analysis of subnetwork: The Biomolecular Interaction Network Database (BIND: <http://bind.ca>) archives biomolecular interaction, complex, and pathway information.¹⁴⁾ The PPI network of DEGs was constructed by BIND, and visualized by Cytoscape software.¹⁵⁾ Afterwards, analysis of the subnetwork was performed using the plugin clusterONE in Cytoscape¹⁶⁾ to identify the most significant subnetwork.

RESULTS

Identification of DEGs: After comparing the gene expression profiles between the MI group and sham group, 5 DEGs at 1-day post-MI were identified, including 4 up-regulated DEGs (TIMP1, ANXA1, HCK, and YWHAG) and one down-regulated DEG (CD8b) (Table I). Five up-regulated DEGs at 7-day post-MI (AQP2, SERPINE1, MMP14, NPTXR, and LNPEP) were identified (Table I). Additionally, 7 DEGs including 5 up-regulated DEGs (CDC20, MMP14, CDK2, CD8b, and LNPEP) and 2 down-regulated DEGs (HCK and YWHAG) were identified via comparison between the MI group and sham group at 1-day and 7-day post-MI (Table II).

As shown in the Venn diagram, 2 genes (TIMP1 and ANXA1) only differentially expressed at 1-day post-MI; 3 genes (AQP2, SERPINE1, and NPTXR) only differentially expressed at 7-day post-MI; 2 genes (CDK2 and CDC20) did not differentially express at either 1-day post-MI or 7-day post-MI (Figure 1). Although the 1-day post-MIs and the 1-day and 7-day shams after operation were relatively scattered, the dif-

Table I. Differentially Expressed Genes at the 2 Time Points of 1-Day and 7-Day Myocardial Infarction

Time points	Probe ID	<i>P</i>	Log ₂ FC	Gene symbol
1-day post-MI	R642	0.00124862	0.517218347	TIMP1
	R265	0.002041964	0.870974333	ANXA1
	RG504	0.005224835	-0.537096566	CD8b
	R680	0.005480698	0.842502643	HCK
	RG233	0.006358907	0.567477971	YWHAG
7-day post-MI	RG433	5.82E-06	0.569868289	AQP2
	R272	9.86E-06	1.094060402	SERPINE1
	R589	0.002510064	0.518138572	MMP14
	R013	0.002750524	0.541958807	NPTXR
	R463	0.005192015	0.650866228	LNPEP

MI indicates myocardial infarction.

Table II. Differentially Expressed Genes Between the Myocardial Infarction and Sham Group at 1-Day and 7-Day Post Myocardial Infarction

Probe ID	<i>P</i>	Log ₂ FC	Gene symbol
R418	0.001528632	1.004053415	CDC20
R680	0.003533637	-1.326077909	HCK
RG233	0.005450354	-0.735260341	YWHAG
R589	0.005467815	0.850697897	MMP14
R080	0.007476241	0.754401283	CDK2
RG504	0.008473219	0.589947733	CD8b
R463	0.009839569	1.052669616	LNPEP

ferences in the expression levels of DEGs between MI and sham at 1-day and 7-day post-MI were clearly observed from the heat map of the cluster analysis (Supplemental Figure).

GO functional enrichment analysis: Among DEGs at 1-day post-MI, 2 (ANXA1 and TIMP1) were enriched in response to cytokine stimulus ($P = 0.036542186$) (Table III). After enrichment analysis in the BP category for DEGs at 7-day post-MI, 3 of these DEGs (NPTXR, SERPINE1, and AQP2) were enriched in response to inorganic substance ($P = 0.00285111$) and 3 DEGs (LNPEP, SERPINE1, and AQP2) were enriched in chemical homeostasis ($P = 0.009386135$). For DEGs at 7-day post-MI in the CC category, 3 of these genes (NPTXR,

MMP14, and AQP2) were enriched in cytoplasmic membrane-bound vesicles ($P = 0.013068004$). For the MF category analysis for DEGs at 7-day post-MI, 3 DEGs (LNPEP, SERPINE1, and MMP14) were enriched in peptidase activity, acting on L-amino acid peptides ($P = 0.010873017$) and peptidase activity ($P = 0.012068486$) (Table III).

For all of the 12 DEGs between the MIs and Sham groups, 3 DEGs (LNPEP, MMP14, and CDK2) were enriched in response to hormone stimulus ($P = 0.023786$) and response to endogenous stimulus ($P = 0.02961$), and 2 DEGs (CDC20 and CDK2) were enriched in mitosis and nuclear division ($P = 0.049091$) (Table III).

Construction of PPI network and subnetwork analysis: The PPI network involved 7 up-regulated DEGs (ANXA1, CDC20, CDK2, AQP2, NPTXR, YWHAG, and SERPINE1) (Figure 2A). The subnetwork (nodes = 8, density = 0.5, quality = 0.609, $P = 0.002$) contained one up-regulated DEG (CDK2) (Figure 2B).

DISCUSSION

In this study, a total of 12 DEGs were identified between the MI group and sham group at 1-day and 7-day post-MI. From the functional enrichment analysis, the DEGs at 1-day and 7-day post-MI were enriched in different functions, which might suggest changed mechanisms during the development of CHF after MI.

The DEGs at 1-day post-MI, such as ANXA1 and TIMP1, were enriched in response to cytokine stimulus, which was not included in the GO terms for DEGs at the 7-day post-MI. As previously reported, 2 cytokines, interleukin (IL)-1 β

Figure 1. Venn diagram of differentially expressed genes (DEGs) in myocardial infarction (MI). The diagram shows 2 DEGs only differentially expressed at 1-day post-MI, 3 DEGs only differentially expressed at 7-day post-MI, and 2 DEGs did not differentially express at either 1-day or 7-day post-MI.

Table III. Functional Enrichment Analysis Results of the Differentially Expressed Genes

DEG	Category	Term	Name	Count	Gene symbol	P
1-day post-MI	BP	GO:0034097	response to cytokine stimulus	2	ANXA1, TIMP1	0.036542186
7-day post-MI	BP	GO:0006979	response to oxidative stress	3	NPTXR, SERPINE1, MMP14	0.001339695
	BP	GO:0010035	response to inorganic substance	3	NPTXR, SERPINE1, AQP2	0.00285111
	BP	GO:0048878	chemical homeostasis	3	LNPEP, SERPINE1, AQP2	0.009386135
	BP	GO:0009725	response to hormone stimulus	3	LNPEP, MMP14, AQP2	0.01006572
	BP	GO:0003014	renal system process	2	LNPEP, AQP2	0.010216609
	BP	GO:0009719	response to endogenous stimulus	3	LNPEP, MMP14, AQP2	0.01261856
	BP	GO:0042592	homeostatic process	3	LNPEP, SERPINE1, AQP2	0.019287634
	BP	GO:0000302	response to reactive oxygen species	2	NPTXR, SERPINE1	0.030091472
	BP	GO:0010033	response to organic substance	3	LNPEP, MMP14, AQP2	0.031801712
	BP	GO:0007568	aging	2	SERPINE1, AQP2	0.048709837
	MF	GO:0070011	peptidase activity, acting on L-amino acid peptides	3	LNPEP, SERPINE1, MMP14	0.010873017
	MF	GO:0008233	peptidase activity	3	LNPEP, SERPINE1, MMP14	0.012068486
	CC	GO:0016023	cytoplasmic membrane-bounded vesicle	3	LNPEP, MMP14, AQP2	0.013068004
	CC	GO:0031988	membrane-bounded vesicle	3	LNPEP, MMP14, AQP2	0.014209055
	CC	GO:0031410	cytoplasmic vesicle	3	LNPEP, MMP14, AQP2	0.01695066
	CC	GO:0031982	vesicle	3	LNPEP, MMP14, AQP2	0.018978087
	CC	GO:0005886	plasma membrane	4	LNPEP, NPTXR, SERPINE1, AQP2	0.036608309
	CC	GO:0000267	cell fraction	3	LNPEP, SERPINE1, AQP2	0.042699138
Between MI and sham groups at 1-day and 7-day post-MI	BP	GO:0009725	response to hormone stimulus	3	LNPEP, MMP14, CDK2	0.023786
	BP	GO:0009719	response to endogenous stimulus	3	LNPEP, MMP14, CDK2	0.02961
	BP	GO:0048167	regulation of synaptic plasticity	2	LNPEP, YWHAG	0.039533
	BP	GO:0007067	mitosis	2	CDC20, CDK2	0.049091
	BP	GO:0000280	nuclear division	2	CDC20, CDK2	0.049091

BP indicates biological process; CC, cellular components; MF, molecular functions; and MI, myocardial infarction.

and IL-6, were strongly induced in the first 3-12 hours after MI in the infarct area of mice, while their expressions were not significantly altered compared to sham-operated controls from the 3rd day.¹⁷⁾ This was in accordance with our study. Annexin 1 (ANXA1) is an endogenous calcium-binding protein endowed with anti-inflammatory functions, and the expression of ANXA1 was up-regulated by cytokine IL-6.^{18,19)} Other cytokines such as IL-1 β were found to be regulated by ANXA1 in the cells sensitive to ANXA1.¹⁸⁾ Furthermore, tissue inhibitor of metalloproteinase (TIMP) 1 was also involved in cardiac dysfunction by regulating the expression of IL-1 β and IL-10.^{20,21)} Transgenic animal models supported the beneficial effects of TIMP1 after MI.²²⁾ This suggests that ANXA1 and TIMP1 might participate in the mechanism of response to cytokine stimulus in the early stage of MI by regulating the transcription levels of cytokines including IL-1 β and IL-10.

Furthermore, DEGs at the 7-day post-MI, including SERPINE1 and AQP2, were enriched in response to inorganic substance and chemical homeostasis. Inorganic substances such as mercury have been suggested to have negative effects on cardiovascular health including MI,²³⁾ thus resulting in the disturbance of homeostasis in humor, including calcium and water homeostasis.²⁴⁻²⁶⁾ Aquaporin 2 (AQP2) water channel protein is essential for the homeostases of extracellular calcium and water.^{27,28)} Increased abundance of inner medulla AQP2 was observed in CHF rat hearts after 7-day.^{29,30)} Moreover, plasmin and plasminogen in urinary tissue were identified to play crucial roles in sodium, potassium, and calcium homeostasis.^{31,32)} In large epidemiological studies, increased plasminogen activator inhibitor (PAI)-1(SERPINE1) levels have been identified as a predictor of MI.³³⁾ MI mice lacking SERPINE1 developed less cardiac fibrosis, suggesting the loss of SERPINE1 was protective against the development of cardiac fibrosis.^{33,34)} This revealed that AQP2 and SERPINE1 might have crucial roles in

maintaining humor homeostasis, which might be disturbed by the inorganic substance or MI disease itself.

Moreover, 7 DEGs between the MI and sham group, such as CDK2 and CDC20, were identified and enriched in mitosis and nuclear division. CDK2 was also enriched in response to hormone stimulus and endogenous stimulus. CDK2 interacted with CCNA1, CCNE1, and KRAS in the PPI network and subnetwork. The cyclin-dependent kinase family members (CDKs) are crucial for progression of the cell cycle as well as DNA replication and synthesis.^{35,36)} The progression of mitosis is controlled by cyclins, a family of proteins activating CDKs.³⁷⁾ Cyclin A1 (CCNA1) and CCNA2 complex and CDK2 are essential for mitosis.^{38,39)} Additionally, cell-division cycle protein 20 (CDC20) regulates mitotic exit by targeting cyclins A and B.⁴⁰⁾ CDK2 was shown to suppress the interaction of p21 with CDC20 and facilitate the degradation of p21, thus regulating mitosis.⁴¹⁻⁴³⁾ KRAS is a member of the p21 small GTPase family and numerous investigations have reported that the overexpression of p21 proteins encoded by KRAS was detected in the cardiovascular disease.⁴⁴⁻⁴⁶⁾ As reported previously, polymorphisms in estrogen receptor (ER)- α were correlated with increased risk of MI, and the activation of ER- α was regulated by p21-activated kinase 1.^{47,48)} Taken together, these studies revealed the essential roles of CDK2, CCNA1, and KRAS in the mitosis of cells, and might demonstrate that they promote the mitosis of cardiac myocytes after MI by participating in these processes, thus providing more information with which to investigate the mechanisms of CHF induced by MI.

Conclusions: Generally speaking, the DEGs at 1-day post-MI were different from the DEGs at 7-day post-MI. Compared with the DEGs at 1-day post-MI (including ANXA1 and TIMP1) which were enriched in response to cytokine stimulus, the DEGs at 7-day post-MI (including AQP2) were enriched in

Figure 2. PPI network and subnetwork of the differentially expressed genes (DEGs). **A:** PPI network of the DEGs. **B:** Significantly clustered subnetwork. The proteins of genes are indicated by diamonds, and the interactions are indicated by edges. Dark grey indicates an up-regulated DEG, and light grey represents DEGs not identified in this study.

response to hormone stimulus and in response to endogenous stimulus, which also were the significantly enriched functions for the DEGs (including CDK2) between the MI and sham groups at 1-day post-MI and 7-day post-MI. These results suggest that the induction of these DEGs at 7-day post-MI might be a persistent response to CHF after MI, thus providing an insight into investigation of CHF induced by MI. However, more investigations should be conducted by more than one experimental test in order to verify whether these candidate genes are responsible for elucidation of the mechanism of cardiovascular disease and whether they should be regarded as therapeutic targets for MI.

DISCLOSURE

The authors report no financial relationships or conflicts of interest regarding the content herein.

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SUPPLEMENTAL FILES

Supplemental Figure
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