Gene microarray analysis of expression profiles in liver ischemia and reperfusion

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Abstract. Liver ischemia and reperfusion (I/R) injury is of primary concern in cases of liver disease worldwide and is associated with hemorrhagic shock, resection and transplantation. Numerous studies have previously been conducted to investigate the underlying mechanisms of liver I/R injury, however these have not yet been fully elucidated. To determine the difference between ischemia and reperfusion in signaling pathways and the relative pathological mechanisms, the present study downloaded microarray data GSE10657 from the Gene Expression Omnibus database. A total of two data groups from 1-year-old mice were selected for further analysis: i) A total of 90 min ischemia; ii) 90 min ischemia followed by 1 h of reperfusion, n=3 for each group. The Limma package was first used to identify the differentially expressed genes (DEGs). DEGs were subsequently uploaded to the Database for Annotation Visualization and Integrated Discovery online tool for Functional enrichment analysis. A protein-protein interaction (PPI) network was then constructed via STRING version 10.0 and analyzed using Cytoscape software. A total of 114 DEGs were identified, including 21 down and 93 upregulated genes. These DEGs were primarily enriched in malaria and influenza A, in addition to the tumor necrosis factor and mitogen activated protein kinase signaling pathways. Hub genes identified in the PPI network were C-X-C motif chemokine ligand (CXCL) 1, C-C motif chemokine ligand (CCL) 2, interleukin 6, Jun proto-oncogene, activator protein (AP)-1 transcription factor subunit, FOS proto-oncogene, AP-1 transcription factor subunit and dual specificity phosphatase 1. CXCL1 and CCL2 may exhibit important roles in liver I/R injury, with involvement in the immune and inflammatory responses and the chemokine-mediated signaling pathway, particularly at the reperfusion stage. However, further experiments to elucidate the specific roles of these mediators are required in the future.

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Abbreviations: I/R, ischemia and reperfusion; DEGs, differentially expressed genes; DAVID, Database for Annotation Visualization and Integrated Discovery; PPI, Protein-protein interaction network; GEO, network Gene Expression Omnibus database; GO, Gene Ontology; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes

Key words: gene microarray, liver, ischemia, reperfusion, C-X-C motif chemokine ligand 1, C-C motif chemokine ligand 2

Introduction

Liver ischemia/reperfusion (I/R) injury is caused by blood deprivation and subsequent reperfusion. It caused the release of biological mediators contributing to liver dysfunction eventually (1). Although Liver IR injury is a main complication of hemorrhagic shock, resection and transplantation, its mechanisms haven't been described adequately (2). The pathophysiology of liver I/R injury may include ATP depletion, caused by decrease in oxidative phosphorylation, ROS (reactive oxygen species) creation, cytokines and chemokines production by kupffer cells, neutrophil accumulation, nitric oxide, apoptosis and necrosis (3). For example, liver I/R can induce Kupffer cell activation releasing TNF α. The increasing serum TNF α levels resulted in not only liver injury but also remote organ insult (4). Effects on hepatic secretory function and microsomal drug metabolizing systems varied in duration of ischemia or reperfusion. These may be related to lipid peroxidation rise (5). A lot of research suggested that liver I/R injury was age-dependent, which may be associated with neutrophil recruitment and function or NF-kB activation (6,7). The age-related mechanism of NF-κB activation in liver I/R injury could be related to recruitment of phosphorylated and ubiquitinylated NF-κB-inhibitoryprotein, IκBα, to the proteasome. This biological process can be stopped by expression decline of proteasome subunit, non-ATPase 4 (PSMD4) (8). Many methods and drugs had been applied to ameliorate liver I/R

injury (9-11). Blood supply restoration was a primary step to treat ischemia damage in clinical work. But reperfusion itself may exacerbate organ injury induced by ischemia alone. Many therapeutic strategies should be considered when applied to reduce tissue injury (12). Nowadays, pathways, pivotal genes or cellular functions about liver ischemia and reperfusion, have not been demonstrated clearly. In order to explore more theoretical information about I/R injury precaution and treatment, we tried to compare different molecular mechanisms between liver ischemia followed by reperfusion and ischemia alone.

Materials and methods

Microarray data. Gene expression profile dataset GSE10657 was obtained from the Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo/), including 30 liver tissue samples (8). The annotation platform was GPL1261 [Mouse430_2] Affymetrix Mouse Genome 430 2.0 Array. A total of 30 liver tissue samples were collected for analysis of whole mouse genome microarrays. We selected the data of two groups (ischemia of 90 min and 90 min of ischemia followed by 1 h of reperfusion) from 1-year-old mice. Each group included 3 mice.

Data processing. The expression data were processed using the R package limma in Bioconductor (http://www.bioconductor.org/), including background correction, quantile normalization, log2 transformed and final probe summarization (13,14). We compared the gene expression of two groups of one-year old mice (ischemia of 90 min and 90 min of ischemia followed by 1 h of reperfusion). The criterion for differentially expressed genes (DEGs) are adjusted P-value < 0.05 and |log2fold-change (FC)|≥1.

Function annotation and KEGG pathway analysis. To explore the biological function of DEGs, we uploaded the target genes to the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david-d.ncifcrf.gov/). Gene Ontology (GO) annotation (15) associated with biological process (BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (16) pathway enrichment analysis were utilized to analyze the function and potential pathways of these DEGs. The P-value <0.05 and gene counts >2 were criteria of the both.

PPI network construction. We aimed to identify the possible interaction networks of DEGs by using STRING version 10.0, which covers over 2,000 organisms and provides direct (physical) and indirect (functional) associations (17). DEGs were put in STRING database to construct a PPI network. The confidence score for selection was ≥0.4. Cytoscape (http://www.cytoscape.org/) software was used to dispose the PPI network for visualization.

Results

Gene expression analysis. After comparing sample records from 1-year-old mice subjected to different conditions (90 min of ischemia followed by 1 h of reperfusion

Table I. Differentially expressed genes.

Gene	logFC	P-value
Upregulated genes		
Hspa1a	4.189803495	4.40E-06
Il6	3.414377201	0.007214798
Hspa1b	2.835249411	0.002570444
Moxd1	2.792333639	0.005880914
Fos	2.635607507	0.008852822
S100a8	2.470587997	0.001612021
Atf3	2.429293274	0.048994575
S100a9	2.382088918	0.00198724
Thbs1	2.366926809	0.002259069
Btg2	2.024032519	0.036787526
Ctla2a	1.987516668	6.94E-07
Gem	1.957385710	3.11E-05
Egr2	1.945932505	0.004711597
Ch25h	1.945386244	0.00116547
Cyr61	1.884283907	0.00418327
Jun	1.834974240	0.02738022
Dnajb1	1.834159651	0.017880087
Tnfaip6	1.783788316	0.000235748
Fgl2	1.713266954	1.40E-05
Rhob	1.649879783	0.01784261
Junb	1.559927741	0.048521262
Nfkbiz	1.502336562	0.02315746
Apol11b	1.465232692	3.76E-05
Pmaip1	1.457536521	1.95E-06
Snca	1.446393501	0.002008527
G530011O06Rik	1.443344075	0.000226511
Plscr1	1.441607653	0.003825377
Dusp1	1.421057162	0.018558258
Hspb1	1.415609621	0.012319322
Gm7173	1.414339093	1.21E-07
Cxcl1	1.410753622	0.044905834
Hbb-b2	1.351033985	0.000672398
Adamts1	1.329196641	0.003830208
Icam1	1.290995380	0.004126491
5730412P04Rik	1.283988337	9.54E-05
Rasl11a	1.282142362	6.19E-07
Maff	1.272068458	0.037153789
2010002N04Rik	1.271499304	1.21E-07
Rgs1	1.251181552	0.003312274
4833405L11Rik	1.247575574	4.51E-05
Zfp36	1.234524892	0.011557402
Lcn2	1.231778515	0.001751743
Klf6	1.218441804	0.012152268
Chka	1.213280213	0.002802906
Olfr1507	1.211292555	0.000350502
D530037H12Rik	1.197701336	6.69E-06
H2-gs10	1.196542823	0.00144295
Fst	1.193249187	0.000621087
Ell3	1.182028693	3.54E-05
P2ry10	1.171525584	0.017352901
2810404M03Rik	1.167787654	5.67E-05

Table I. Continued.

Gene	logFC	P-value
Ccl2	1.163350947	0.002313274
Hsd17b1	1.158145456	0.000301852
Il33	1.142985472	0.000393507
C76533	1.142247522	4.89E-05
Ppbp	1.137612231	0.011227964
Id3	1.132539137	0.03950213
Ier3	1.130341285	0.014790191
1700016K19Rik	1.128388132	0.000105373
D9Ertd596e	1.117817849	1.34E-05
1200016E24Rik	1.106239555	0.040908811
Sele	1.106222576	0.002874116
Fam19a1	1.097266221	4.82E-06
Slfn4	1.091663043	2.98E-05
Snhg3	1.090971444	0.002765684
4833419O12Rik	1.087463982	1.21E-07
Defa21	1.081747821	0.000252684
Gm10309	1.081623512	3.19E-05
Spin2	1.081365088	6.39E-07
3300002A11Rik	1.078493847	8.17E-06
Pf4	1.078344289	1.21E-07
4930469G21Rik	1.074420496	0.000104134
9530006C21Rik	1.067140075	0.003784627
Procr	1.060112014	3.41E-05
Cebpd	1.056892489	0.009736321
Olfr315	1.054905005	1.28E-06
Vpreb1	1.049741265	6.09E-07
Fabp5	1.043946536	0.046394908
Hbegf	1.041917009	0.002254356
Akr1b7	1.038117758	0.029888552
1700010N08Rik	1.032603816	4.55E-05
D9Wsu90e	1.031933317	0.013273235
S100a6	1.025678192	1.46E-05
Arid5a	1.023214775	5.14E-05
Srgap1	1.020638687	1.71E-07
Dusp5	1.020438070	1.17E-05
Gm14085	1.018206352	0.000563399
H3f3b	1.010059710	0.003098606
Cytip	1.004873236	0.025437274
B830004H01Rik	1.004683417	0.000757856
Glipr1	1.003361817	0.010390201
Apol7b	1.002402297	9.71E-05
Cpne9	1.000076745	1.21E-07
Downregulated genes		
Cyp4a14	-1.491686323	0.046208079
Igsf6	-1.357451711	1.21E-07
Cacna1s	-1.339250877	0.000204248
Gucy2c	-1.310550662	1.88E-07
Emr4	-1.269668108	0.000244481
C030010L15Rik	-1.235720965	3.00E-05
1500015A07Rik	-1.137485728	2.58E-05
AW125324	-1.097415017	7.03E-05
BC023202	-1.096162784	1.21E-07
Gm11818	-1.083128913	1.21E-07
2810404F17Rik	-1.081564746	1.21E-07
BC151093	-1.076581311	0.005019689
1700011B04Rik	-1.044376574	0.000123774
Otx2os1	-1.042838602	0.001185282
Ttc26	-1.039636118	8.52E-07
4933437I04Rik	-1.036693263	1.21E-07
4921513H07Rik	-1.032871132	3.95E-06
2010003K10Rik	-1.031683970	1.21E-07
Gm9748	-1.026995861	1.92E-06
Adam18	-1.024258697	1.21E-07
9430082L08Rik	-1.006312751	1.21E-07

FC, fold-change.

or ischemia of 90 min) (n=3 each group), 114 DEGs were selected to further analysis with the standard of|log2fold change (FC)|≥1 and adjusted P-values <0.05. (Table I and Fig. 1) Among the DEGs, 21 genes were downregulated, while another 93 were upregulated. Cyp4a14, Igsf6 and Cacna1 s were most notably changed of the 21 downregulated genes. Hspa1a, Il6, Hspa1b, Moxd1, Fos, S100a8, Atf3, S100a9, Thbs1 and Btg2 were the top ten increased of the 93 DEGs.

GO analysis and KEGG pathway. According to function annotation, the most significant biological processes included immune response (GO:0006955, P=1.37E-05), leukocyte migration involved in inflammatory response (GO:0002523, P=1.48E-05), inflammatory response (GO:0006954, P=5.96E-05), skeletal muscle cell differentiation (GO:0035914, P=1.08E-04), chemotaxis (GO:0006935, P=1.82E-04), response to lipopolysaccharide (GO:0032496, P=3.58E-04), positive regulation of transcription from RNA polymerase II promoter (GO:0045944, P=4.11E-04), and positive regulation of apoptotic process (GO:0043065, P=4.96E-04) (Table II and Fig. 2).

As for highly enriched pathways, TNF signaling pathway (P=1.57E-06), Malaria (P=5.41E-06), Influenza A (P=3.28E-05), and MAPK signaling pathway (P=3.72E-04) were detected (Table III).

Interaction network construction. All 114 DEGs were put in the String database. A PPI network included 94 nodes and 145 edges was constructed. We analyzed the network by Cytoscape. (Fig. 3) To get more useful information, PPI sub-networks were generated. Nodes with edges more than 6 were CCL2, JUN, CYR61, DUSP1, KLF6, BTG2, ZFP36, IL6, CXCL1, JUNB, NFKBIZ, MAFF, FOS, EGR2 and ATF3 (Fig. 4). Genes with interaction combined-score ≥0.9 were selected to form a PPI sub-network (Fig. 5). Hub proteins were FOS, CCL2, CXCL1, JUN, IL6 and DUSP1, all of which were upregulated.

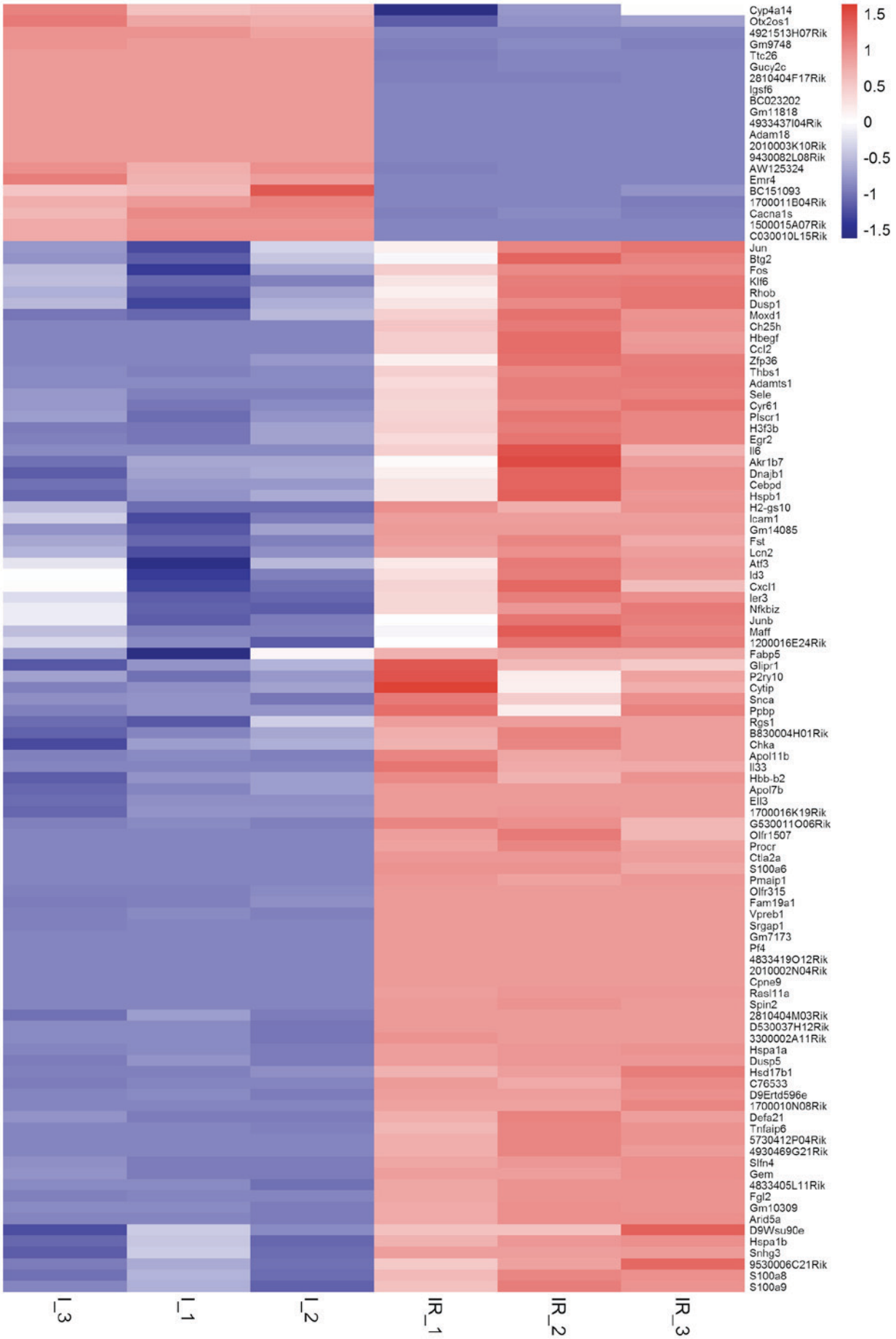

Figure 1. Heat map of DEGs. I_ (1-3): 90 min of Ischemia; IR_(1-3): 90 min of ischemia followed by 1 h of reperfusion. Colors from blue to red mean increasing expression of DEGs between two groups. DEGs, differentially expressed genes.

Table II. GO biological process for DEGs (top 10).

GO ID	GO Term	Count	P-value
GO:0006955	Immune response	9	1.37E-05
GO:0002523	Leukocyte migration involved in inflammatory response	4	1.48E-05
GO:0006954	Inflammatory response	9	5.96E-05
GO:0035914	Skeletal muscle celldifferentiation	5	1.08E-04
GO:0006935	Chemotaxis	6	1.82E-04
GO:0032496	Response to lipopolysaccharide	6	3.58E-04
GO:0045944	Positive regulation oftranscription from RNApolymerase II promoter	14	4.11E-04
GO:0043065	Positive regulation of apoptoticprocess	8	4.96E-04
GO:0006366	Transcription from RNApolymerase II promoter	7	8.04E-04
GO:0070098	Chemokine-mediatedsignaling pathway	4	0.001213338

Count, the number of DEGs involved in GO terms. DEGs, differentially expressed genes; GO, Gene Ontology

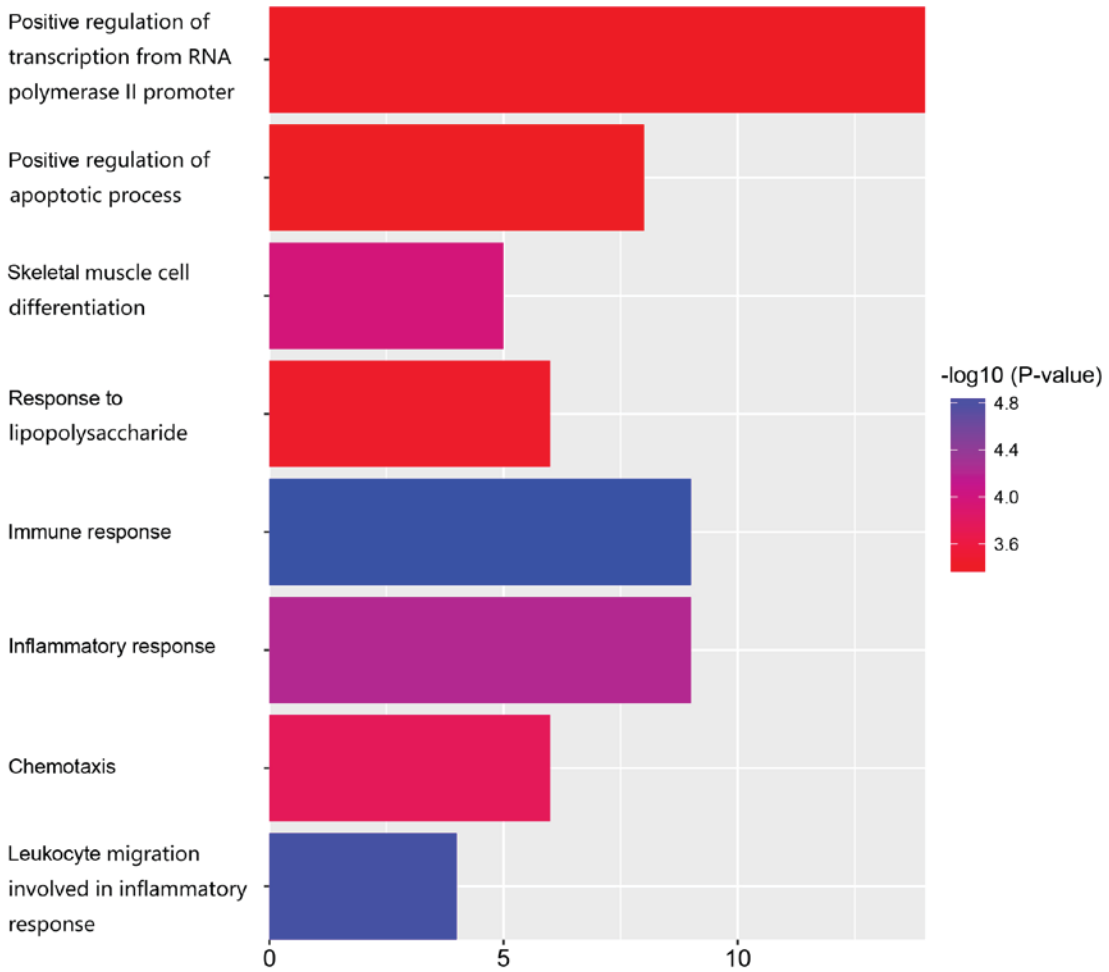

Figure 2. GO biological process for DEGs. P-value are shown in different colors, from red to blue, meaning decreasing P-value. The number of DEGs involved in GO terms are shown in *X*-axle. DEGs, differentially expressed genes; GO, Gene Ontology.

Discussion

In the current study, 114 DEGs were recognized in the liver tissue from two groups of 1-year-old mice. The expression was significantly different between 90 min of ischemia and 90 min of ischemia followed by 1 h of reperfusion. Based on the pathway enrichment analysis, most DEGs enriched in immune response, leukocyte migration involved in inflammatory response, and inflammatory response, including genes like CXCL1, PLSCR1, IL6, CCL2, PROCR, PPBP, VPREB2,

Table III. KEGG pathway analysis for DEGs (top 10).

KEGG ID	KEGG Term	Count	P-value
mmu04668	TNF signaling pathway	8	1.57E-06
mmu05144	Malaria	6	5.41E-06
mmu05164	Influenza A	8	3.28E-05
mmu04010	MAPK signaling pathway	8	3.72E-04
mmu05166	HTLV-I infection	8	6.71E-04
mmu05143	African trypanosomiasis	4	8.72E-04
mmu05323	Rheumatoid arthritis	5	8.93E-04
mmu04915	Estrogen signaling pathway	5	0.001899238
mmu05134	Legionellosis	4	0.003588248
mmu05169	Epstein-Barr virus infection	6	0.005803772

Count, the number of DEGs involved in KEGG terms. KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

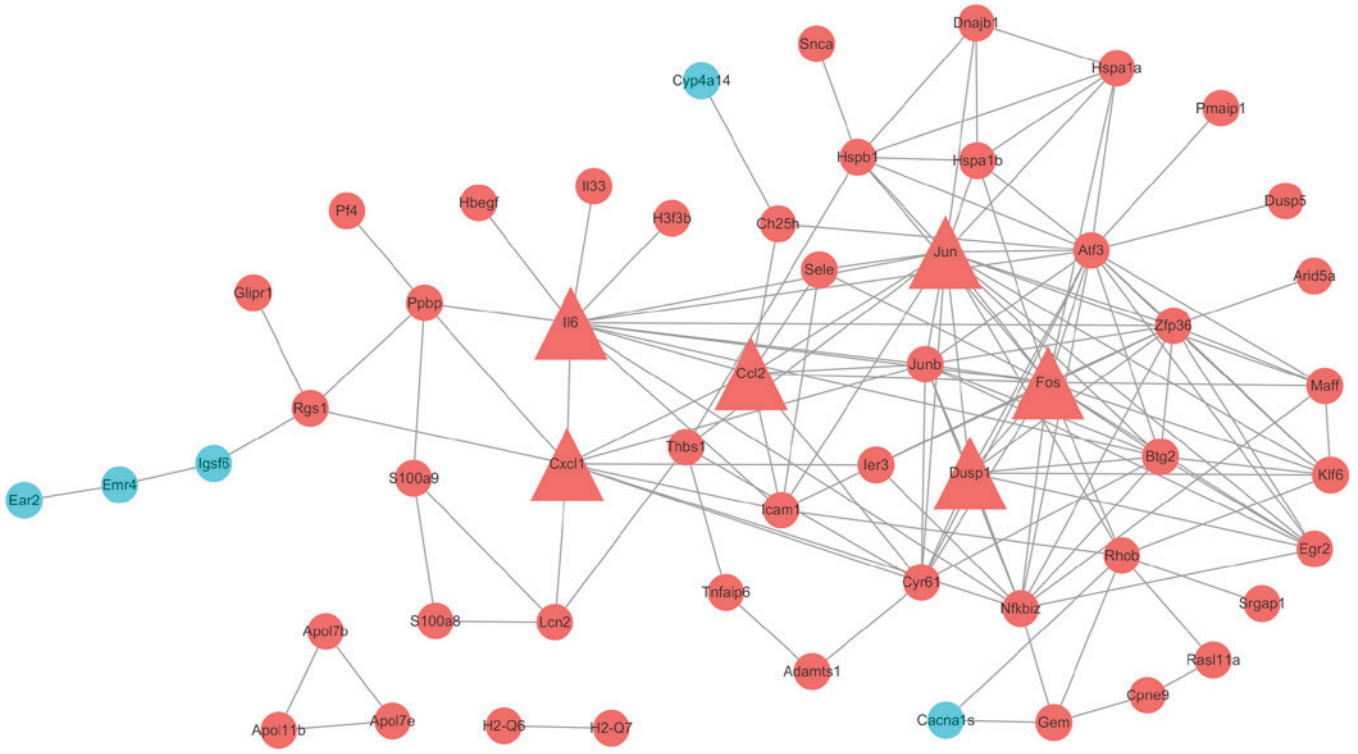

Figure 3. Target genes interaction network in liver ischemia and reperfusion. Hub genes are labeled by triangles. Upregulated and downregulated expression are shown in red and blue severally.

VPREB1, PF4, S100A8, S100A9, NFKBIZ, THBS1, and SELE. TNF signaling pathway and MAPK signaling pathway were recognized with highest count and low P-value. In PPI network, CXCL1, CCL2, IL6, JUN, FOS and DUSP1 were hub proteins.

In our results, the expression of CXCL1 and IL6 increased rapidly in 90 min of ischemia followed by 1 h of reperfusion, suggesting that reperfusion could induce severer damage or more organs dysfunction. CXCL1, also known as GRO-α, could be a therapeutic target with further research. For instance, depletion of CXCL1 can lessen angiogenesis activity and reduce tumor growth. AS a member of the CXC chemokine family, it involved in recruitment of leukocytes and their migration, and many other inflammatory conditions (18). Gomez-Rodriguez *et al* (19) discovered that the expression of CXCL1 can be regulated by MMP-10. The latter was necessary for tissue repair by inhibiting CXCL1. *In vivo*, pre-emptive hypoxia-regulated Haem oxygenase-1 (pHRE-HO-1) could reduce the level of IL6 and CXCL. It was helpful for tissue regeneration and thus alleviating critical limb ischemia injury (20). Ahuja *et al* (21) first proved that serum IL6 had an essential role in AKI-mediated lung neutrophil accumulation

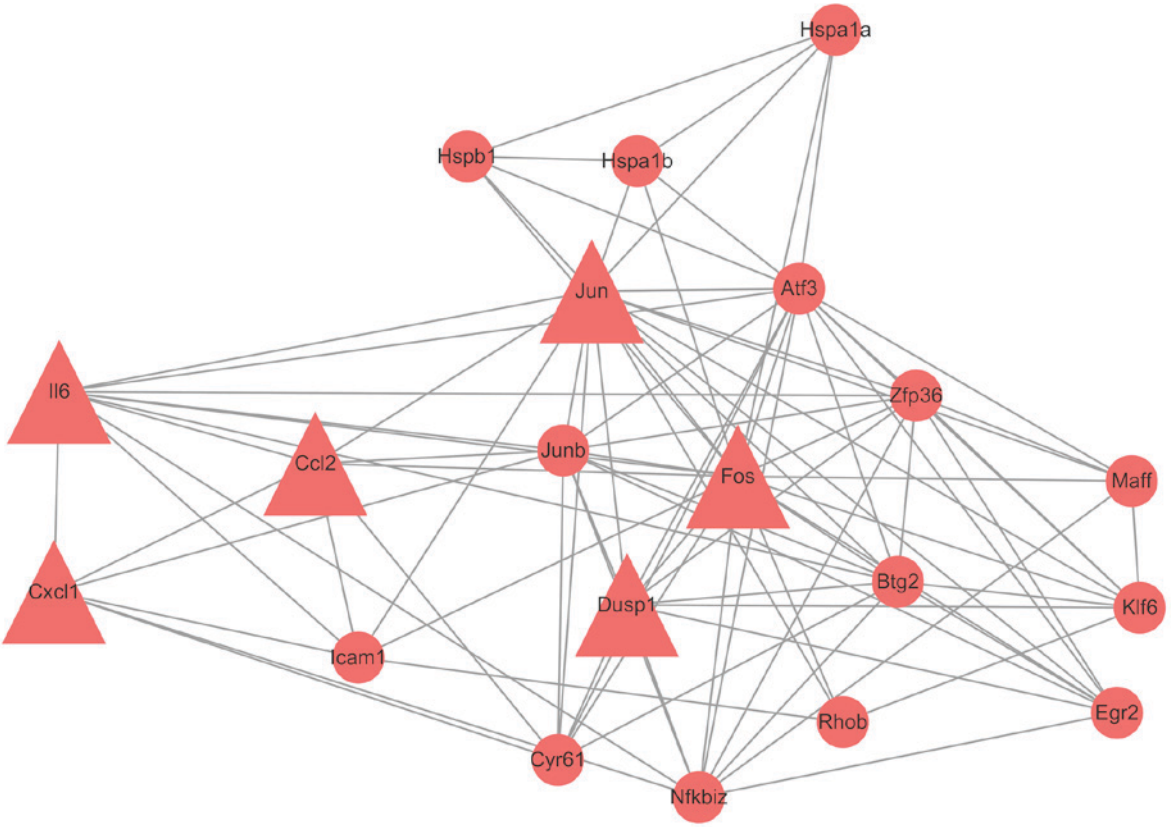

Figure 4. Network of target genes with node degree ≥6.

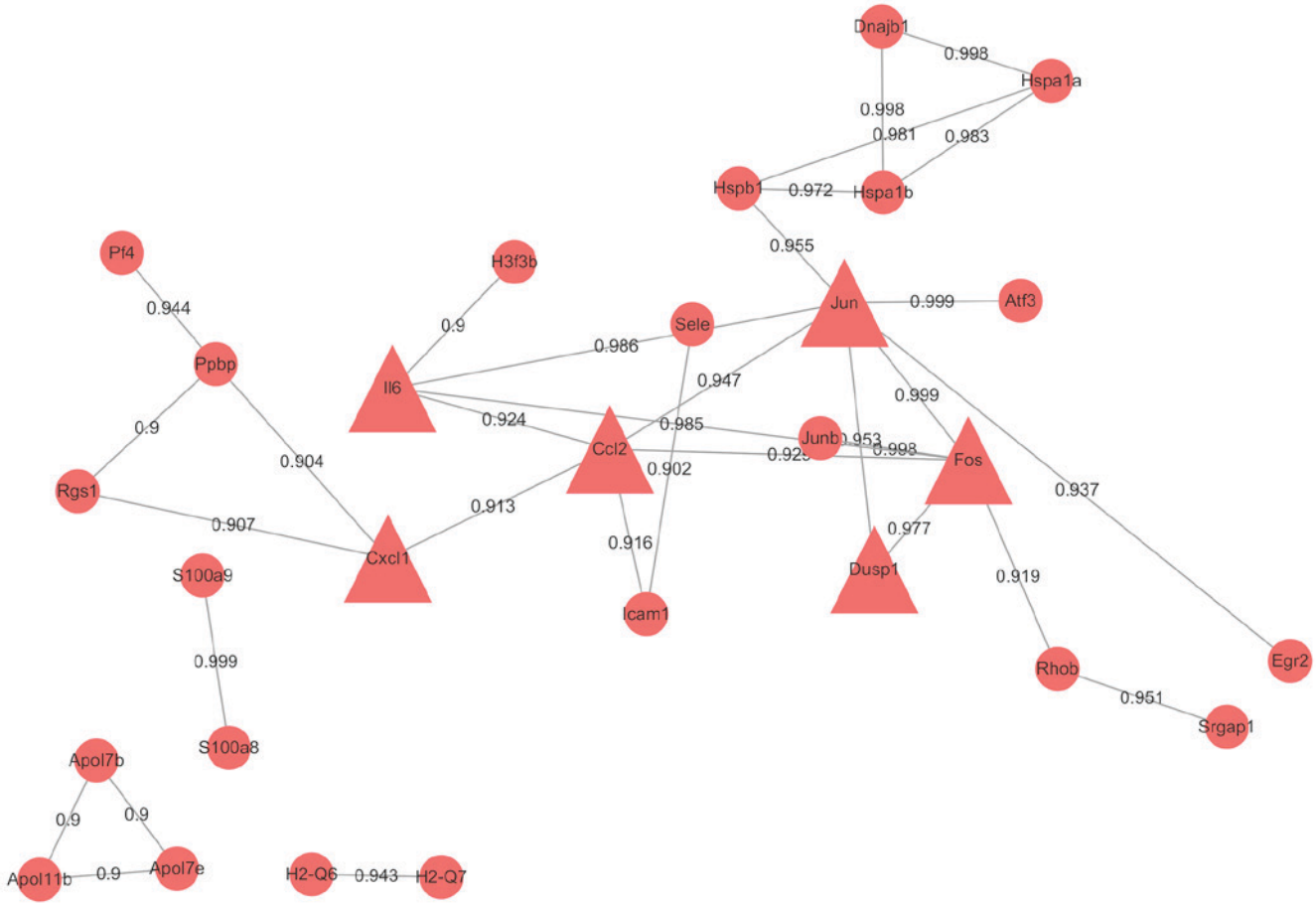

Figure 5. Network of target genes with edge combined score ≥0.9.

and lung injury by stimulating CXCL1 production in lung, which indicated that inhibition of CXCL1 may be a possible therapy of lung injury after AKI. Hepatic stellate cells (HSCs) had a significant effect on I/R- and endotoxin-induced acute hepatocyte injury. When suppressing the function of HSCs, the expression of TNF α, neutrophil chemoattractant CXCL1 and endothelin-A receptor were all decreased (22).

Our study also identified that CCL2 was upregulated in I/R group. It might indicate that reperfusion could aggravate inflammation reaction. Much research had tried to confirm the relationship between CCL2 and inflammation. For example, CCL2-CCR2 signaling could accelerate liver I/R injury, for the reason that CCL2 attracted inflammatory monocytes and CCR2-expressing neutrophil to move into liver from bone marrow (23). Heil *et al* (24) stated that CCL2, was related to the accumulation of macrophages in growing collateral vessels. In mouse femoral artery excision model, CCL2 and CCR2, played an important role in post-ischemic regenerative processes of skeletal muscle (25). CCL2/CCR2 dominated post-ischemic vessel growth (26). Zhang *et al* (27) found that in retinal vascular inflammation, the production of CCL2 required NAD (P) H oxidase activity.

The other three key genes in this study are JUN, FOS and DUSP1. Expression of FOS and DUSP1 were substantially elevated in stroke patients (28).

We analyzed the gene microarray data from a new point, the damage of reperfusion per se, while Huber *et al* (8) studied liver I/R injury emphasizing on the impact of age. There were some limitations of our study. Firstly, for the lack of preconditioning data, we can't continue to mine biological function under the circumstance of precondition or other more relations. Kapoor *et al* (29) proposed that liver ischemic preconditioning activated MAPK signaling pathway, permitting hepatocytes to sustain secondary damage. Oyaizu *et al* (30) suggested that in rat pulmonary ischemia-reperfusion models, Src PTK activation was the major reason for reperfusion-induced lung injury but not gene expression alteration. Secondly, GSE10657 only consisted of reperfusion data of one time point. We couldn't compare gene expression changes between different time points of reperfusion.

In conclusion, our study provides supplementary evidence for the hypothesis that Reperfusion itself creates injury during liver I/R. We identified 114 DEGs between Reperfusion following Ischemia and Ischemia alone. CXCL1, CCL2, IL6, JUN, FOS and DUSP1 were key genes in I/R injury. These genes may be the potential therapeutic target. However, more experimental researches are needed to verify.

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References

1. Li Y, Yang Y, Feng Y, Yan J, Fan C, Jiang S and Qu Y: A review of melatonin in hepatic ischemia/reperfusion injury and clinical liver disease. Ann Med 46: 503-511, 2014.
2. Zhai Y, Petrowsky H, Hong JC, Busuttil RW and Kupiec-Weglinski JW: Ischaemia-reperfusion injury in liver transplantation-from bench to bedside. Nat Rev Gastroenterol Hepatol 10: 79-89, 2013.
3. Papadopoulos D, Siempis T, Theodorakou E and Tsoulfas G: Hepatic ischemia and reperfusion injury and trauma: Current concepts. Arch Trauma Res 2: 63-70, 2013.
4. Tanaka Y, Maher JM, Chen C and Klaassen CD: Hepatic ischemia-reperfusion induces renal heme oxygenase-1 via NF-E2-related factor 2 in rats and mice. Mol Pharmacol 71: 817-825, 2007.
5. Lee SM, Park MJ, Cho TS and Clemens MG: Hepatic injury and lipid peroxidation during ischemia and reperfusion. Shock 13: 279-284, 2000.
6. Calkins CM, Bensard DD, Moore EE, McIntyre RC, Silliman CC, Biffl W, Harken AH, Partrick DA and Offner PJ: The injured child is resistant to multiple organ failure: A different inflammatory response? J Trauma 53: 1058-1063, 2002.
7. Okaya T, Blanchard J, Schuster R, Kuboki S, Husted T, Caldwell CC, Zingarelli B, Wong H, Solomkin JS and Lentsch AB: Age-dependent responses to hepatic ischemia/reperfusion injury. Shock 24: 421-427, 2005.
8. Huber N, Sakai N, Eismann T, Shin T, Kuboki S, Blanchard J, Schuster R, Edwards MJ, Wong HR and Lentsch AB: Age-related decrease in proteasome expression contributes to defective nuclear factor-kappaB activation during hepatic ischemia/reperfusion. Hepatology 49: 1718-1728, 2009.
9. Jaeschke H and Woolbright BL: Current strategies to minimize hepatic ischemia-reperfusion injury by targeting reactive oxygen species. Transplant Rev (Orlando) 26: 103-114, 2012.
10. Abu-Amara M, Yang SY, Seifalian A, Davidson B and Fuller B: The nitric oxide pathway-evidence and mechanisms for protection against liver ischaemia reperfusion injury. Liver Int 32: 531-543, 2012.
11. Zaouali MA, Boncompagni E, Reiter RJ, Bejaoui M, Freitas I, Pantazi E, Folch-Puy E, Abdennebi HB, Garcia-Gil FA and Roselló-Catafau J: AMPK involvement in endoplasmic reticulum stress and autophagy modulation after fatty liver graft preservation: A role for melatonin and trimetazidine cocktail. J Pineal Res 55: 65-78, 2013.
12. Eltzschig HK and Collard CD: Vascular ischaemia and reperfusion injury. Br Med Bull 70: 71-86, 2004.
13. Cai J, Perkumas KM, Qin X, Hauser MA, Stamer WD and Liu Y: Expression profiling of human schlemm's canal endothelial cells from eyes with and without glaucoma. Invest Ophthalmol Vis Sci 56: 6747-6753, 2015.
14. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK: limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids Res 43: e47, 2015.
15. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, *et al*: Gene ontology: Tool for the unification of biology. The gene ontology consortium. Nat Genet 25: 25-29, 2000.
16. Kanehisa M, Goto S, Sato Y, Furumichi M and Tanabe M: KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Res 40 (Database issue): D109-D114, 2012.
17. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, *et al*: STRING v10: Protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 43 (Database issue): D447-D452, 2015.
18. Bechara C, Chai H, Lin PH, Yao Q and Chen C: Growth related oncogene-alpha (GRO-alpha): Roles in atherosclerosis, angiogenesis and other inflammatory conditions. Med Sci Monit 13: RA87-RA90, 2007.
19. Gomez-Rodriguez V, Orbe J, Martinez-Aguilar E, Rodriguez JA, Fernandez-Alonso L, Serneels J, Bobadilla M, Perez-Ruiz A, Collantes M, Mazzone M, *et al*: Functional MMP-10 is required for efficient tissue repair after experimental hind limb ischemia. FASEB J 29: 960-972, 2015.
20. Jazwa A, Stepniewski J, Zamykal M, Jagodzinska J, Meloni M, Emanueli C, Jozkowicz A and Dulak J: Pre-emptive hypoxia-regulated HO-1 gene therapy improves post-ischaemic limb perfusion and tissue regeneration in mice. Cardiovasc Res 97: 115-124, 2013.
21. Ahuja N, Andres-Hernando A, Altmann C, Bhargava R, Bacalja J, Webb RG, He Z, Edelstein CL and Faubel S: Circulating IL-6 mediates lung injury via CXCL1 production after acute kidney injury in mice. Am J Physiol Renal Physiol 303: F864-F872, 2012.

22. Stewart RK, Dangi A, Huang C, Murase N, Kimura S, Stolz DB, Wilson GC, Lentsch AB and Gandhi CR: A novel mouse model of depletion of stellate cells clarifies their role in ischemia/reperfusion- and endotoxin-induced acute liver injury. J Hepatol 60: 298-305, 2014.
23. Zhang J, Xu P, Song P, Wang H, Zhang Y, Hu Q, Wang G, Zhang S, Yu Q, Billiar TR, *et al*: CCL2-CCR2 signaling promotes hepatic ischemia/reperfusion injury. J Surg Res 202: 352-362, 2016.
24. Heil M, Ziegelhoeffer T, Wagner S, Fernández B, Helisch A, Martin S, Tribulova S, Kuziel WA, Bachmann G and Schaper W: Collateral artery growth (arteriogenesis) after experimental arterial occlusion is impaired in mice lacking CC-chemokine receptor-2. Circ Res 94: 671-677, 2004.
25. Contreras-Shannon V, Ochoa O, Reyes-Reyna SM, Sun D, Michalek JE, Kuziel WA, McManus LM and Shireman PK: Fat accumulation with altered inflammation and regeneration in skeletal muscle of CCR2-/-mice following ischemic injury. Am J Physiol Cell Physiol 292: C953-C967, 2007.
26. Cochain C, Rodero MP, Vilar J, Récalde A, Richart AL, Loinard C, Zouggari Y, Guérin C, Duriez M, Combadière B, *et al*: Regulation of monocyte subset systemic levels by distinct chemokine receptors controls post-ischaemic neovascularization. Cardiovasc Res 88: 186-195, 2010.
27. Zhang W, Rojas M, Lilly B, Tsai NT, Lemtalsi T, Liou GI, Caldwell RW and Caldwell RB: NAD(P)H oxidase-dependent regulation of CCL2 production during retinal inflammation. Invest Ophthalmol Vis Sci 50: 3033-3040, 2009.
28. Adamski MG, Li Y, Wagner E, Yu H, Seales-Bailey C, Soper SA, Murphy M and Baird AE: Expression profile based gene clusters for ischemic stroke detection. Genomics 104: 163-169, 2014.
29. Kapoor S, Berishvili E, Bandi S and Gupta S: Ischemic preconditioning affects long-term cell fate through DNA damage-related molecular signaling and altered proliferation. Am J Pathol 184: 2779-2790, 2014.
30. Oyaizu T, Fung SY, Shiozaki A, Guan Z, Zhang Q, dos Santos CC, Han B, Mura M, Keshavjee S and Liu M: Src tyrosine kinase inhibition prevents pulmonary ischemia-reperfusion-induced acute lung injury. Intensive Care Med 38: 894-905, 2012.