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Original Article

Comparative study of gene expression profiles rooted in acute myocardial infarction and ischemic/reperfusion rat models

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Abstract: Mining data in depth of genome-wide sequencing data generated from pathological target tissues under disease conditions is necessary for seeking novel functional genes, and developing more biological study directions for the field. Based on our previous published RNA-seq data generated from acute myocardial ischemia and ischemia-reperfusion in rat heart, we re-analysed these two data sets using bioinformatics tools. All these raw fastq files were extracted from Illumina BCL using the Illumina CASAVA program. Four groups were obtained: UD (genes up-regulated in MI but down-regulated in I/R injury), DU (genes down-regulated in MI but up-regulated in I/R injury), UU (genes both up-regulated in MI and I/R injury), and DD (genes both down-regulated in MI and I/R injury) groups. The results showed that 304 common genes in the UD group, 236 common genes in the DU group, 318 common genes in the UU group, and 159 common genes in the DD group detected by comparing data sets of the MI and the I/R injury. We then listed the top 30 DEGs for each group, and carried out GO and KEGG analyses for enrichment and pathway studies for those top expressed genes. Further analysis of INTERPRO Protein Domains and Features enriched by DEGs showed that 20% of the Domains enriched were related to c-type lectin, and 17% of these domains are related to neurotransmitter-gated ion-channel. 15% of PFAM Protein Domains were about Neurotransmitter-gated ion-channel. There were only 8 SMART Protein Domains DEGs enriched and 37.5% of which were concerned about leucine-rich. Collagen involvement in Reactome Pathways accounted for 22.7%. We found that only a few DEGs in these two disease conditions have been reported in the literatures, suggesting that there are many new genes would be considered in the future studies. These analyses would provide some information for seeking more novel targets of these two clinic diseases, acute myocardial ischemia and myocardial ischemia/reperfusion.

Keywords: Comparative study, gene expression profiles, myocardial infarction, myocardial ischemia-reperfusion, bioinformatics

Introduction

Acute myocardial infarction (MI) is a common critical disease with high morbidity and mortality worldwide [1]. MI results from the abrupt interruption of blood supply to a part of the heart, and lead to ischemia and even death of the affected cardiac tissue [2]. The treatment principle of acute myocardial infarction is to resume blood reperfusion on myocardial ischemia as soon as possible [3]. Primary percutaneous coronary intervention (PPCI), coronary artery bypass surgery (also known as coronary artery bypass graft, CABG) and drug thrombolytic are the three most effective therapies to recover ischemic myocardial blood flow [4-7]. In ischemic myocardial reperfusion, the status of myocardial ischemia might be corrected and cardiac functions are preserved. However, with blood reperfusion, part of the patients may suffer from lethal arrhythmias, cardiomyocyte death, and even death. It is difficult to accurate-

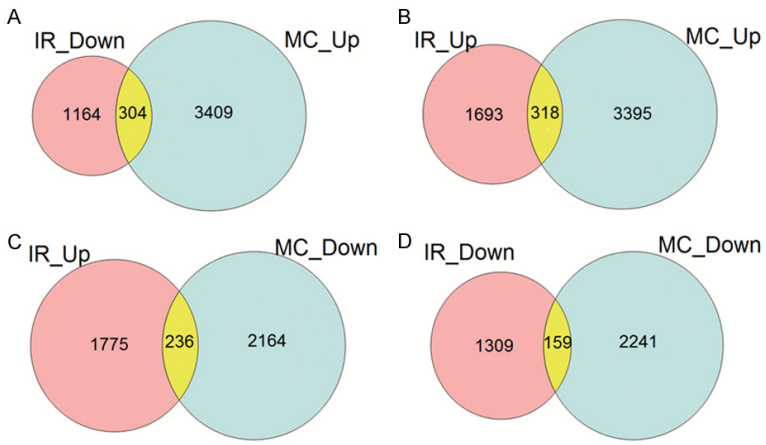


Figure 1. Venn diagrams and clustering analysis of RNA-seq results. (A-D) Venn diagrams were drawn based on the gene expression profiles of MI (numbered GSE54132) and I/R (numbered GSE61840) generated by our previous studies. Comparing the 3,713 up-regulated genes in myocardial infarction (MI) with the 1,468 down-regulated genes in ischemia/reperfusion (I/R), 304 common genes (UD group) were obtained. Furthermore, 236 common genes (DU group) were down-regulated in MI and up-regulated in I/R, 318 common genes (UU group) were both up-regulated in MI and I/R, and 159 common genes (DD group) were both down-regulated in MI and I/R. Red circles indicate the numbers of genes up- (B, C) or down-regulated (A, D) in I/R group; green circles represent the numbers of up- (A, B) or down-regulated (C, D) genes in MI group.

Table 1. Differentially expressed genes (DEGs) across four groups

Category	Group	DEGs
MI-Up vs. IR-Down	UD	304
MI-Up vs. IR-Up	UU	318
MI-Down vs. IR-Up	DU	236
MI-Down vs. IR-Down	DD	159

Note: Comparing the 3,713 up-regulated genes in myocardial infarction (MI) with the 1,468 down-regulated genes in ischemia/reperfusion (I/R), 304 common genes (UD group) were obtained. Furthermore, 236 common genes (DU group) were down-regulated in MI and up-regulated in I/R, 318 common genes (UU group) were both up-regulated in MI and I/R, and 159 common genes (DD group) were both down-regulated in MI and I/R.

ly determine the boundaries between MI injury and I/R injury in clinic and the mechanism is not yet fully understood.

Expression profile analysis has been used to predict myocardial stress response to acute MI and myocardial I/R injury for many years [8, 9]. In fact, molecular biology studies have confirmed 27 genetic variants that are concerned with the increased risk of MI [10]. In addition, recent progress in genotyping technology has made available newer and more powerful tools for the identification of susceptibility genes that in turn may provide new opportunities to evaluate the individual cardiovascular risk profile, detect novel disease pathways, and develop innovative therapeutic approaches. Some gene expression profile studies have made progress in dealing with acute MI and I/R injury, as well as the functional study of a single gene or few genes [11-14]. Thus, many research efforts continue to address the identification of acquired and inherited risk factors of these complex diseases. Many studies have hammered at I/R injury, ranging from basic research to clinical studies [15, 16]. Furthermore, all these studies, especially for basic research, have come to some conclusions, but could not provide a full understanding of the molecular biology mechanism of MI and I/R injury. Therefore, it is significant to seek for new molecular biological evidence to distinguish the progress of MI and I/R injury and guide clinical practice.

In the present study, the gene expression profiles of MI (numbered GSE54132) and I/R (numbered GSE61840) in rats, which were generated by our previous studies, were compared, and uniquely and commonly up- or down-regulated genes under MI and I/R injury were screened [17, 18]. Thus, four groups of differentially expressed genes (DEGs) were obtained: UD (genes up-regulated in MI but down-regulated in I/R injury), DU (genes down-regulated in MI but up-regulated in I/R injury), UU (genes both up-regulated in MI and I/R injury), and DD (genes both down-regulated in MI and I/R injury) groups. Database for Annotation, Visualization and Integrated Discovery (DAVID) tool was used in performing the Gene Ontology (GO) analysis and KEGG pathway analysis of each group of genes; and the Search Tool for Retrieval of Interacting Genes (STRING) database was performed to seek for protein-protein interaction (PPI) network constructions [19, 20]. We preliminarily analyzed all the DEGs and

Table 2. Research situations of the top 30 differentially expressed genes of the four groups analyzed

Items	UD	UU	DU	DD
Reported to be related to MI or I/R	*Cpz, Scn9a, Kcne1, Tnfrsf12a, Has1, Camp, Micb, Cyr61, Hmox1*	*Itga4, Msr1, Cybb*	*Cyp1a1, Kcnma1, Cd69, Il1a*	*Treml1, Alox15, vnn3, Gata1, Pbx4*
Not reported to be related to MI & I/R injury	*Cldn23, Gsc, Cldn4, Ankrd2, Prss3, Tbx15, Ngp, Ntf4, Gbx2, Cd177, Scn3a, Hunk, P2rx2, Olfm2, Msln, P4ha3, Sulf2, Rhbdl2, UDUpd1, Kcnc1, Areg, Sh2d1a*	*Glra2, Gpr65, Klra5, Gpr34, Ipcef1, Tm4sf4, LOC500948, Trpv6, Kynu, Bank1, Galnt5, Dixdc1, Rnase11, Ndst3, Hs6st2, Mctp2, Inhba, Slc27a6, Clec4a2, Rims2, Zdhhc23, C1ql3, Gpr160, Scel, Trat1, Lilra5, Ly49si1*	*Tuba3a, Klre1, Nxph1, Btla, Crygf, Dmrta1, Sh2d1a, Abcg3l4, Clec12b, RGD1311251, Cntn1, Agr2, Hapln1, Slc13a5, Glb1l3, MGC105567, Il24, Dppa3, Pde1c, Cpvl, Rsph1, Zfp68, Klrk1, Jph3, Tectb, Hpgds*	*Pnliprp1, Sgpp2, Enpp6, Hoxb9, Vpreb1, RGD1308775, Hist2h4, Spdef, Klk1c9, Trim43a, Atp6v1g2, Vdac3, Mybphl, Add2, Car1, Adam11, Vpreb2, Dlk1, Guca1b, Cbln1, Oc90, Akr1c1, Akap3, Tecta, Atp6ap1l*

Note: UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R. Only 9 genes in UD group have been reported related to MI and I/R injury before, and only 3 in UU group, 4 in DU group, and 5 in DD group. But a total of nearly 100 common genes in relating to MI or I/R have not been reported in the four groups.

Table 4. Items of the Gene Ontology (GO) enrichment analysis of the four groups

Common GO id and Description	Groups and Categories	Common GO id and Description	Groups and Categories
GO:0008083~growth factor activity	UD-Molecular Function, UU-Molecular Function	GO:0072562~blood microparticle	UD-Cellular Component, UU-Cellular Component
GO:0005125~cytokine activity	UD-Molecular Function, UU-Molecular Function, DU-Molecular Function	GO:0030246~carbohydrate binding	DU-Molecular Function, UU-Molecular Function
GO:0005509~calciumion binding	UD-Molecular Function, UU-Molecular Function	GO:0005249~voltage-gated potassium channel activity	DU-Molecular Function, UU-Molecular Function
GO:0005102~receptor binding	UD-Molecular Function, UU-Molecular Function	GO:0009897~external side of plasma membrane	DU-Cellular Component, UU-Cellular Component
GO:0020037~heme binding	UD-Molecular Function, DD-Molecular Function	GO:0070374~positive regulation of ERK1 and ERK2 cascade	UD-Biological Process, DD-Biological Process
GO:0005615~extracellular space	UD-Cellular Component, DD-Cellular Component, UU-Cellular Component	GO:0044344~cellular response to fibroblast growth factor stimulus	UD-Biological Process, DD-Biological Process
GO:0005576~extracellular region	UD-Cellular Component, DD-Cellular Component	GO:0043627~response to estrogen	UD-Biological Process, DD-Biological Process
GO:0005578~proteinaceous extracellular matrix	UD-Cellular Component, UU-Cellular Component	GO:0042593~glucose homeostasis	UD-Biological Process, DD-Biological Process
GO:0016324~apical plasma membrane	UD-Cellular Component, UU-Cellular Component	GO:0007010~cytoskeleton organization	UD-Biological Process, DD-Biological Process
GO:0005887~integral component of plasma membrane	UD-Cellular Component, DU-Cellular Component, UU-Cellular Component	GO:0051591~response to cAMP	UD-Biological Process, DD-Biological Process
GO:0009986~cell surface	UD-Cellular Component, UU-Cellular Component		

Note: UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R. There are totally 21 Common Gene Ontology IDs and Descriptions which concerned different groups.

Table 3. Items of the Gene Ontology enrichment analysis of the four groups

Items	UD	UU	DU	DD	Total
Molecular Function (MF)	22	18	10	8	58
Cellular Component (CC)	22	15	5	6	48
Biological Process (BP)	106	49	32	31	218

Note: UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R. The Gene Ontology (GO) enrichment analysis showed that most genes in each group concentrated in Biological Process.

items of the GO and KEGG pathway enrichment analysis, as well as PPI network constructions, and confirmed that these analysis tools are the directions of further research in the future.

Materials and methods

Data resources preprocessing and grouping

The gene expression profiles rooted in our previous studies and the raw data (GSE54132 and GSE61840) have been deposited onto the Gene Expression Omnibus database (GEO; http://www.ncbi.nlm.nih.gov/geo/) [17, 18]. After ranking the up- and down-regulated genes in the MI-Control group and IR-SO (sham operation) group according to expression level from high to low, the four groups of DEGs (P<0.01 and Log_2 fold change (FC) > |±1|) were compared. Following four groups of DEGs were obtained: UD, UU, DU and DD.

GO function analysis

The GO enrichment analysis of DEGs of the four groups was performed with DAVID, which provided a comprehensive set of functional annotation tools for investigators to understand the biological meaning behind the large list of genes [19]. GO terms were enriched with a threshold of P<0.01, according to the principle of hyper-geometric distribution.

Pathway enrichment analysis

Pathway analysis was used to identify related proteins within a pathway or build pathway *de novo* from the proteins of interest, which would help to study the differential expression of a gene in a disease or analyze any Omics dataset with a large number of proteins. It was used to carry out finding distinct cell processes (cellular processes), diseases or signaling pathways that are statistically associated with the selection of DEGs between two samples. In our study, the DAVID tool was also utilized for KEGG pathway analysis of the screened four groups of DEGs [19].

PPI network construction

In molecular biology, STRING 10.0, which is a tool for known and predicted protein-protein interactions based on a biological database and web resource, was performed to construct PPI networks in these four groups of DEGs [21]. 'Nodes' and 'edges' comprised the PPI network and a node represented a protein, while an edge revealed the interaction of pairwise proteins. In this study, String 10.0 was operated to search the PPI network construction for four groups of DEGs.

Statistical analysis

Data were presented as means ± standard deviation (SD). Statistics analysis was performed using SPSS 18.0; a one-way ANOVA analysis of variance followed by the Turkey HSD test is used for multiple group comparisons. P<0.05 was considered statistically significant.

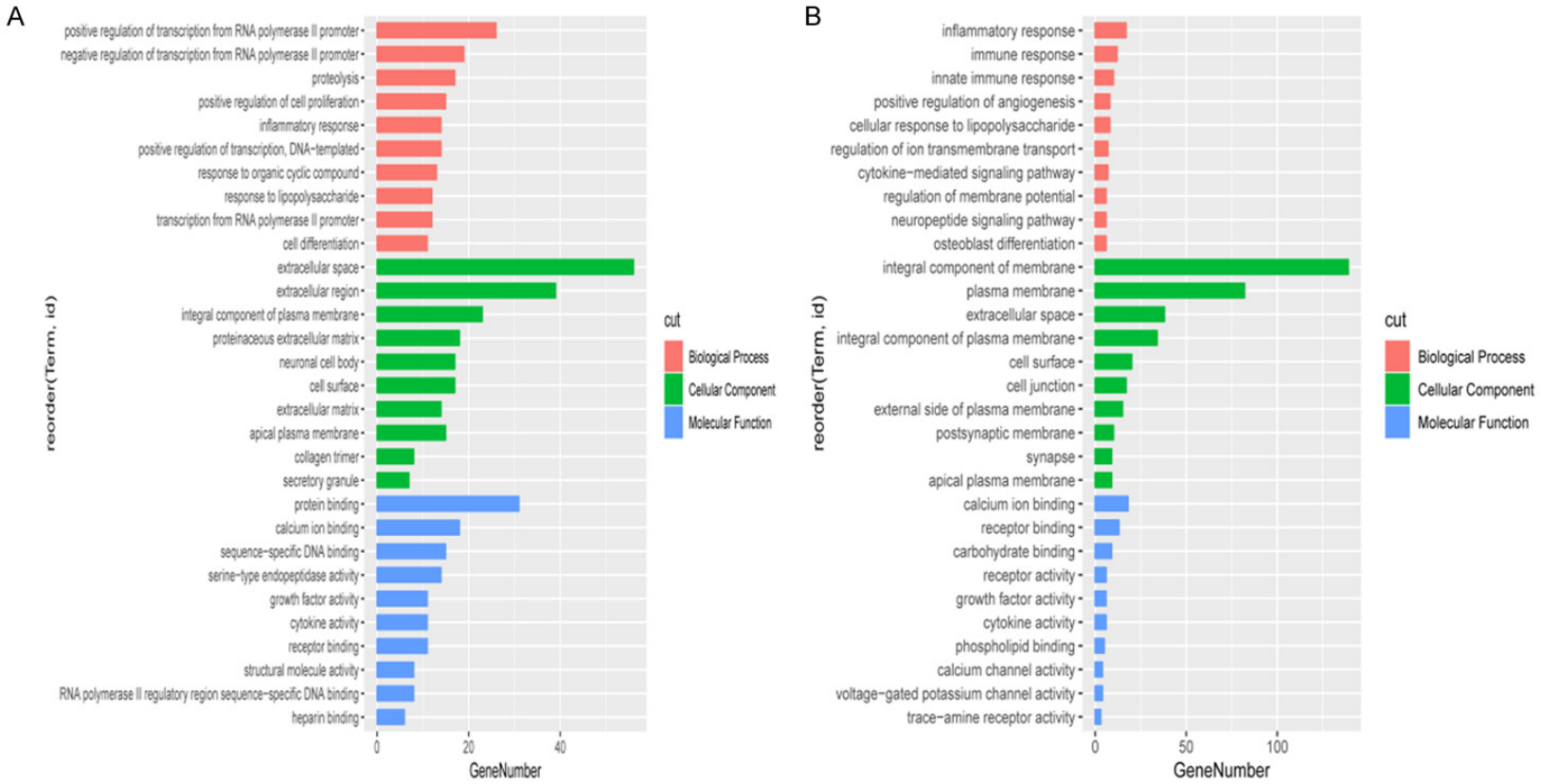
Results

Data preprocessing

After comparing the 3,713 up-regulated genes in the MI with the 1,468 down-regulated genes in the I/R injury, 304 common genes (UD group) were obtained according to their relative expression levels. Furthermore, 236 common genes (DU group) were down-regulated in the MI and up-regulated in the I/R injury, 318 common genes (UU group) were both up-regulated in the MI and the I/R injury, and 159 common genes (DD group) were both down-regulated in the MI and the I/R injury. All four comparisons were listed and analyzed with Venn Diagrams as **Figure 1**. The grouping situation is shown in **Table 1**.

The top 30 DEGs from the four groups analyzed

The top 30 DEGs with the highest fold changes in the UD group were listed in Table S1. We found that some genes listed in this top 30 DEGs were previously reported, such as *Cpz,*


A
positive regulation of transcription from RNA polymerase II promoter
negative regulation of transcription from RNA polymerase II promoter
proteolysis
positive regulation of cell proliferation
inflammatory response
positive regulation of transcription, DNA-templated
response to organic cyclic compound
response to lipopolysaccharide
transcription from RNA polymerase II promoter
cell differentiation
extracellular space
extracellular region
integral component of plasma membrane
proteinaceous extracellular matrix
neuronal cell body
cell surface
extracellular matrix
apical plasma membrane
collagen trimer
secretory granule
protein binding
calcium ion binding
sequence-specific DNA binding
serine-type endopeptidase activity
growth factor activity
cytokine activity
receptor binding
structural molecule activity
RNA polymerase II regulatory region sequence-specific DNA binding
heparin binding
reorder(Term, id)
0
20
40
GeneNumber
cut
Biological Process
Cellular Component
Molecular Function
B
inflammatory response
immune response
innate immune response
positive regulation of angiogenesis
cellular response to lipopolysaccharide
regulation of ion transmembrane transport
cytokine-mediated signaling pathway
regulation of membrane potential
neuropeptide signaling pathway
osteoblast differentiation
integral component of membrane
plasma membrane
extracellular space
integral component of plasma membrane
cell surface
cell junction
external side of plasma membrane
postsynaptic membrane
synapse
apical plasma membrane
calcium ion binding
receptor binding
carbohydrate binding
receptor activity
growth factor activity
cytokine activity
phospholipid binding
calcium channel activity
voltage-gated potassium channel activity
trace-amine receptor activity
reorder(Term, id)
0
50
100
GeneNumber
cut
Biological Process
Cellular Component
Molecular Function

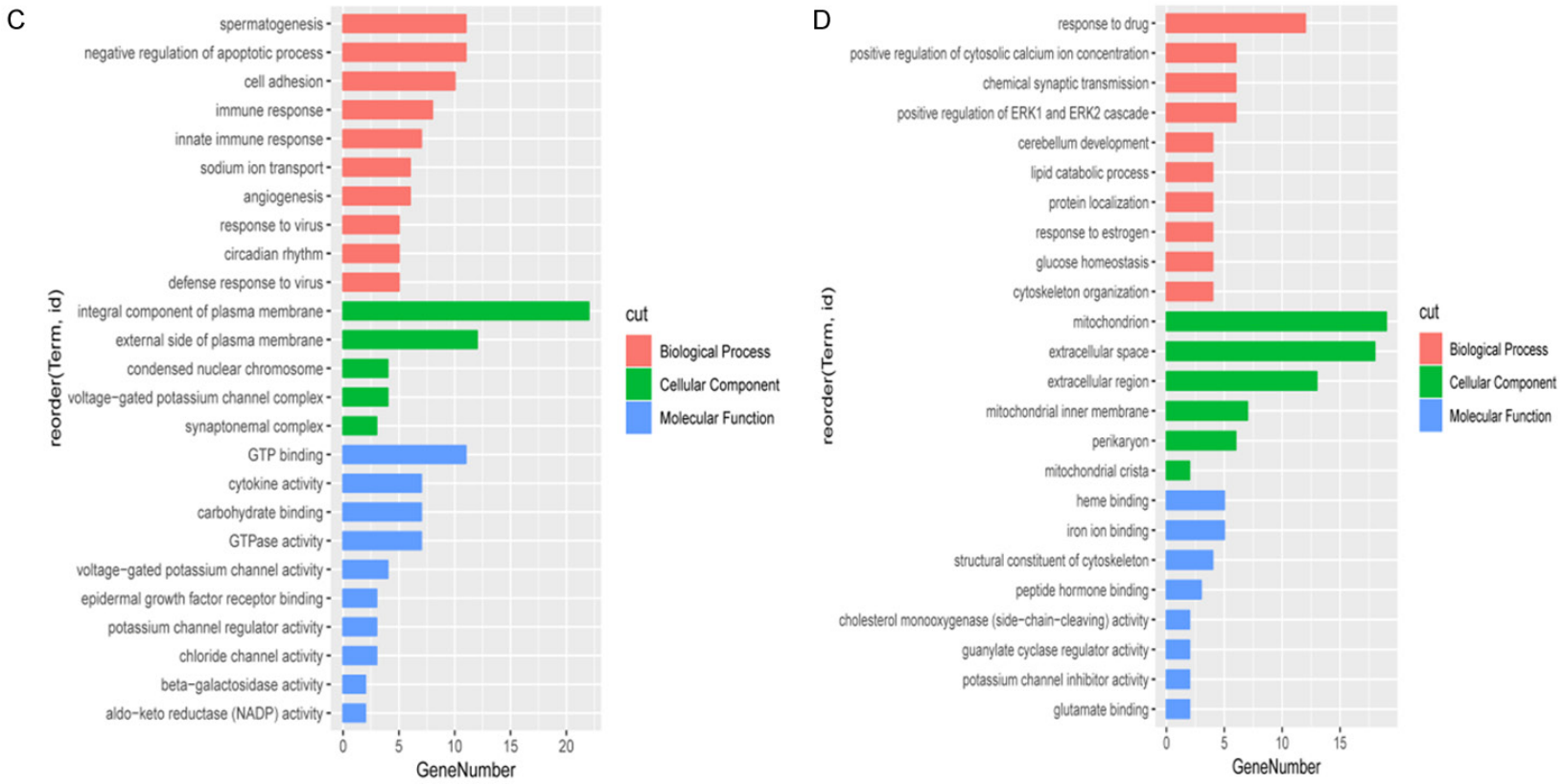


Figure 2. Gene Ontology enrichment analyses of DEGs of the four groups were performed with the DAVID. A-D. Correspond to UD, UU, DU, and DD group separately. (*P<0.05) UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R.

Table 5. Items of the KEGG pathway analysis of the four groups

Items	UD	UU	DU	DD
Amount of KEGG pathways	44	42	29	31

Note: UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R.

Scn9a, Kcne1, Tnfrsf12a, Has1, Camp, Micb, Cyr61 and Hmox1 with MI or I/R injury [22-31]. In the top 30 DEGs in the DU group (Table S2), only *Cyp1a1, Kcnma1, Cd69* and *Il1a* have been reported [32-35]. None of the rest of the 28 genes was reported in relation to MI or I/R injury. In addition, only few studies stated the functions of *Itga4, Msr1 and Cybb* in MI or I/R injury in the top 30 DEGs in the UU group (Table S3) [36-38]. In the top 30 DEGs in the DD group (Table S4), *Treml1, Alox15, vnn3, Gata1* and *Pbx4* have been reported in relation to MI or I/R injury with extreme limitations [39-43]. All research situations were shown in **Table 2**.

GO enrichment analysis

GO enrichment analysis of DEGs of the four groups were performed with the DAVID tool (**Table 3**). In the UD group, there were a total of 22 items on Molecular Function, 22 items on Cellular Component and 106 items on Biological Process. In the UU group, there were 18 items on Molecular Function, 15 items on Cellular Component and 49 items on Biological Process. In the DU group, there were 10 items on Molecular Function, five items on Cellular Component and 32 items on Biological Process. In the DD group, there were eight items on Molecular Function, six items on Cellular Component and 31 items on Biological Process. The comparison of the GO enrichment analysis data of the four groups revealed that 21 GO ids were in common in gene ontological domains among different groups. In the UD group, five GO ids were in common with other groups in the Molecular Function domain, seven GO ids were in common with other groups in the Cellular Component domain, and six GO ids were in common with other groups in the Biological Process domain. All items of the GO enrichment analyses of these four groups were shown in **Table 4**. The GO enrichment analysis items of top 30 differentially expressed genes of four groups were shown in **Figure 2**.

KEGG pathway analysis

Furthermore, the DAVID tool was also utilized for the KEGG pathway analysis of the four groups of screened DEGs. In the UD group, 44 KEGG pathways were enriched by DEGs with the smallest *P*-value (1.37E-04) and FDR (0.17). The pathway with the biggest fold enrichment (6.92, rno04974: protein digestion and absorption) was associated with the metabolism of protein. The complement and coagulation cascades pathway (rno04610, *P*=0.002247062) is concerned about the complement system and coagulation system. The 42 KEGG pathways were enriched by DEGs in the UU group, in which the term with the smallest *P*-value (4.73E-05) and FDR (0.058523675) was neuroactive ligand-receptor interaction (rno04080). In the DU group, 29 KEGG pathways were enriched by DEGs, in which renin secretion pathway (rno04924) had the smallest *P*-value (0.005544226) and FDR (6.416363241). In the DD group, 31 KEGG pathways were enriched by DEGs, in which the term with the smallest *P*-value (0.002628711) and FDR (2.949112652) was the cAMP signaling pathway (rno04024). All pathways added up to 146, which are listed in **Table 5**.

When deeply looking into all the KEGG pathway analysis results, 30 (20.5%) common KEGG pathway terms among the four groups were picked out and shown in **Table 6**. Neuroactive ligand-receptor interaction (rno04080) was the common pathway among the four groups. Furthermore, 17 common pathways were concerned about the UD or DU group (56.7%), 21 common pathways were concerned about the UU group (70%), and 15 common pathways were concerned about group DD (50%). The KEGG pathways analysis items of top 30 differentially expressed genes of four groups were shown in **Figure 3**.

PPI network construction

After operating String 11.0 (https://string-db.org/), the Protein-protein interaction network of the four groups DEGs was constructed. In the UD group, the PPI network contained 95 nodes, 104 edges and 53 expected edges, with 2.19 average node degrees (PPI enrichment *P*-value: 3.04e-10). The PPI network of DEGs in the UU group contained 192 nodes, 441 edges and 316 expected edges, with 4.59 average node degrees (PPI enrichment *P*-value: 2.08e-11). In

Table 6. Distribution of the common KEGG pathway analysis in the four groups

Common KEGG pathway term	Groups	Common KEGG pathway term	Groups
rno04080:Neuroactive ligand-receptor interaction	UD, UU, DU, DD	rno04630:Jak-STAT signaling pathway	UD, UU, DU
rno04610:Complement and coagulation cascades	UD, UU	rno04512:ECM-receptor interaction	UD, UU, DU
rno04060:Cytokine-cytokine receptor interaction	UD, UU, DU	rno05032:Morphine addiction	UU, DU
rno04640:Hematopoietic cell lineage	UU, DU, DD	rno00250:Alanine, aspartate and glutamate metabolism	UU, DD
rno03320:PPAR signaling pathway	UD, UU	rno04915:Estrogen signaling pathway	UD, UU
rno00590:Arachidonic acid metabolism	UU, DD	rno00260:Glycine, serine and threonine metabolism	UU, DD
rno04725:Cholinergic synapse	UD, UU	rno04510:Focal adhesion	UD, DD
rno04750:Inflammatory mediator regulation of TRP channels	UD, UU	rno04020:Calcium signaling pathway	UD, DD
rno04514:Cell adhesion molecules (CAMs)	UD, UU, DU	rno04916:Melanogenesis	UD, DU
rno04978:Mineral absorption	UD, UU	rno04024:cAMP signaling pathway	UD, DU, DD
rno04151:PI3K-Akt signaling pathway	UD, UU, DU	rno04540:Gap junction	DU, DD
rno04730:Long-term depression	UU, DU	rno04022:cGMP-PKG signaling pathway	DU, DD
rno04014:Ras signaling pathway	UU, DD	rno04975:Fat digestion and absorption	DU, DD
rno05133:Pertussis	UU, DD	rno04145:Phagosome	DU, DD
rno04970:Salivary secretion	UD, UU, DU	rno04923:Regulation of lipolysis in adipocytes	DU, DD

Note: UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R. There are totally 30 common KEGG pathways analysis which concerned two or more groups.

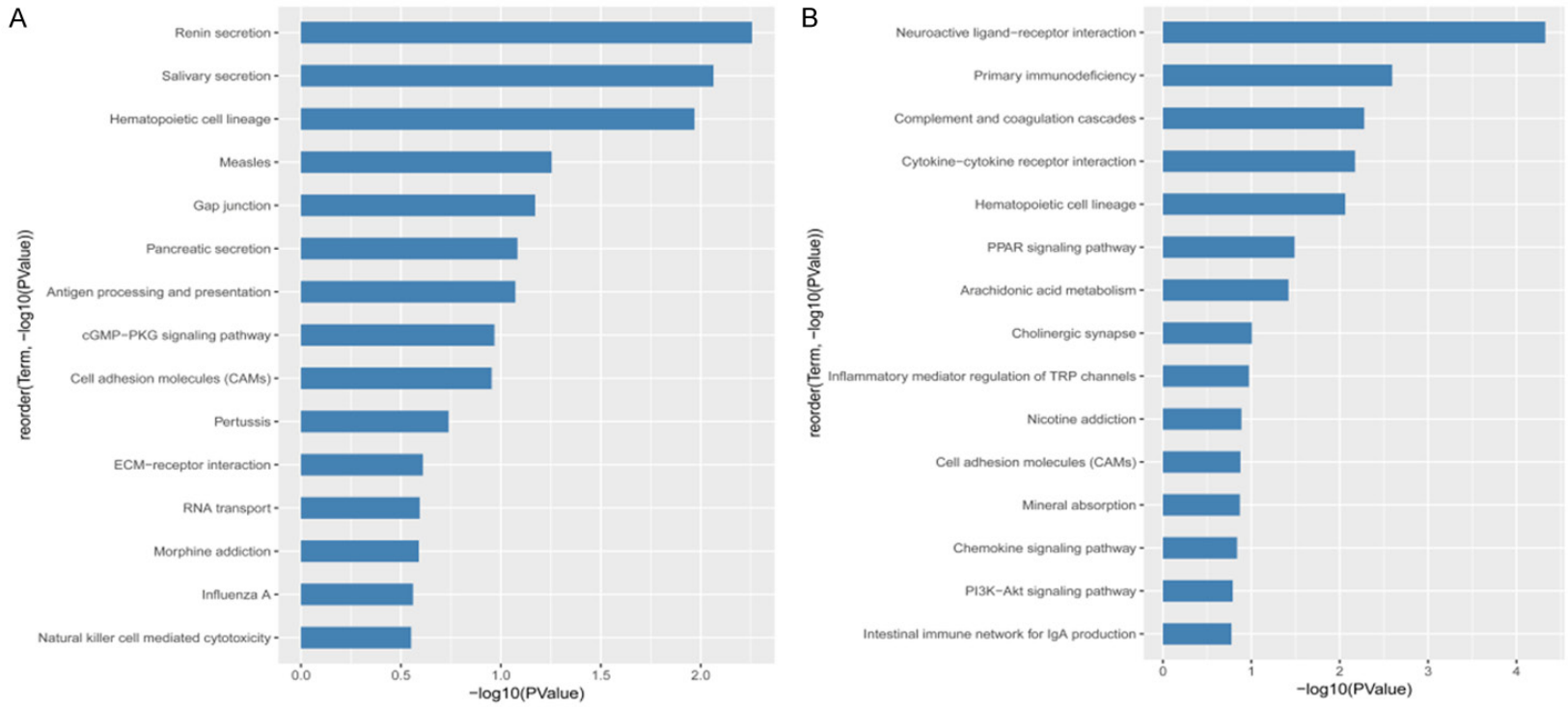
the DU group, the PPI network contained 181 nodes, 309 edges and 225 expected edges, with 3.41 average node degrees (PPI enrichment *P*-value: 7.49e-08). Analogously, the PPI network of DEGs in the DD group contained 159 nodes, 278 edges and 175 expected edges, with 3.50 average node degrees (PPI enrichment *P*-value: 3.84e-13) (**Table 7**). INTERPRO Protein Domains and Features showed 35 domains significantly enriched in the four DEGs groups (**Table 7**). All the domains mainly included Voltage-dependent channel domain superfamily, Toll/interleukin-1 receptor homology (TIR) domain superfamily, Neurotransmitter-gated ion-channel ligand-binding domain superfamily, Neurotransmitter-gated ion-channel transmembrane domain superfamily, Neurotransmitter-gated ion-channel ligand-binding domain, Toll/interleukin-1 receptor homology (TIR) domain, Immunoglobulin-like domain superfamily, Natural killer cell receptor-like, and C-type lectin-like domain. All PPI network construction pictures were shown in **Figure 4**.

Discussion

MI and I/R injury are both common diseases in clinic and have heavily threatened patients with cardiovascular disease. In 2013, more than 8.6 million people suffered from MI worldwide [44]. Although there are numerous studies on MI and I/R injury, the molecular mechanisms of MI and I/R injury are not fully understood. Preliminary genetic sequencing shows that acute MI or I/R can all lead to the increase in gene expression or cut [45, 46]. However; studies in this field are far from adequacy.

In order to further investigate genes related to MI and I/R injury and their relationship with these two diseases, we studied four different groups of genes in rat models, and found that most DEGs in the four groups had not been studied in relation to MI or I/R injury.

In the top 30 genes in the UD group, 10 genes (*Cpz, Scn9a, Kcne1, Tnfrsf12a, Has1, Camp, Micb, Cyr61, Hmox1* and *Sh2d1a*) have been reported to be concerned about MI or I/R injury. *Hmox1* and *Tnfrsf12a* both have been proven to have a direct relationship between MI and I/R. *Hmox1*, which belongs to the hemeoxygenase family, was considerably up-regulated with 2.9351 of Log_2 FC by MI and down-regulated with -2.22313 of Log_2 FC. Causey *et al*. has reported that *Tnfrsf12a*, as a member of TNF family, play a crucial role in apoptosis, cell death and angiogenesis, as well as cardiac remodeling and vascular development [47]. *Cldn23* and *Cldn4* are all encoded members of the claudin family. However, studies about two genes were all the association of these with tumors, whereas no report was found on the correlation with acute MI or I/R injury [48]. *Cyr61*, which was outstandingly up-regulated


A
Renin secretion
Salivary secretion
Hematopoietic cell lineage
Measles
Gap junction
Pancreatic secretion
Antigen processing and presentation
cGMP−PKG signaling pathway
Cell adhesion molecules (CAMs)
Pertussis
ECM−receptor interaction
RNA transport
Morphine addiction
Influenza A
Natural killer cell mediated cytotoxicity
reorder(Term, −log10(PValue))
0.0
0.5
1.0
1.5
2.0
−log10(PValue)
B
Neuroactive ligand−receptor interaction
Primary immunodeficiency
Complement and coagulation cascades
Cytokine−cytokine receptor interaction
Hematopoietic cell lineage
PPAR signaling pathway
Arachidonic acid metabolism
Cholinergic synapse
Inflammatory mediator regulation of TRP channels
Nicotine addiction
Cell adhesion molecules (CAMs)
Mineral absorption
Chemokine signaling pathway
PI3K−Akt signaling pathway
Intestinal immune network for IgA production
reorder(Term, −log10(PValue))
0
1
2
3
4
−log10(PValue)

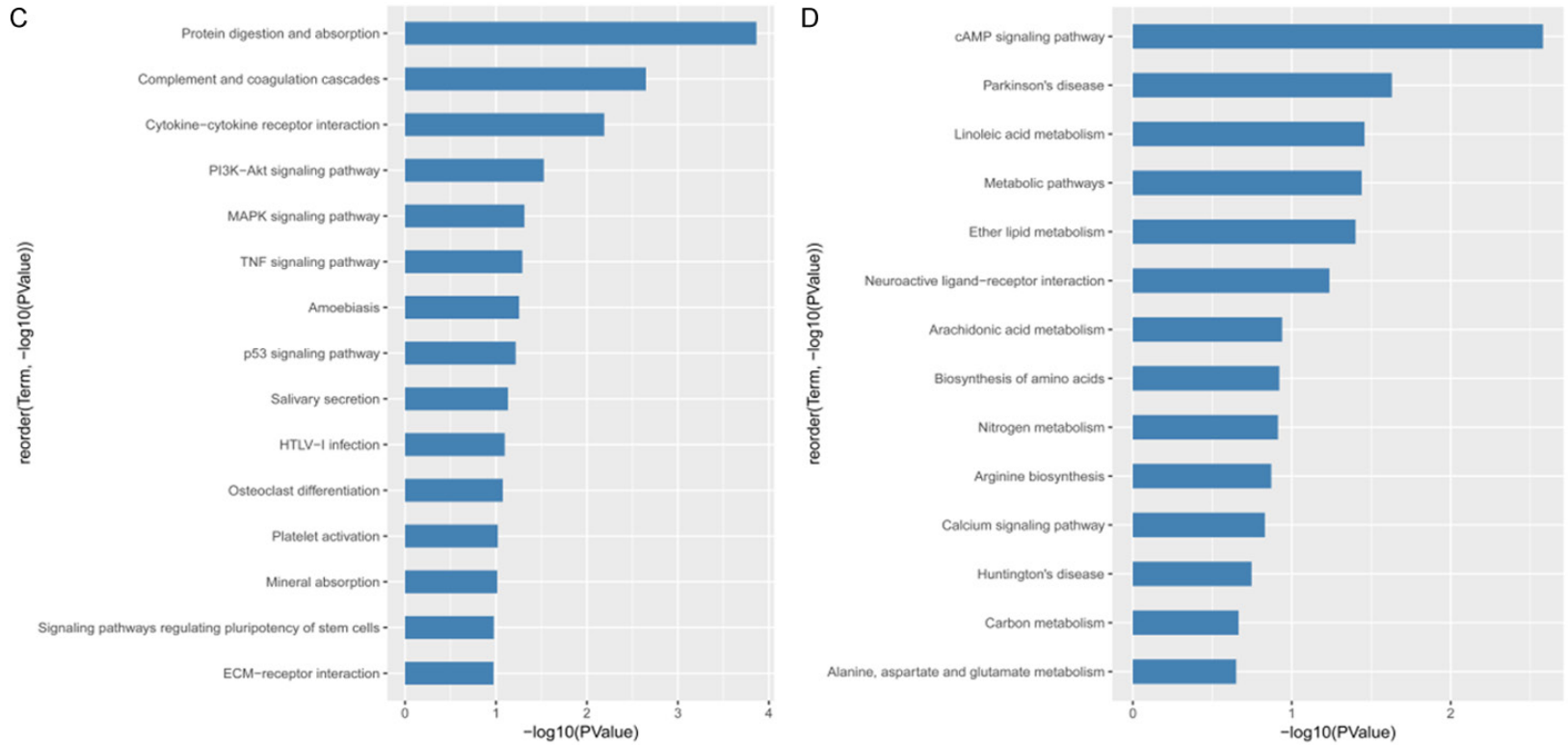


Figure 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of four groups of DEGs. A-D. Correspond to UD, UU, DU, and DD group separately. (*P<0.05) UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R.

Table 7. Items of the protein-protein interaction (PPI) network constructions of the four groups

Items	Number of nodes	Number of edges	Average node degree	Clustering coefficient	Expected number of edges	PPI enrichment *P*-value
UD	95	104	2.19	0.672	53	3.04E-10
UU	192	441	4.59	0.382	316	2.08E-11
DU	181	309	3.41	0.577	225	7.49E-08
DD	159	278	3.5	0.568	175	3.84E-13

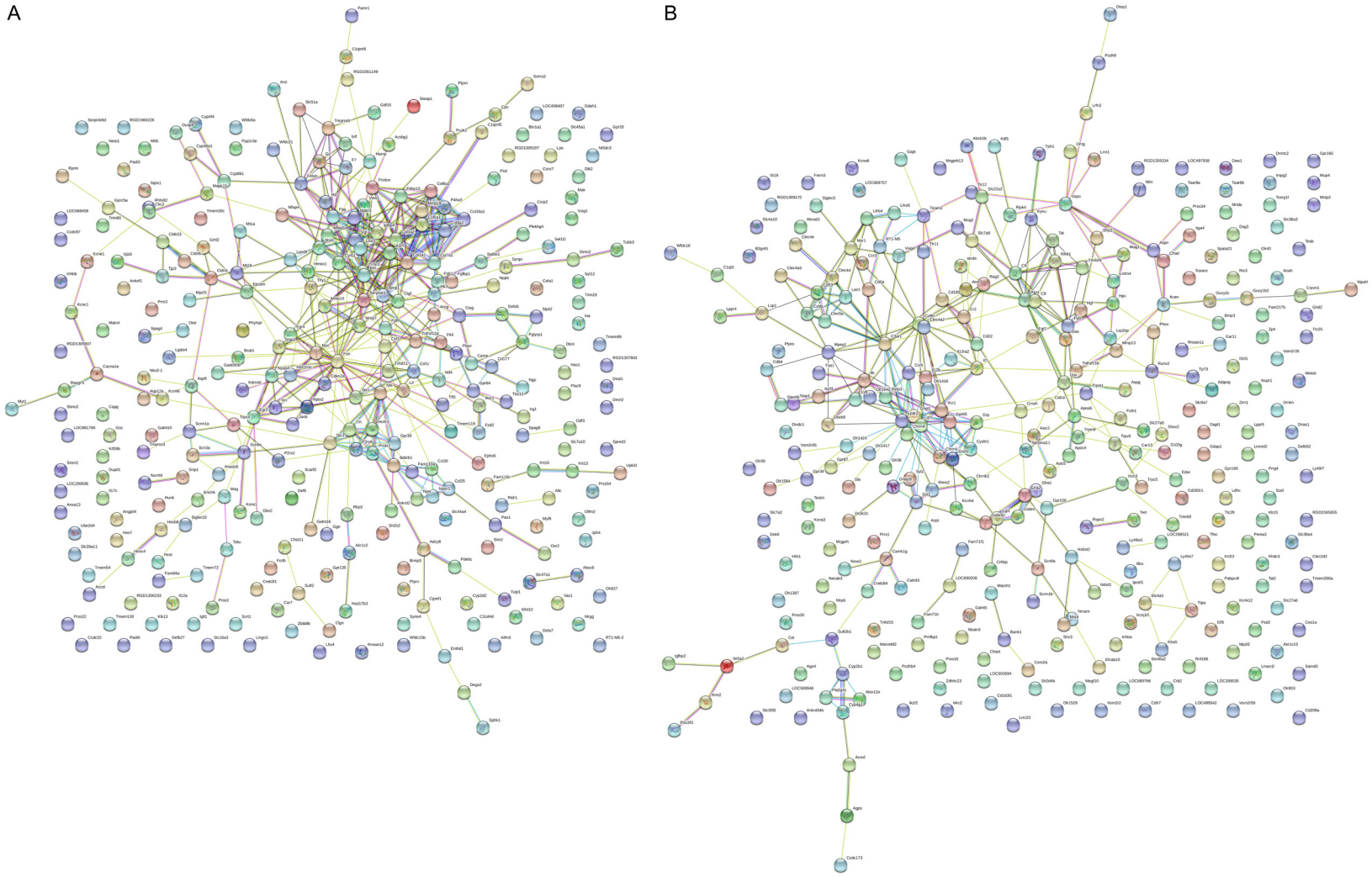
Note: UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R.

with 1.168 of Log_2 FC by MI and down-regulated with -2.22702 of Log_2 FC, is a member of the growth factor-inducible immediate early genes and can modulate inflammation. Bonda TA, *et al*. found that the expression of *Cyr61* protein was only slightly attenuated and had no influence in *IL-6* KO mice after MI, but *Cyr61* distribution was significantly changed when blockading the AT1 receptor [27]. It is noteworthy that the biological effects of these genes, being up-regulated genes in MI with the down-regulated genes in IR such as *Gsc, Cpz, Ankrd2, Prss3,* and *Tbx15* have not been fully researched. All these findings by this comparative study may shed light on the MI and I/R research filed.

In the top 30 genes in the DU group, five genes (*Cyp1a1, Abcg3l4, Kcnma1, Cd69* and *Il1a*) had been proven to be related to MI or I/R injury. *Kcnma1* (potassium large conductance calcium-activated channel, subfamily M, alpha member 1, MaxiK) is a protein coding gene. In our study, the expression of *Kcnma1* was outstandingly down-regulated with 1.727 of Log_2 FC on MI and up-regulated with -2.22702 of Log_2 FC on IR, which signified that the damaged biological functions of MaxiK may be recovered with the reperfusion of blood, which was also confirmed by many other researches [33]. Fretwell L *et al.* found that BK (Ca) channels participated in adenosine A_1 receptor-induced pharmacological post-conditioning in a cell model system by reducing H/R-induced LDH release [49]. Tomás M *et al.* discovered that the Ca^{2+}-dependent potassium channel could control human blood pressure and impact cardiovascular disease, and the genetic variation in the *Kcnma1* potassium channel alpha subunit could severely increase systolic hypertension and general hypertension [50].

In the top 30 genes in the UU group, only three genes (*Itga4, Msr1* and *Cybb*) have been studied in relation to acute MI or (and) I/R injury. *Itga4* encodes a member of the integrin alpha chain family of proteins, which come into play in cell motility and migration. Wingerd KL *et al.* found that *Itga4* plays an important role in axons development and innervations, which was expressed on developing axons *in vivo* [51]. *Msr1* can encode class A macrophage scavenger receptors. Tsujita K *et al.* found that the risk of cardiac rupture with MI was increased when targeted knocking out the class A macrophage scavenger receptor [52]. Shantsila E *et al.* described that the expression level of *Msr1* on *Mon1* was related to tissue type plasminogen activator levels, and thus, inferred *CXCR4* positive and angiogenic monocytes in MI [53]. *Cybb* has been proposed as a primary component of the microbicidal oxidase system of phagocytes. Almeida SA *et al.* found that the mechanism of improving functional cardiac parameters subjected to MI of exercise training might be concerned with the decreasing expression of both *Cybb* and AT_1 receptor [37]. Xu J *et al.* reported that the increased protein expression of *Cybb* and transforming growth factor β1 resulted in ventricular dilatation, as well as dysfunction, interstitial fibrosis and myocardial hypertrophy, which was caused by MI.

In the top 30 genes in the DD group, five genes (*Treml1, Alox15, vnn3, Gata1* and *Pbx4*) were detected. *Treml1* and *Alox15* were studied in relation to MI or I/R injury. *Treml1* is a triggering receptor expressed on myeloid cells like 1. Recent researches suggested that the soluble triggering receptor could facilitate atherothrombosis, which helps understand atherothrombosis-associated acute coronary syndrome [42]. Recent studies have shown that *Alox15* is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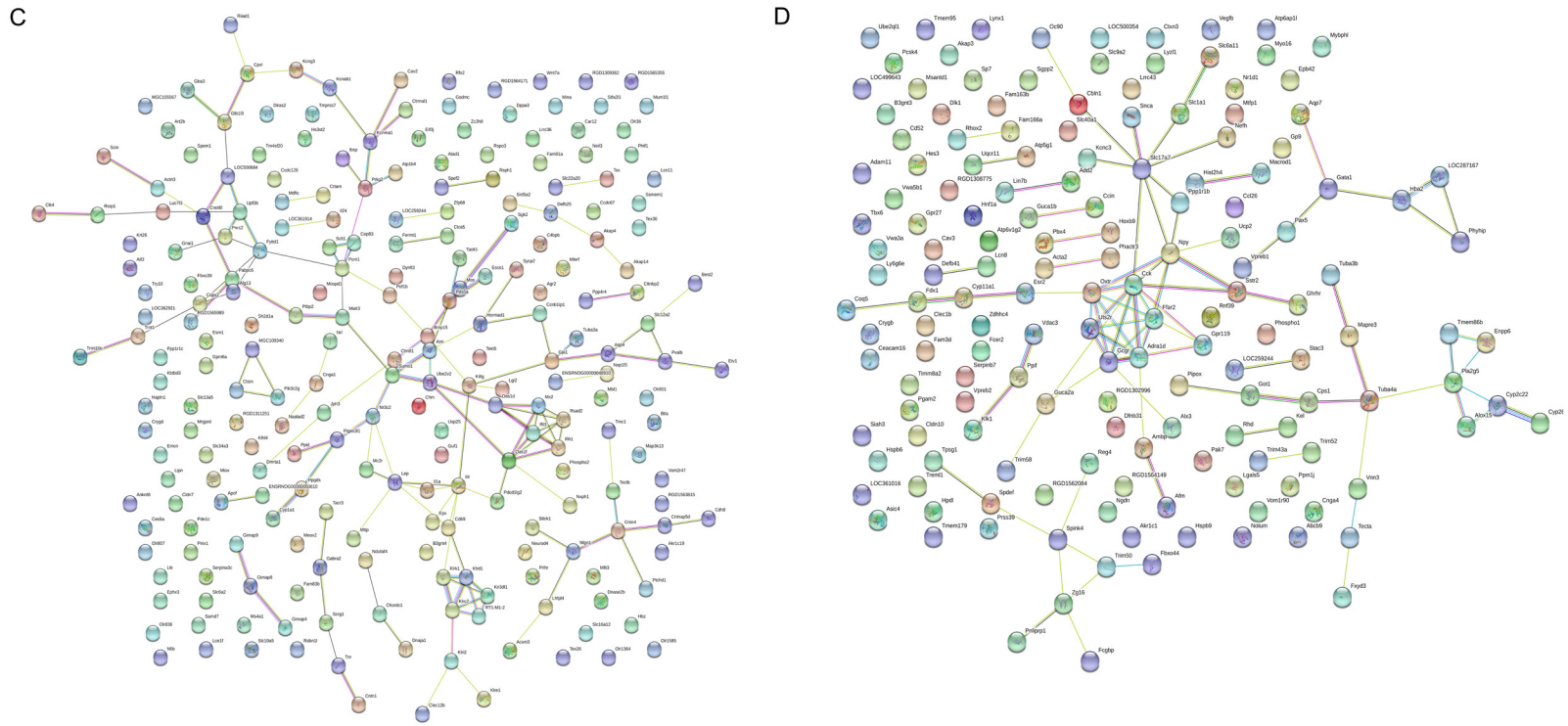

Figure 4. Protein-protein interaction (PPI) network construction was connected with the Search Tool for Retrieval of Interacting Genes (STRING) database. A-D. Correspond to UD, UU, DU, and DD group separately. (*P<0.05) UD: genes up-regulated in myocardial infarction (MI) but down-regulated in ischemia/reperfusion (I/R); DU: genes down-regulated in MI but up-regulated in I/R; UU: genes both up-regulated in MI and I/R; DD: genes both down-regulated in MI and I/R.

involved in inflammation and have been implicated in atherosclerosis, and then related to stroke, MI, and coronary artery disease (CAD) [41].

At the same time, we found many issues on the GO enrichment analysis of DEGs in the four groups worthy of further study. First, there were a total of 58 GO items on Molecular Function, 48 GO items on Cellular Component and 218 GO items on Biological Process. When MI and I/R injury occurred, myocardial cells, including the subsidiary of the neurovascular, produced a series of molecular biological changes; and these changes would be reflected in the results of the GO enrichment analysis [54]. Second, some GO terms were commonly shared in different groups, and a total of 21 common GO terms were found. Some common GO terms, such as growth factor activity (GO:0008083), cytokine activity (GO:0005125) and cellular response to fibroblast growth factor stimulus (GO:0044344), played important roles in inflammation and cell apoptosis [55, 56].

Similarly, a total of 148 KEGG pathways were found from the DEGs of the four groups. Many pathways such as the TNF signaling pathway (rno04668), p53 signaling pathway (rno04115), and leukocyte transendothelial migration (rno04670) had been proven to be of significance in the molecular mechanisms of MI or I/R injury [57, 58]. However, the overwhelming majority of these pathways had not been researched in relation to MI or I/R injury. It is worth noting that there were 30 common pathways of many different groups. Neuroactive ligand-receptor interaction (rno04080) was a common pathway in the four groups, but no studies with MI or I/R injury have been reported. With pathway analysis, we can find distinct cell processes, diseases or signaling pathways that are statistically associated with selection of deferentially expressed genes between two samples [59]. Common pathways in different groups meant different physiological and pathological process share common molecular mechanisms. Meanwhile, the results of the PPI network constructions of the four groups revealed that a total of 159 nodes, 278 edges, and 175 expected edges. However, very limited studies have been conducted on these PPI networks so far.

Conclusion

In conclusion, by comparing the gene expression profiles of MI and I/R injury models, we obtained four groups of differentially expressed genes, and GO function and KEGG pathway enrichment analyses were carried out from the four groups of DEGs. All these results provide vast areas for the subsequent research in finding mechanism of MI and I/R injury and developing new therapeutic targets.

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Disclosure of conflict of interest

None.

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References

[1] Sen SK, Barb JJ, Cherukuri PF, Accame DS, Elkahloun AG, Singh LN, Lee-Lin SQ, Program NCS, Kolodgie FD, Cheng Q, Zhao X, Chen MY, Arai AE, Green ED, Mullikin JC, Munson PJ and Biesecker LG. Identification of candidate genes involved in coronary artery calcification by transcriptome sequencing of cell lines. BMC Genomics 2014; 15: 198.

[2] Suchal K, Malik S, Gamad N, Malhotra RK, Goyal SN, Chaudhary U, Bhatia J, Ojha S and Arya DS. Kaempferol attenuates myocardial ischemic injury via inhibition of MAPK signaling pathway in experimental model of myocardial ischemia-reperfusion injury. Oxid Med Cell Longev 2016; 2016: 7580731.

[3] Sim DS, Jeong MH, Ahn Y, Kim YJ, Chae SC, Hong TJ, Seong IW, Chae JK, Kim CJ, Cho MC, Rha SW, Bae JH, Seung KB and Park SJ; Korea Acute Myocardial Infarction Registry (KAMIR) Investigators. Pharmacoinvasive strategy versus primary percutaneous coronary intervention in patients with ST-segment-elevation myocardial infarction: a propensity score-matched analysis. Circ Cardiovasc Interv 2016; 9.

[4] Abuzaid AA, Zaki M and Al Tarief H. Potential risk factors for surgical site infection after isolated coronary artery bypass grafting in a bahrain cardiac centre: a retrospective, case-controlled study. Heart Views 2015; 16: 79-84.

[5] Andjic M, Spiroski D, Ilic Stojanovic O, Vidakovic T, Lazovic M, Babic D, Ristic A, Mazic S, Zdravkovic M and Otasevic P. Effects of short-term exercise training in patients following acute myocardial infarction treated with primary percutaneous coronary intervention. Eur J Phys Rehabil Med 2016; 52: 364-9.

[6] Iqbal MB, Robinson SD, Ding L, Fung A, Aymong E, Chan AW, Hodge S, Della Siega A and Nadra IJ; British Columbia Cardiac Registry Investigators. Intra-aortic balloon pump counterpulsation during primary percutaneous coronary intervention for st-elevation myocardial infarction and cardiogenic shock: insights from the british columbia cardiac registry. PLoS One 2016; 11: e0148931.

[7] Russhard P, Al Janabi F, Parker M and Clesham GJ. Patterns of ST segment resolution after guidewire passage and thrombus aspiration in primary percutaneous coronary intervention (PPCI) for acute myocardial infarction. Open Heart 2016; 3: e000430.

[8] Topol EJ, Smith J, Plow EF and Wang QK. Genetic susceptibility to myocardial infarction and coronary artery disease. Hum Mol Genet 2006; 15 Spec No 2: R117-123.

[9] Arab S, Konstantinov IE, Boscarino C, Cukerman E, Mori A, Li J, Liu PP, Redington AN and Coles JG. Early gene expression profiles during intraoperative myocardial ischemia-reperfusion in cardiac surgery. J Thorac Cardiovasc Surg 2007; 134: 74-81, 81, e1-2.

[10] O'Donnell CJ and Nabel EG. Genomics of cardiovascular disease. N Engl J Med 2011; 365: 2098-2109.

[11] Wang Q. Advances in the genetic basis of coronary artery disease. Curr Atheroscler Rep 2005; 7: 235-241.

[12] Kullo IJ and Ding K. Mechanisms of disease: the genetic basis of coronary heart disease. Nat Clin Pract Cardiovasc Med 2007; 4: 558-569.

[13] Franchini M, Peyvandi F and Mannucci PM. The genetic basis of coronary artery disease: from candidate genes to whole genome analysis. Trends Cardiovasc Med 2008; 18: 157-162.

[14] Lippi G, Franchini M and Targher G. Arterial thrombus formation in cardiovascular disease. Nat Rev Cardiol 2011; 8: 502-512.

[15] Ichimura K, Matoba T, Nakano K, Tokutome M, Honda K, Koga J and Egashira K. A translational study of a new therapeutic approach for acute myocardial infarction: nanoparticle-mediated delivery of pitavastatin into reperfused myocardium reduces ischemia-reperfusion injury in a preclinical porcine model. PLoS One 2016; 11: e0162425.

[16] Evonuk KS, Prabhu SD, Young ME and DeSilva TM. Myocardial ischemia/reperfusion impairs neurogenesis and hippocampal-dependent learning and memory. Brain Behav Immun 2017; 61: 266-273.

[17] Fu SP, He SY, Xu B, Hu CJ, Lu SF, Shen WX, Huang Y, Hong H, Li Q, Wang N, Liu XL, Liang F and Zhu BM. Acupuncture promotes angiogenesis after myocardial ischemia through H3K9 acetylation regulation at VEGF gene. PLoS One 2014; 9: e94604.

[18] Huang Y, Lu SF, Hu CJ, Fu SP, Shen WX, Liu WX, Li Q, Wang N, He SY, Liang FR and Zhu BM. Electro-acupuncture at Neiguan pretreatment alters genome-wide gene expressions and protects rat myocardium against ischemia-reperfusion. Molecules 2014; 19: 16158-16178.

[19] Huang da W, Sherman BT and Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.

[20] Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C and Jensen LJ. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013; 41: D808-815.

[21] De Las Rivas J and Fontanillo C. Protein-protein interactions essentials: key concepts to building and analyzing interactome networks. PLoS Comput Biol 2010; 6: e1000807.

[22] Kallner G. Release and effects of calcitonin gene-related peptide in myocardial ischaemia. Scand Cardiovasc J Suppl 1998; 49: 1-35.

[23] Estacion M, Waxman SG and Dib-Hajj SD. Effects of ranolazine on wild-type and mutant hNav1.7 channels and on DRG neuron excitability. Mol Pain 2010; 6: 35.

[24] Xia S, Wang Y, Zhang Y, Deng SB, Du JL, Wang XC and She Q. Dynamic changes in HCN2, HCN4, KCNE1, and KCNE2 expression in ventricular cells from acute myocardial infarction rat hearts. Biochem Biophys Res Commun 2010; 395: 330-335.

[25] Hsieh YY, Chang CC, Hsu CM, Chen SY, Lin WH and Tsai FJ. Major histocompatibility complex class I chain-related gene polymorphisms: associated with susceptibility to Kawasaki disease and coronary artery aneurysms. Genet Test Mol Biomarkers 2011; 15: 755-763.

[26] Solskov L, Magnusson NE, Kristiansen SB, Jessen N, Nielsen TT, Schmitz O, Botker HE and Lund S. Microarray expression analysis in delayed cardioprotection: the effect of exercise, AICAR, or metformin and the possible role of AMP-activated protein kinase (AMPK). Mol Cell Biochem 2012; 360: 353-362.

[27] Bonda TA, Taranta A, Kaminski KA, Dziemidowicz M, Litvinovich S, Kozuch M, Bialuk I, Chyc-

zewski L and Winnicka MM. CCN1 expression in interleukin-6 deficient mouse kidney in experimental model of heart failure. Folia Histochem Cytobiol 2013; 51: 84-91.

[28] Muller J, Gorressen S, Grandoch M, Feldmann K, Kretschmer I, Lehr S, Ding Z, Schmitt JP, Schrader J, Garbers C, Heusch G, Kelm M, Scheller J and Fischer JW. Interleukin-6-dependent phenotypic modulation of cardiac fibroblasts after acute myocardial infarction. Basic Res Cardiol 2014; 109: 440.

[29] Borg N, Alter C, Gorldt N, Jacoby C, Ding Z, Steckel B, Quast C, Bonner F, Friebe D, Temme S, Flogel U and Schrader J. CD73 on T-cells orchestrates cardiac wound healing after myocardial infarction by purinergic metabolic reprogramming. Circulation 2017; 136: 297-313.

[30] Guo N, Zhang N, Yan L, Cao X, Wang J and Wang Y. Correlation between genetic polymorphisms within the MAPK1/HIF-1/HO-1 signaling pathway and risk or prognosis of perimenopausal coronary artery disease. Clin Cardiol 2017; 40: 597-604.

[31] Park ES, Kang DH, Kang JC, Jang YC, Lee MJ, Chung HJ, Yi KY, Kim DE, Kim B and Shin HS. Cardioprotective effect of KR-33889, a novel PARP inhibitor, against oxidative stress-induced apoptosis in H9c2 cells and isolated rat hearts. Arch Pharm Res 2017; 40: 640-654.

[32] Lakshmi SV, Naushad SM, Saumya K, Rao DS and Kutala VK. Role of CYP1A1 haplotypes in modulating susceptibility to coronary artery disease. Indian J Biochem Biophys 2012; 49: 349-355.

[33] Soltysinska E, Bentzen BH, Barthmes M, Hattel H, Thrush AB, Harper ME, Qvortrup K, Larsen FJ, Schiffer TA, Losa-Reyna J, Straubinger J, Kniess A, Thomsen MB, Bruggemann A, Fenske S, Biel M, Ruth P, Wahl-Schott C, Boushel RC, Olesen SP and Lukowski R. KCNMA1 encoded cardiac BK channels afford protection against ischemia-reperfusion injury. PLoS One 2014; 9: e103402.

[34] Arvind P, Jayashree S, Jambunathan S, Nair J and Kakkar VV. Understanding gene expression in coronary artery disease through global profiling, network analysis and independent validation of key candidate genes. J Genet 2015; 94: 601-610.

[35] Lugrin J, Parapanov R, Rosenblatt-Velin N, Rignault-Clerc S, Feihl F, Waeber B, Muller O, Vergely C, Zeller M, Tardivel A, Schneider P, Pacher P and Liaudet L. Cutting edge: IL-1alpha is a crucial danger signal triggering acute myocardial inflammation during myocardial infarction. J Immunol 2015; 194: 499-503.

[36] Maiwald S, Zwetsloot PP, Sivapalaratnam S and Dallinga-Thie GM. Monocyte gene expression and coronary artery disease. Curr Opin Clin Nutr Metab Care 2013; 16: 411-417.

[37] Almeida SA, Claudio ER, Mengal V, Oliveira SG, Merlo E, Podratz PL, Gouvea SA, Graceli JB and de Abreu GR. Exercise training reduces cardiac dysfunction and remodeling in ovariectomized rats submitted to myocardial infarction. PLoS One 2014; 9: e115970.

[38] Ma F, Li T, Zhang H and Wu G. MiR-30s family inhibit the proliferation and apoptosis in human coronary artery endothelial cells through targeting the 3'UTR region of ITGA4 and PLCG1. J Cardiovasc Pharmacol 2016; 68: 327-333.

[39] Wang L, Fan C, Topol SE, Topol EJ and Wang Q. Mutation of MEF2A in an inherited disorder with features of coronary artery disease. Science 2003; 302: 1578-1581.

[40] Holmen OL, Zhang H, Fan Y, Hovelson DH, Schmidt EM, Zhou W, Guo Y, Zhang J, Langhammer A, Lochen ML, Ganesh SK, Vatten L, Skorpen F, Dalen H, Zhang J, Pennathur S, Chen J, Platou C, Mathiesen EB, Wilsgaard T, Njolstad I, Boehnke M, Chen YE, Abecasis GR, Hveem K and Willer CJ. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. Nat Genet 2014; 46: 345-351.

[41] Wuest SJ, Horn T, Marti-Jaun J, Kuhn H and Hersberger M. Association of polymorphisms in the ALOX15B gene with coronary artery disease. Clin Biochem 2014; 47: 349-355.

[42] Esponda OL, Hunter R, Del Rio JR and Washington AV. Levels of soluble TREM-like transcript 1 in patients presenting to the emergency department with chest pain. Clin Appl Thromb Hemost 2015; 21: 30-34.

[43] Park HJ, Noh JH, Eun JW, Koh YS, Seo SM, Park WS, Lee JY, Chang K, Seung KB, Kim PJ and Nam SW. Assessment and diagnostic relevance of novel serum biomarkers for early decision of ST-elevation myocardial infarction. Oncotarget 2015; 6: 12970-12983.

[44] Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injury in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015; 386: 743-800.

[45] Ounzain S, Micheletti R, Beckmann T, Schroen B, Alexanian M, Pezzuto I, Crippa S, Nemir M, Sarre A, Johnson R, Dauvillier J, Burdet F, Iberson M, Guigo R, Xenarios I, Heymans S and Pedrazzini T. Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. Eur Heart J 2015; 36: 353-368a.

[46] Eicher JD, Wakabayashi Y, Vitseva O, Esa N, Yang Y, Zhu J, Freedman JE, McManus DD and Johnson AD. Characterization of the platelet transcriptome by RNA sequencing in patients with acute myocardial infarction. Platelets 2016; 27: 230-239.

[47] Chorianopoulos E, Heger T, Lutz M, Frank D, Bea F, Katus HA and Frey N. FGF-inducible 14-kDa protein (Fn14) is regulated via the RhoA/ROCK kinase pathway in cardiomyocytes and mediates nuclear factor-kappaB activation by TWEAK. Basic Res Cardiol 2010; 105: 301-313.

[48] Maryan N, Statkiewicz M, Mikula M, Goryca K, Paziewska A, Strzalkowska A, Dabrowska M, Bujko M and Ostrowski J. Regulation of the expression of claudin 23 by the enhancer of zeste 2 polycomb group protein in colorectal cancer. Mol Med Rep 2015; 12: 728-736.

[49] Fretwell L and Dickenson JM. Role of large-conductance Ca(2+) -activated potassium channels in adenosine A(1) receptor-mediated pharmacological preconditioning in H9c2 cells. Eur J Pharmacol 2009; 618: 37-44.

[50] Tomas M, Vazquez E, Fernandez-Fernandez JM, Subirana I, Plata C, Heras M, Vila J, Marrugat J, Valverde MA and Senti M. Genetic variation in the KCNMA1 potassium channel alpha subunit as risk factor for severe essential hypertension and myocardial infarction. J Hypertens 2008; 26: 2147-2153.

[51] Wingerd KL, Wayne WC, Jackson DY and Clegg DO. Involvement of alpha4 integrins in maintenance of cardiac sympathetic axons. Auton Neurosci 2005; 122: 58-68.

[52] Tsujita K, Kaikita K, Hayasaki T, Honda T, Kobayashi H, Sakashita N, Suzuki H, Kodama T, Ogawa H and Takeya M. Targeted deletion of class A macrophage scavenger receptor increases the risk of cardiac rupture after experimental myocardial infarction. Circulation 2007; 115: 1904-1911.

[53] Shantsila E, Tapp LD, Wrigley BJ, Montoro-Garcia S and Lip GY. CXCR4 positive and angiogenic monocytes in myocardial infarction. Thromb Haemost 2013; 109: 255-262.

[54] Ward CW, Reiken S, Marks AR, Marty I, Vassort G and Lacampagne A. Defects in ryanodine receptor calcium release in skeletal muscle from post-myocardial infarct rats. FASEB J 2003; 17: 1517-1519.

[55] Ortiz VD, de Castro AL, Campos C, Fernandes RO, Bonetto JH, Siqueira R, Conzatti A, Fernandes TR, Bello-Klein A and Araujo AS. Effects of thyroid hormones on aortic tissue after myocardial infarction in rats. Eur J Pharmacol 2016; 791: 788-793.

[56] Yang F, Liu W, Yan X, Zhou H, Zhang H, Liu J, Yu M, Zhu X and Ma K. Effects of mir-21 on Cardiac microvascular endothelial cells after acute myocardial infarction in rats: role of phosphatase and tensin homolog (PTEN)/vascular endothelial growth factor (VEGF) signal pathway. Med Sci Monit 2016; 22: 3562-3575.

[57] Li C, Wang J, Wang Q, Zhang Y, Zhang N, Lu L, Wu Y, Zhang Q, Wang W, Wang Y and Tu P. Qishen granules inhibit myocardial inflammation injury through regulating arachidonic acid metabolism. Sci Rep 2016; 6: 36949.

[58] Li X, Yang J, Yang J, Dong W, Li S, Wu H and Li L. RP105 protects against myocardial ischemia-reperfusion injury via suppressing TLR4 signaling pathways in rat model. Exp Mol Pathol 2016; 100: 281-286.

[59] Garcia-Campos MA, Espinal-Enriquez J and Hernandez-Lemus E. Pathway analysis: state of the art. Front Physiol 2015; 6: 383.

Table S1. The Top 30 DEGs in IR-Down vs. MI-Up

Gene Name	Description	IR_Down	MC_Up
Cldn23	claudin 23 (Cldn23)	-4.98983	1.48019
Gsc	goosecoid homeobox (Gsc)	-4.15069	1.80807
Cldn4	claudin 4 (Cldn4)	-3.2359	3.5827
Cpz	carboxypeptidase Z (Cpz)	-3.22203	5.03364
Ankrd2	ankyrin repeat domain 2 (Ankrd2)	-3.17035	1.8644
Prss3	protease, serine, 3 (Prss3)	-3.13265	2.27034
Tbx15	T-box 15 (Tbx15)	-3.07141	1.03717
Scn9a	sodium voltage-gated channel alpha subunit 9 (Scn9a)	-3.06093	1.15072
Ngp	neutrophilic granule protein (Ngp)	-2.77955	2.14764
Ntf4	neurotrophin 4 (Ntf4)	-2.77464	2.34842
Kcne1	potassium voltage-gated channel subfamily C member 1 (Kcnc1)	-2.67308	1.08412
Tnfrsf12a	TNF receptor superfamily member 12A (Tnfrsf12a)	-2.67119	1.37161
Gbx2	gastrulation brain homeobox 2 (Gbx2)	-2.66992	3.32557
Cd177	CD177 molecule (Cd177)	-2.61228	3.04557
Scn3a	sodium voltage-gated channel alpha subunit 3 (Scn3a)	-2.60126	1.06723
Hunk	hormonally upregulated Neu-associated kinase (Hunk)	-2.50992	1.88404
P2rx2	purinergic receptor P2X 2 (P2rx2)	-2.50069	3.79439
Olfm2	olfactomedin 2 (Olfm2)	-2.49983	3.53349
Msln	mesothelin (Msln)	-2.47484	7.43791
Has1	hyaluronan synthase 1 (Has1)	-2.47068	2.17646
Camp	cathelicidin antimicrobial peptide (Camp)	-2.45008	2.16603
P4ha3	prolyl 4-hydroxylase subunit alpha 3 (P4ha3)	-2.31742	6.23556
Sulf2	sulfatase 2(Sulf2)	-2.27236	1.55823
Rhbdl2	rhomboid like 2 (Rhbdl2)	-2.26384	2.28603
Dupd1	dual specificity phosphatase and pro isomerase domain containing 1 (Dupd1)	-2.24677	1.35337
Micb	MHC class I polypeptide-related sequence B (Micb)	-2.22821	2.71254
Cyr61	cysteine-rich, angiogenic inducer, 61 (Cyr61)	-2.22702	1.16812
Hmox1	heme oxygenase 1 (Hmox1)	-2.22313	2.9351
Kcnc1	potassium voltage-gated channel subfamily C member 1 (Kcnc1)	-2.21502	4.69107

Table S2. The Top 30 DEGs in IR_ Up vs. MI_Down

Gene Name	Description	IR_ Up	MC_Down
Tuba3a	tubulin, alpha 3A (Tuba3a)	7.97123	-1.41982
Klre1	killer cell lectin-like receptor, family E, member 1 (Klre1)	4.57254	-1.87144
Nxph1	neurexophilin 1 (Nxph1)	4.28732	-1.01639
Btla	B and T lymphocyte associated (Btla)	3.74001	-1.54052
Crygf	crystallin, gamma F (Crygf)	3.17531	-3.53847
Dmrta1	DMRT-like family A1 (Dmrta1)	2.97884	-1.21621
Kcnma1	potassium calcium-activated channel subfamily M alpha 1 (Kcnma1)	2.73868	-1.72746
Sh2d1a	SH2 domain containing 1A (Sh2d1a)	2.70863	-1.14008
Clec12b	C-type lectin domain family 12, member B (Clec12b)	2.6421	-3.52224
RGD1311251	similar to RIKEN cDNA 4930550C14 (RGD1311251)	2.54664	-2.46956
Cntn1	contactin 1 (Cntn1)	2.45939	-2.65511
Cd69	Cd69 molecule (Cd69)	2.42243	-1.08642
Agr2	anterior gradient 2, protein disulphide isomerase family member (Agr2)	2.4138	-1.16561
Hapln1	hyaluronan and proteoglycan link protein 1 (Hapln1)	2.38836	-2.46949

Il1a	interleukin 1 alpha (Il1a)	2.33131	-1.67084
Slc13a5	solute carrier family 13 member 5 (Slc13a5)	2.31643	-1.7592
Glb1l3	galactosidase, beta 1-like 3 (Glb1l3)	2.27469	-2.14909
MGC105567	similar to cDNA sequence BC023105 (MGC105567)	2.23869	-1.3377
Il24	interleukin 24 (Il24)	2.22417	-1.07852
Dppa3	developmental pluripotency-associated 3 (Dppa3)	2.22224	-1.57427
Pde1c	phosphodiesterase 1C (Pde1c)	2.20213	-1.1112
Cpvl	carboxypeptidase, vitellogenic-like (Cpvl)	2.19853	-1.50399
Rsph1	radial spoke head 1 homolog (Rsph1)	2.17924	-2.24894
Zfp68	zinc finger protein 68 (Zfp68)	2.06104	-1.14011
Cyp1a1	cytochrome P450, family 1, subfamily a, polypeptide 1 (Cyp1a1)	2.0229	-3.94734
Klrk1	killer cell lectin like receptor K1 (Klrk1)	1.99757	-1.4125
Jph3	junctophilin 3 (Jph3)	1.99028	-1.11857
Tectb	tectorin beta (Tectb)	1.98392	-3.16903
Abcg3l4	ATP-binding cassette, subfamily G (WHITE), member 3-like 4 (Abcg3l4)	1.97834	-1.28188

Table S3. The Top 30 DEGs in IR_ Up vs. MI_Up

Gene Name	Description	IR_ Up	MC_Up
Glra2	glycine receptor, alpha 2 (Glra2)	4.73692	1.25026
Gpr65	G-protein coupled receptor 65 (Gpr65)	4.02074	1.57515
Klra5	killer cell lectin-like receptor, subfamily A, member 5 (Klra5)	3.85014	5.1647
Ly49si1	immunoreceptor Ly49si1 (Ly49si1)	3.55151	3.61855
Gpr34	G protein-coupled receptor 34 (Gpr34)	3.36847	1.13594
Ipcef1	interaction protein for cytohesin exchange factors 1 (Ipcef1)	3.30216	2.21145
Tm4sf4	transmembrane 4 L six family member 4 (Tm4sf4)	3.21824	1.6097
LOC500948	LRRGT00073 (LOC500948)	3.13422	2.94307
Trpv6	transient receptor potential cation channel, subfamily V, member 6 (Trpv6)	2.88998	2.81786
Kynu	kynureninase (Kynu)	2.81773	1.0404
Itga4	integrin subunit alpha 4 (Itga4)	2.69614	1.59508
Bank1	B-cell scaffold protein with ankyrin repeats 1 (Bank1)	2.69335	1.09357
Galnt5	polypeptide N-acetylgalactosaminyltransferase 5 (Galnt5)	2.62212	1.21011
Dixdc1	DIX domain containing 1 (Dixdc1)	2.61816	1.00189
Rnase11	ribonuclease A family member 11 (Rnase11)	2.61678	2.34305
Ndst3	N-deacetylase and N-sulfotransferase 3 (Ndst3)	2.56268	2.06962
Hs6st2	heparan sulfate 6-O-sulfotransferase 2 (Hs6st2)	2.56139	1.28589
Mctp2	multiple C2 and transmembrane domain containing 2 (Mctp2)	2.5529	2.08467
Inhba	inhibin beta A subunit (Inhba)	2.54028	1.03816
Slc27a6	solute carrier family 27 member 6 (Slc27a6)	2.47714	1.03876
Msr1	macrophage scavenger receptor 1 (Msr1)	2.47693	2.2882
Clec4a2	C-type lectin domain family 4, member A2 (Clec4a2)	2.4523	1.18844
Rims2	regulating synaptic membrane exocytosis 2 (Rims2)	2.44415	1.47808
Zdhhc23	zinc finger, DHHC-type containing 23 (Zdhhc23)	2.44339	1.60627
C1ql3	complement C1q like 3 (C1ql3)	2.43299	1.78869
Cybb	cytochrome b-245 beta chain (Cybb)	2.41474	1.59634
Gpr160	G protein-coupled receptor 160 (Gpr160)	2.41251	1.16048
Scel	sciellin (Scel)	2.40078	1.20501
Trat1	T cell receptor associated transmembrane adaptor 1 (Trat1)	2.38184	1.40893

Table S4. The Top 30 DEGs in IR_Down vs. MI_Down

Gene Name	Description	IR_Down	MC_Down
Pnliprp1	pancreatic lipase-related protein 1 (Pnliprp1)	-3.28899	-1.72661
Sgpp2	sphingosine-1-phosphate phosphatase 2 (Sgpp2)	-2.94869	-1.90397
Enpp6	ectonucleotide pyrophosphatase/phosphodiesterase 6 (Enpp6)	-2.7406	-2.32045
Hoxb9	homeo box B9 (Hoxb9)	-2.6857	-2.99594
Vpreb1	pre-B lymphocyte 1 (Vpreb1)	-2.67402	-2.18896
RGD1308775	similar to RIKEN cDNA 4921536K21 (RGD1308775)	-2.63141	-1.01678
Hist2h4	histone cluster 2, H4 (Hist2h4)	-2.54091	-1.70127
Spdef	SAM pointed domain containing ets transcription factor (Spdef)	-2.52697	-1.56354
Klk1c9	kallikrein 1-related peptidase C9 (Klk1c9)	-2.4468	-1.95554
Treml1	triggering receptor expressed on myeloid cells-like 1 (Treml1)	-2.24371	-1.16141
Trim43a	tripartite motif-containing 43A (Trim43a)	-2.19518	-1.6797
Atp6v1g2	ATPase H+ transporting V1 subunit G2 (Atp6v1g2)	-2.1851	-1.34582
Vdac3	voltage-dependent anion channel 3 (Vdac3)	-2.16511	-1.3123
Mybphl	myosin binding protein H-like (Mybphl)	-2.08168	-1.63613
Add2	adducin 2 (Add2)	-1.99755	-1.48643
Car1	carbonic anhydrase I (Car1)	-1.98694	-1.02856
Adam11	ADAM metallopeptidase domain 11 (Adam11)	-1.953	-1.10552
Vnn3	vanin 3 (Vnn3)	-1.94433	-2.74579
Alox15	arachidonate 15-lipoxygenase (Alox15)	-1.93693	-2.66977
Vpreb2	pre-B lymphocyte gene 2 (Vpreb2)	-1.83754	-2.18896
Dlk1	delta like non-canonical Notch ligand 1 (Dlk1)	-1.82955	-1.24563
Guca1b	guanylate cyclase activator 1B (Guca1b)	-1.80228	-1.80736
Cbln1	cerebellin 1 precursor (Cbln1)	-1.79002	-4.3118
Gata1	GATA binding protein 1 (Gata1)	-1.78246	-1.12702
Oc90	otoconin 90 (Oc90)	-1.76323	-2.18899
Akr1c1	aldo-keto reductase family 1, member C1 (Akr1c1)	-1.72742	-1.38391
Akap3	A-kinase anchoring protein 3 (Akap3)	-1.71387	-1.48443
Tecta	tectorin alpha (Tecta)	-1.69632	-1.10592
Atp6ap1l	ATPase H+ transporting accessory protein 1 like (Atp6ap1l)	-1.64534	-1.59076