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Bioinformatics Analysis Reveals Hub Genes That May Reduce Inflammation and Complications After Cardiopulmonary Bypass

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ABSTRACT

Cardiopulmonary bypass (CPB), though indispensable in many cardiac surgery procedures, has several undesirable consequences. The aim of this study was to identify potential genes that may reduce the inflammatory response and complications after CPB. The GSE132176 dataset was selected from the Gene Expression Omnibus (GEO) database and included 10 patients with tetralogy of Fallot and 10 patients with an atrial septal defect who underwent CPB surgery. TSV files were downloaded after GEO2R processing. Protein-protein interaction analysis of common differentially expressed genes (DEGs) was performed using the Search Tool for the Retrieval of Interacting Genes. Gene modules and hub genes were visualized in the protein-protein interaction network using Cytoscape. Enrichment analysis was performed for all important DEGs, modular genes, and hub genes. A total of 72 DEGs were screened, including two functional and one hub gene module. FOS modular genes were primarily enriched in NGF-stimulated transcription, spinal cord injury, and PID AP1 pathway. The ATF3 modular gene was mainly enriched in cytomegalovirus infection and transcriptional misregulation in cancer. Hub gene modules were primarily enriched in the PID AP1 pathway, positive regulation of pri-miRNA transcription by RNA polymerase II, and the PID ATF2 pathway. FOS, JUN, ATF3, and EGR1 were the four most important hub genes; the top three hub genes were involved in the formation of AP-1 and enriched in the AP-1 pathway. Finally, we measured the expression levels of these four genes in patients undergoing CPB via qRT-PCR, and the results were consistent with those obtained in bioinformatic analysis. FOS, JUN, ATF3, and EGR1 and the AP-1 pathway may play key roles in inflammation and complications caused by CPB.

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INTRODUCTION

The development and application of cardiopulmonary bypass (CPB) is regarded as one of the most important medical advancements in the second half of the 20th century. In 1953, John Gibbon, Jr. was the first to perform open-heart surgery with CPB in clinical practice. The CPB procedure has undergone significant changes in its nearly 70-year history [Hessel 2014]. Although new technologies, such as endovascular interventions and off-pump coronary artery bypass grafting, are emerging, CPB continues to be essential in many cardiac surgery operations [Paparella 2002]. Advances in extracorporeal circulation technology, surgery, and anesthesia have markedly reduced the morbidity and mortality of heart surgery in children with congenital heart disease [McRobb 2014]. However, owing to the contact of blood components with the non-physiological surface of the CPB machine, ischemia-reperfusion (IR) injury, endotoxemia, hypothermia, blood transfusion, and surgical trauma, CPB can cause systemic inflammatory response syndrome (SIRS) [McRobb 2014; Squiccimarro 2019]. In fact, nearly one-third of children with a pediatric congenital heart disease develop SIRS after CPB [Boehne 2017]. In addition, a study by Squiccimarro et al. [Squiccimarro 2019] found that 142/502 patients (28.3%) met the SIRS criteria following their CPB procedure. This inflammatory syndrome also may lead to postoperative complications, including dysfunction of the heart, lungs, kidneys, liver, and other organs. In severe cases, it can even lead to multiple organ failure. The occurrence of complications, such as neurological dysfunction and bleeding disorders, also threatens the lives of patients [Paparella 2002].

Inflammation is the body's protective response to damaged fluids and cells. Early inflammation in CPB is caused by blood components in contact with the CPB pathway, whereas late inflammation is driven by IR and endotoxemia. Inflammation occurs owing to the activation of complement pathways, cytokines, coagulation-fibrinolysis, endothelial cells, neutrophils, macrophages, and monocytes, among other cells. The role of specific cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-8, as well as complement and cell activation, in inflammation has been extensively studied [Durandy 2014]. In this study, we used bioinformatics to analyze the datasets retrieved from the Gene Expression Omnibus (GEO) database and conducted a series of expression profiling to precisely identify potential Hub genes. These genes could be promising molecular targets to effectively treat

postoperative inflammation and prevent other commonly-occurring complications in patients undergoing CPB surgery.

MATERIALS AND METHODS

Dataset information: The GEO database is maintained by the National Center for Biotechnology Information. It contains multiple omics raw data of various diseases for users to download and study. The search term we used was ("cardiopulmonary bypass" [MeSH Terms] OR "Cardiopulmonary bypass" [All Fields]) AND "Homo sapiens" [porgn]. We chose a dataset containing samples of patients before CPB and samples of patients after CPB: GSE132176. The GSE132176 dataset was generated on the GPL13158 platform ([HT_HG-U133_Plus_PM] Affymetrix HT HG-U133+ PM Array Plate). GSE132176 samples include 10 patients with atrial septal defect (ASD) and 10 patients with tetralogy of Fallot (TOF).

Data processing and screening of DEGs: We analyzed the preoperative and postoperative data for each patient and used the GEO database tool GEO2R to compare the analysis results of ASD patients and TOF patients in GSE132176. We used the R language software (https://www.r-project.org/) to screen and map DEGs.

Ordinary DEG enrichment analysis: We used the DAVID website (https://david.ncifcrf.gov/tools.jsp) to perform gene ontology (GO) analysis of DEGs and the clusterProfiler package in the R software to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis on DEGs. KEGG analysis parameters were set to pvalueCutoff = 0.05 and qvalueCutoff = 0.05. Enrichment analysis is a common method to identify molecular function (MF), cellular component (CC), and biological process (BP) characteristics of genome or transcriptome data. KEGG mainly includes system information, genomic information, and chemical information, which can be used to annotate the genome and integrate related effects of known protein products.

PPI network analysis: We used an online search tool (STRING; https://string-db.org/) to search for interacting genes for PPI network analysis. This tool can be used to evaluate the correlation between two or more protein products. In addition, the MCC method in the CytoHubba plug-in in Cytoscape (v3.7.2) (https://cytoscape.org/) was used to detect the top 10 important genes in the PPI network. Then, the MCODE plug-in was used to detect the relevant modules of the differential genes, and the parameters were set as degree cutoff = 2, maximum depth = 100, k-core = 2, and node score cutoff = 0.2.

Enrichment analysis of hub genes: The Metascape database (https://metascape.org/) was used for enrichment analysis of the genes in the module. This database integrates multiple authoritative data resources, such as GO, KEGG, UniProt, and DrugBank, which can better complete the path enrichment and annotation of biological processes. Then, we used the GeneCards database (https://www.genecards.org/) to perform additional analysis on the main hub genes.

qRT-PCR: Six patients who underwent the cardiopulmonary bypass (CPB) procedure between October 21 and 30, 2021, were randomly selected from the Department of Cardiac Surgery at our hospital. The gene expression levels of the four hub genes from the right atrium were estimated using qRT-PCR. Samples for gene expression evaluation were collected from each patient 5 min before aortic cross-clamping and 15 min after the end of aortic cross-clamping. The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. Written informed consent was obtained from all patients stating their voluntary participation.

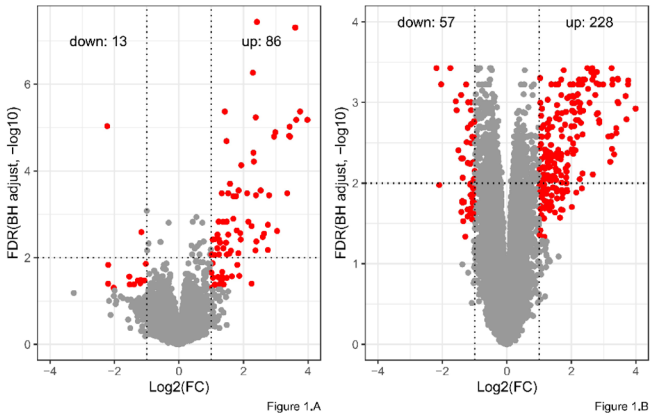

Figure 1. Screening results of differentially expressed genes. Gene expression volcano map of the dataset. Red and black data points represent genes with and without significant differences in expression ($P < 0.05$), respectively. (A) Differentially expressed genes in atrial septal defect (ASD). (B) Differentially expressed genes in tetralogy of Fallot (TOF).

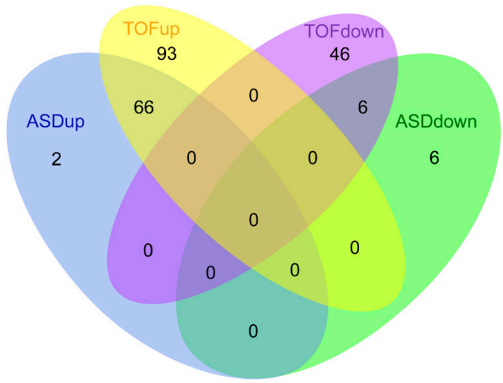

Figure 2. Venn diagrams of commonly differentially expressed genes in atrial septal defect (ASD) and tetralogy of Fallot (TOF).

RESULTS

Screening of DEGs in myocardial tissues of patients before and after CPB: By comparing the data of ASD patients and TOF patients, we found 86 upregulated and 13 downregulated genes in ASD patients, and 228 upregulated and 57 downregulated genes in TOF patients. Afterwards, the Venn diagram software was used to screen all upregulated

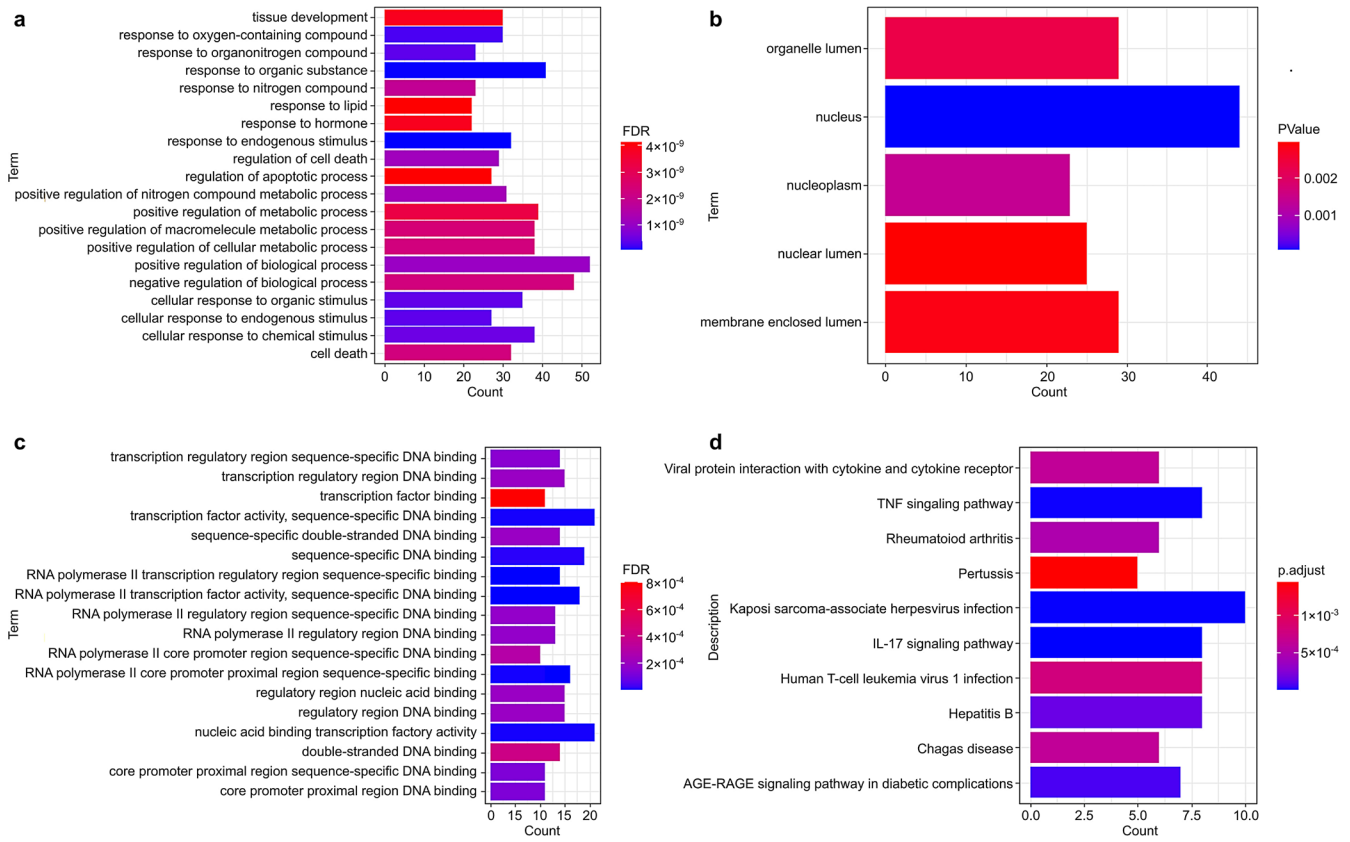

Figure 3. Gene ontology (GO) and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway enrichment analyses for common differentially expressed genes. (A) Biological process analyses. (B) Cellular component analyses. (C) Molecular function analyses. (D) KEGG pathway enrichment analyses.

and downregulated DEGs to determine the common DEGs. After filtering out some useless ribosomal genes, we screened a total of 72 DEGs, of which 66 were upregulated and six were downregulated. Then, we used the ggplot2 package of the R Studio to draw the volcano map and Venn diagram of the DEGs. (Figure 1) (Figure 2)

GO of common DEGs and enrichment analysis of KEGG pathway: The results of GO enrichment analysis are shown in Figure 3. (Figure 3) In BP analysis, DEGs primarily were involved in the cell's response to cytokine stimulation, the response to organic compounds, and the regulation of cells. In CC analysis, DEGs were enriched in the nucleus and organelles. In MF analysis, DEGs were mainly enriched in the activation of transcription factors and specific DNA binding. The main KEGG analysis results showed that DEGs were mainly enriched in the TNF signaling pathway, IL-17 signaling pathway, fluid shear stress and atherosclerosis, human T-cell leukemia virus type 1 infection, AGE-RAGE signaling pathway in diabetic complications, mineral absorption, and Kaposi's sarcoma-associated herpesvirus infection pathway.

PPI network and co-expression analysis: We imported 72 DEGs into the STRING online search tool to obtain the PPI relationship (score > 0.4), exported DEGs in TSV format, and imported the resulting file into the Cytoscape software. The obtained PPI network had 52 nodes and 345 edges (Figure 4A). (Figure 4) The plug-in CytoHubba was used to screen the key genes (hub genes), followed by MCC algorithm analysis, and the top 10 genes were selected as the hub genes. Two gene-related modules were obtained through the MCODE plug-in (Figure 4B).

Further enrichment analysis of central genes: The modular genes were further analyzed with the Metascape database, which integrated GO function and KEGG pathway analyses. The results showed that the first modular gene was primarily enriched in NGF-stimulated transcription, spinal cord injury, the PID AP1 pathway, PID REG pathway, corticotropin-releasing hormone signaling pathway, and ST differentiation pathway in PC12 cells. KEGG pathway enrichment analysis further revealed that the first module gene was significantly enriched in the TNF signaling pathway, hepatitis B, and parathyroid hormone synthesis, secretion, and action (Figure 5A and 5B). (Figure 5)

The second module gene primarily was enriched in the PID AP1 pathway, PERK-mediated unfolded protein response, IL-4 and IL-13 signaling, IL-10 signaling, hypertrophy model, and cell cycle arrest. The KEGG pathway enrichment analysis showed that the second module gene was enriched in human cytomegalovirus infection and transcriptional misregulation in cancer (Figure 6A and 6B). (Figure 6) GO analysis was performed on the top four hub genes.

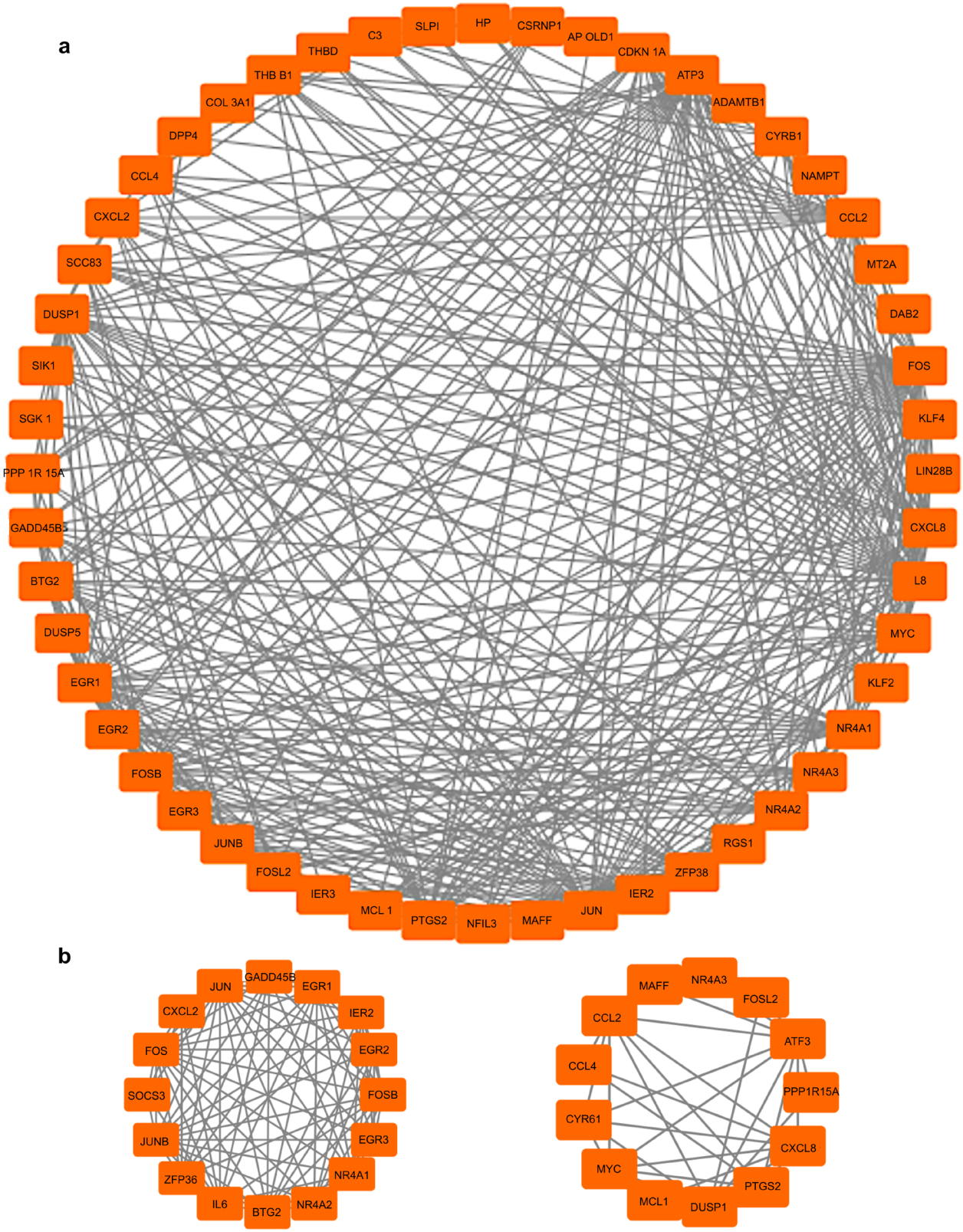

Figure 4. Protein-protein interaction (PPI) analysis. (A) PPI networks of the common differentially expressed genes. (B) Two gene modules.

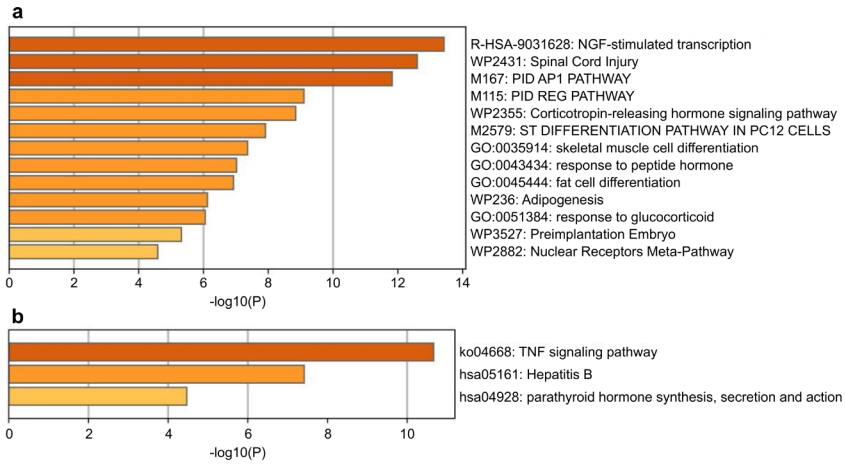

Figure 5. Enrichment analysis and KEGG analysis of the first set of modular genes. (A) Enrichment analysis of the first set of modular genes. (B) KEGG analysis of the first set of modular genes.

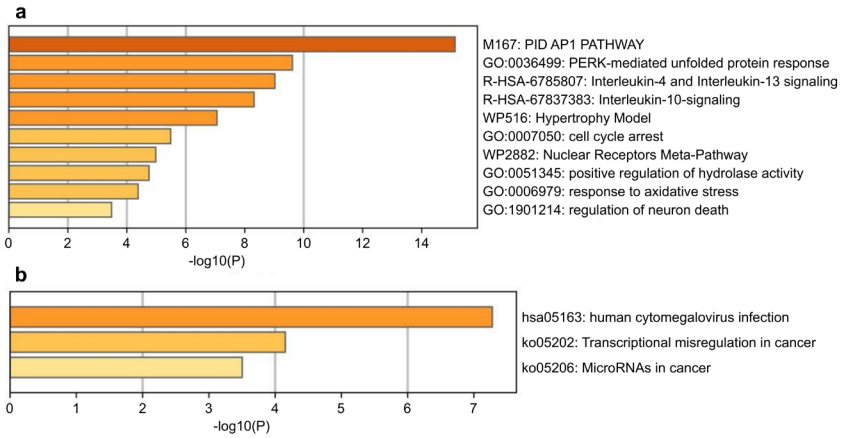

Figure 6. Enrichment analysis and KEGG analysis of the second group of modular genes. (A) Enrichment analysis of the second set of modular genes. (B) KEGG analysis of the second set of modular genes.

In the BP analysis, FOS was mainly enriched in the regulation of transcription and response of cells to extracellular stimuli. Meanwhile, JUN was primarily associated with the regulation of transcription, angiogenesis, and cell proliferation. Activating transcription factor 3 (ATF3) was primarily enriched in the regulation of transcription and the regulation of cell proliferation. Early growth response 1 (EGR1) was mainly enriched in the regulation of transcription and the ischemia-hypoxia response.

Moreover, the CC analysis showed that FOS and JUN were primarily enriched in the nucleus and cytoplasm, as well as in the transcription factor activator protein-1 (AP-1) complex. ATF3 and EGR1 were enriched in the nucleus. In the MF analysis, the four hub genes were all enriched in transcription regulatory region sequence-specific DNA binding, DNA binding, protein binding, and transcription factor binding.

The main enrichment results of the TOP4 hub gene are shown in Figure 7. (Figure 7) The first four hub genes were primarily enriched in the PID AP1 pathway, positive regulation of pri-miRNA transcription by RNA polymerase II, the PID ATF2 pathway, skeletal muscle cell differentiation, and regulation of transcription from RNA polymerase II promoter in response to stress. Among them, the AP1 pathway had the lowest *P*-value (Figure 8). (Figure 8)

Evaluation of expression levels of the hub genes via qRT-PCR: Total RNA was extracted from the six pairs of tissue samples collected from respective patients using the TRIzol Invitrogen reagent (Cat# 15596-026) following the manufacturer's instructions. The concentrations of the extracted RNA were quantified using an ultra-micro UV-Vis spectrophotometer (Denovix, USA). Corresponding cDNAs were synthesized from RNA samples via reverse transcription using the HiFiScriptT Rapid Genomic cDNA First Strand Removal Synthesis Kit (cwbiotech, Cat# CW2582M). The reaction mixture was prepared following the manufacturer's protocol provided with the SuperReal PreMix Plus (SYBR Green) PCR kit (monadbiotech, Cat# MQ00401S). Next, qRT-PCR was performed using a fluorescence qPCR

Genes	Biological process	Cellular component	Molecular function
FOS	GO:0001661 conditioned taste aversion	GO:0000790 nuclear chromatin	GO:0000976 transcription regulatory region sequence-specific DNA binding
	GO:0006306 DNA methylation	GO:0005634 nucleus	GO:0000978 RNA polymerase II proximal promoter sequence-specific DNA binding
	GO:0006355 regulation of transcription, DNA-templated	GO:0005654 nucleoplasm	GO:0000979 RNA polymerase II core promoter sequence-specific DNA binding
	GO:0006357 regulation of transcription by RNA polymerase II	GO:0005667 transcription factor complex	GO:0000981 DNA-binding transcription factor activity, RNA polymerase II-specific
	GO:0006366 transcription by RNA polymerase II	GO:0005737 cytoplasm	GO:0001102 RNA polymerase II activating transcription factor binding
	GO:0006954 inflammatory response	GO:0005783 endoplasmic reticulum	GO:0001228 DNA-binding transcription activator activity, RNA polymerase II-specific
JUN	GO:0000122 negative regulation of transcription by RNA polymerase II	GO:0000228 nuclear chromosome	GO:0000976 transcription regulatory region sequence-specific DNA binding
	GO:0001525 angiogenesis	GO:0000790 nuclear chromatin	GO:0000978 RNA polymerase II proximal promoter sequence-specific DNA binding
	GO:0001774 microglial cell activation	GO:0005634 nucleus	GO:0000981 DNA-binding transcription factor activity, RNA polymerase II-specific
	GO:0001836 release of cytochrome c from mitochondria	GO:0005654 nucleoplasm	GO:0001102 RNA polymerase II activating transcription factor binding
	GO:0001889 liver development	GO:0005667 transcription factor complex	GO:0001228 DNA-binding transcription activator activity, RNA polymerase II-specific
	GO:0001938 positive regulation of endothelial cell proliferation	GO:0005719 nuclear euchromatin	GO:0003677 DNA binding
	GO:0003151 outflow tract morphogenesis	GO:0005829 cytosol	GO:0003682 chromatin binding
	GO:0006355 regulation of transcription, DNA-templated	GO:0035976 transcription factor AP-1 complex	GO:0003700 DNA-binding transcription factor activity
ATF3	GO:0000122 negative regulation of transcription by RNA polymerase II	GO:0000790nuclear chromatin	GO:0000976 transcription regulatory region sequence-specific DNA binding
	GO:0006094 gluconeogenesis	GO:0005634nucleus	GO:0000977 RNA polymerase II regulatory region sequence-specific DNA binding
	GO:0006355 regulation of transcription, DNA-templated	GO:0005654nucleoplasm	GO:0000978 RNA polymerase II proximal promoter sequence-specific DNA binding
	GO:0006357 regulation of transcription by RNA polymerase II	GO:0005730nucleolus	GO:0000981 DNA-binding transcription factor activity, RNA polymerase II-specific
	GO:0008284 positive regulation of cell proliferation	GO:1990622CHOP-ATF3 complex	GO:0001227 DNA-binding transcription repressor activity, RNA polymerase II-specific
	GO:0010628 positive regulation of gene expression		GO:0001228 DNA-binding transcription activator activity, RNA polymerase II-specific
EGR1	GO:0000122 negative regulation of transcription by RNA polymerase II	GO:0000790nuclear chromatin	GO:0000976 transcription regulatory region sequence-specific DNA binding
	GO:0001666 response to hypoxia	GO:0005634nucleus	GO:0000977 RNA polymerase II regulatory region sequence-specific DNA binding
	GO:0002931 response to ischemia	GO:0005654nucleoplasm	GO:0000981 DNA-binding transcription factor activity, RNA polymerase II-specific
	GO:0006355 regulation of transcription, DNA-templated	GO:0005737cytoplasm	GO:0001228 DNA-binding transcription activator activity, RNA polymerase II-specific
	GO:0006357 regulation of transcription by RNA polymerase II		GO:0003676 nucleic acid binding
	GO:0006366 transcription by RNA polymerase II		GO:0003677 DNA binding

Figure 7. GO enrichment analysis of the top four hub genes.

instrument (Bio-Rad). The following conditions were set in the thermocycler: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec, 60°C for 30 sec, and 72°C for 30 sec. The internal control used in this experiment was β-actin. The samples for each gene were prepared in triplicate and divided in three wells. Melting curves were analyzed from 60°C to 95°C with a 0.5% increment. Expression levels of the key genes were measured following the 2-ΔΔCt method, according to the following formula:

ΔΔCt = CT (target gene) - CT (internal control), ΔΔCt = ΔΔCt (experimental group) - ΔΔCt (control group).

The sequences of the four key genes are provided in Table 1. (Table 1) Graphs were plotted and statistical analysis were performed using GraphPad Prism 8.0.2 software. Differences with $P < 0.05$ were considered statistically significant. The expression of JUN ($P = 0.0031$), FOS ($P = 0.0009$), ATF3 ($P= 0.0067$), and EGR1 ($P = 0.0007$) was significantly elevated in samples collected after 15 mins of aortic cross-clamping compared to that in samples collected prior to the aortic cross-clamping procedure (Figure 9). (Figure 9) The results of qRT-PCR were consistent with those obtained from bioinformatic analysis.

DISCUSSION

In the present study, a dataset of patients undergoing CPB surgery was selected from the GEO database, and a

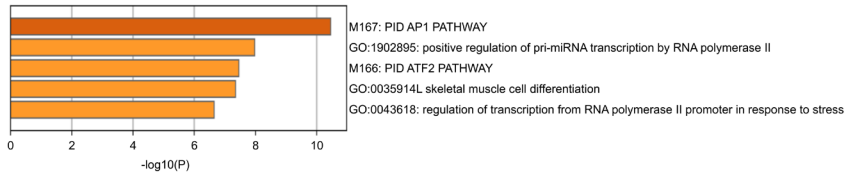

Figure 8. Enrichment analysis of the first four genes.

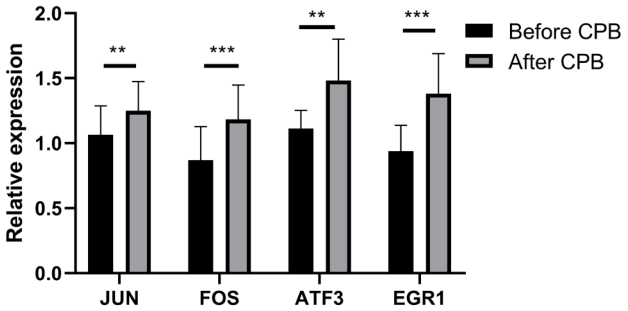

Figure 9. Representation of qRT-PCR results for the four hub genes in a histogram.

total of 72 DEGs were identified, of which 10 hub genes were selected for further analysis. FOS, JUN, ATF3, and EGR1 were the four highest-scoring genes, all of which have also been found to be associated with many other inflammatory diseases and molecules. JUN can mediate intervertebral disk inflammation by regulating the expression of cyclooxygenase 2 (COX2) and PGE2 production [Li 2016]. Moreover, elevated JUN expression in macrophages regulates COX2 and arginase-1 levels, thus, promoting the development of arthritis [Hannemann 2017]. Meanwhile, Uluçkan et al. [Uluckan 2015] found that FOS, JUN, and AP1 are related to inflammatory diseases of the skin and can be used as potential therapeutic targets for these diseases. Cao et al. [Cao 2019] found that ATF3 can prevent colitis by regulating T follicular helper cells in the intestine, and the expression of ATF3 is negatively correlated with the severity of ulcerative colitis. Additionally, overexpression of EGR1 in mature cells can modulate the inflammatory response by reducing cytokine secretion, blocking stimulatory ligands, such as CD86, and increasing CTLA4 and PD-1 ligands to blunt the activation of macrophages [Trizzino 2021]. Collectively, these findings provide insightful information and elucidate the role of hub genes in inflammation. Our study identifies potential therapeutic targets to effectively manage inflammation, treat postoperative complications, and ameliorate the clinical outcomes by increasing the chances of patient survival.

FOS is a proto-oncogene of the immediate early transcription factor family and a part of the AP-1 transcription factor. It is only expressed in a limited number of cell types and is involved in cell proliferation, differentiation, apoptosis, and transformation. Moreover, it can regulate a variety of cell functions when induced by some stimuli [Jariel-Encontre 1997; Kang 2011]. Zhu et al. [Zhu 2008] found that the IR of rat myocardium can stimulate the expression of FOS. Kingma [Kingma 1999] found that the longer the ischemic time, the higher the expression level of FOS. The same results were obtained even after a brief coronary occlusion and ischemic preconditioning [Kingma 1999]. Other studies found that the inhibition of inflammatory response by mesenchymal stromal cell-derived exosomes may be related to the inhibition of FOS [Wei 2019]. These studies are consistent with our findings. Our results showed that FOS was highly expressed in patients after CPB. Moreover, this gene had the highest score. FOS may be a potential therapeutic target to reduce systemic inflammation after CPB.

Mammalian JUN proteins include c-JUN, JunB, and JunD [Leppa 1999]. JUN is expressed in a variety of cell types, and its transcription is rapid and transient under various stimuli, such as TNF-α, IL-1β, ultraviolet radiation, ionizing radiation, and hydrogen peroxide. Moreover, it is also related to complex cell responses, such as growth, differentiation, and programmed cell death [Wisdom 1999; Aebert 1997; Sawai 2013]. The induction of FOS and JUN expression in animal heart tissues was found to be a response to hypoxia, ischemia, neurohormonal stimulation, or vascular wall stress. FOS and JUN can also act as transcription factors to regulate other genes [Aebert 1997]. The regulation of JUN is complex, involving both the increase of c-JUN protein levels and the phosphorylation of specific serine residues (63 and 73) by c-JUN N-terminal kinase [Wisdom 1999]. Wang et al. [Wang 1999] showed that c-JUN is a pro-apoptotic molecule in vascular endothelial cells, which can lead endothelial cells into prethrombotic and pro-inflammatory states. This can result in angiogenesis, inflammation, thrombosis, and atherosclerosis [Wang 1999]. Ruel et al. [Ruel 2003] found that the expression of FOS and JUN in the right atrial subendometrial cardiomyocytes and endothelial cells was upregulated after cold crystal arrest. The JUN gene was the second-ranked gene in our hub genes. The above evidence also indicated that JUN serves as a potential therapeutic target for alleviating complications after CPB.

ATF3 is a stress-induced transcription factor that belongs to the AP-1 transcription factor family. Transcription factors regulate cell responses to various environmental stress conditions. This factor plays an important role in regulating metabolism, immunity, and tumorigenesis. Under the stimulation of hypoxia, cytokines, endoplasmic reticulum stress, and chemokines, ATF3 forms homodimers or heterodimers with other members of the AP-1 family to inhibit or activate transcription [Ku 2020; Rohini 2018; Tanaka 2011]. The expression level of ATF3 is very low under normal conditions;

Table 1. Sequences of the four hub genes

Gene name	NCBI GeneID	GenBank Accession	Sequence
JUN	3725	NM_002228	Forward: 5'-AACCGCATCGCTGCCTCCAA-3', Reverse: 5'-GCTGTGCCACCTGTTCCCTG-3'
FOS	2353	NM_005252	Forward: 5' -GGGGCAAGGTGGAACAGTTAT-3', Reverse: 5'-CCGCTTGGAGTGTATCAGTCA-3'
ATF3	467	NM_001206486	Forward: 5' -CCTCTGCGCTGGAATCAGTC-3', Reverse: 5'-TTCTTTCTCGTCGCCTCTTTTT-3'
EGR1	1958	NM_001964	Forward: 5' -GGTCAGTGGCCTAGTGAGC-3', Reverse: 5'-GTGCCGCTGAGTAAATGGGA-3'

however, its expression substantially increases when the heart is stressed. In patients with heart failure, the expression of ATF3 is very high [Soraya 2021]. ATF3 expression is also related to the cell's response to damage, including those in the heart, kidney, and brain tissue [Lin 2018]. Several studies have shown that acute hypoxia in the cardiovascular system can lead to increased expression of ATF3 in vascular endothelial cells, thereby preventing apoptosis of rat cardiomyocytes [Rohini 2018]. ATF3 plays a leading role in cardiac hypertrophy, cardiac fibrosis, cardiac dysfunction, and death [Soraya 2021]. Koren et al. [Koren 2015] found that ATF3 is necessary for the growth response of cardiomyocytes to endothelin-1 stimulation, and that it plays a protective role in pressure overload and maladaptive cardiomyocyte hypertrophy. ATF3 was ranked third in our hub gene group. ATF3 may be used as a potential therapeutic target to reduce the damage to cardiomyocytes and reduce the stimulation of inflammation after CPB.

EGR1 gene is an immediate early gene, a member of the zinc finger transcription factor family [Gao 2009; Huang 2009], and an important transcription factor that can recruit multiple downstream target genes to express IL-1β, transforming growth factor β, and intercellular adhesion molecule-1 tissue factor, among others [Zhou 2010; Zhang 2008]. EGR1 also actively regulates inflammation, thrombosis, and apoptosis through direct and indirect mechanisms [Bhindi 2012]. Some studies have found that EGR1 may be the main switch that triggers the pathogenesis of lung IR injury, and inhibiting the expression of EGR1 can also prevent liver IR injury [Zhang 2008]. Yan et al. [Yan 2006] showed that the activation of EGR1 is related to acute and chronic vascular emergencies, such as hypoxia, IR, atherosclerosis, and acute vascular injury. Wu et al. [Wu 2013] found that the expression of EGR1 is upregulated after multiple organ IR injury. Knockout of EGR1 or antisense oligonucleotides to block the effect of EGR1 can prevent or reduce IR damage onset. These results indicated that the upregulation of EGR1 expression is a common feature of IR injury, and EGR-1 may be a potential target for its treatment [Zhang 2008; Wu 2013]. Wang et al. [Wang 2014] showed that EGR1 plays a role in acute myocardial injury and can protect the heart from myocardial injury caused by reperfusion. EGR1 was ranked fourth in our hub gene group. It plays a role in reperfusion injury and myocardial injury and can be used as a potential therapeutic target to reduce complications after CPB.

AP-1 is composed of a variety of dimers, which include a group of structural and functionally related members of the JUN, FOS, ATF, and MAF protein families [Trop-Steinberg 2017; Maulik 1999; Raivich 2006]. AP-1 is involved in the regulation of cell proliferation, apoptosis, differentiation, migration, and transformation, and can also regulate the transcription of specific genes at different levels [Trop-Steinberg 2017]. The activation of AP-1 may mediate the acceleration of cell proliferation and differentiation after IR [Yeh 2000]. Maulik et al. [Maulik 1999] found that an increase in the expression of AP-1 components is related to cell apoptosis. Ischemic myocardial reperfusion leads to the activation of AP-1 and, at the same time, leads to cell apoptosis [Maulik 1999]. Alfonso-Jaume et al. [Alfonso-Jaume 2006] found that the content of AP-1 components JunB and FosB increases in the myocardial cell nucleus in tissues under IR injury. This suggests that the activation of AP-1 transcription factor is related to the extracellular signal-regulated kinase 1/2. Phosphorylation is related to the nuclear accumulation of FOS and JUN proto-oncogenes in hypoxic cardiomyocytes [Alfonso-Jaume 2006]. In our study, JUN, FOS, and ATF were the top three hub genes, which are all involved in the formation of AP-1 and are enriched in the AP-1 pathway. AP-1 is likely involved in the IR injury and systemic inflammation caused by CPB.

A limitation of our research is that it only included one GEO dataset. Additionally, we were unable to analyze the severity of inflammation in each patient, thus preventing us from determining whether the degree of gene enrichment in each patient correlates with the degree of inflammation. We estimated the expression levels of the four hub genes from the patients undergoing CPB via qRT-PCR, and the results validated our findings from the previous analysis. However, we hope to verify whether the higher degree of inflammation correlates with the observed gene enrichment in future vitro studies.

CONCLUSION

We used a GEO dataset and applied bioinformatics analysis to determine that JUN, FOS, ATF3, and EGR1 are the potential top central genes involved in inflammation and various complications after CPB. Moreover, the AP-1 pathway may play an important role in the occurrence of IR injury and systemic inflammation caused by CPB. Although these observations still need to be verified in the future, the results of this study may help to further reduce inflammation and complications after CPB in the clinic.

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REFERENCES

Aebert H, Cornelius T, Ehr T, et al. 1997. Expression of immediate early genes after cardioplegic arrest and reperfusion. Ann Thorac Surg. 63: 1669-75.

Alfonso-Jaume MA, Bergman MR, Mahimkar R, et al. 2006. Cardiac ischemia-reperfusion injury induces matrix metalloproteinase-2 expression through the AP-1 components FosB and JunB. American Journal of Physiology-Heart and Circulatory Physiology. 291: H1838-H46.

Bhindi R, Fahmy RG, McMahon AC, Khachigian LM, Lowe HC. 2012. Intracoronary delivery of DNAzymes targeting human EGR-1 reduces infarct size following myocardial ischaemia reperfusion. J Pathol. 227: 157-64.

Boehne M, Sasse M, Karch A, et al. 2017. Systemic inflammatory response syndrome after pediatric congenital heart surgery: Incidence, risk factors, and clinical outcome. J Card Surg. 32: 116-25.

Cao Y, Yang Q, Deng H, et al. 2019. Transcriptional factor ATF3 protects against colitis by regulating follicular helper T cells in Peyer's patches. Proc Natl Acad Sci U S A. 116: 6286-91.

Durandy Y. 2014. Minimizing systemic inflammation during cardiopulmonary bypass in the pediatric population. Artif Organs. 38: 11-8.

Gao FF, Jia QY, Guo FX, et al. 2009. Egr-1, a central and unifying role in cardioprotection from ischemia-reperfusion injury? Cell Physiol Biochem. 24: 519-26.

Hannemann N, Jordan J, Paul S, et al. 2017. The AP-1 Transcription Factor c-Jun Promotes Arthritis by Regulating Cyclooxygenase-2 and Arginase-1 Expression in Macrophages. J Immunol. 198: 3605-14.

Hessel EA, 2nd. 2014. A Brief History of Cardiopulmonary Bypass. Semin Cardiothorac Vasc Anesth. 18: 87-100.

Huang Z, Li H, Guo F, et al. 2009. Egr-1, the potential target of calcium channel blockers in cardioprotection with ischemia/reperfusion injury in rats. Cell Physiol Biochem. 24: 17-24.

Jariel-Encontre I, Salvat C, Steff AM, et al. 1997. Complex mechanisms for c-fos and c-jun degradation. Mol Biol Rep. 24: 51-6.

Kang SM, Lim S, Won SJ, et al. 2011. c-Fos regulates hepatitis C virus propagation. FEBS Lett. 585: 3236-44.

Kingma JG, Jr. 1999. Cardiac adaptation to ischemia-reperfusion injury. Ann N Y Acad Sci. 874: 83-99.

Koren L, Alishekevitz D, Elhanani O, et al. 2015. ATF3-dependent cross-talk between cardiomyocytes and macrophages promotes cardiac maladaptive remodeling. International Journal of Cardiology. 198: 232-40.

Ku HC, Cheng CF. 2020. Master Regulator Activating Transcription Factor 3 (ATF3) in Metabolic Homeostasis and Cancer. Front Endocrinol (Lausanne). 11: 556.

Leppa S, Bohmann D. 1999. Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. Oncogene. 18: 6158-62.

Li JK, Nie L, Zhao YP, et al. 2016. IL-17 mediates inflammatory reactions via p38/c-Fos and JNK/c-Jun activation in an AP-1-dependent manner in human nucleus pulposus cells. J Transl Med. 14: 77.

Lin H, Cheng CF. 2018. Activating transcription factor 3, an early cellular adaptive responder in ischemia/reperfusion-induced injury. Ci Ji Yi Xue Za Zhi. 30: 61-65.

Maulik N, Goswami S, Galang N, Das DK. 1999. Differential regulation of Bcl-2, AP-1 and NF-kappaB on cardiomyocyte apoptosis during myocardial ischemic stress adaptation. FEBS Lett. 443: 331-6.

McRobb CM, Mejak BL, Ellis WC, et al. 2014. Recent Advances in Pediatric Cardiopulmonary Bypass. Semin Cardiothorac Vasc Anesth. 18: 153-60.

Paparella D, Yau TM, Young E. 2002. Cardiopulmonary bypass induced inflammation: pathophysiology and treatment. An update. Eur J Cardiothorac Surg. 21: 232-44.

Raivich G, Behrens A. 2006. Role of the AP-1 transcription factor c-Jun in developing, adult and injured brain. Prog Neurobiol. 78: 347-63.

Rohini M, Haritha Menon A, Selvamurugan N. 2018. Role of activating transcription factor 3 and its interacting proteins under physiological and pathological conditions. Int J Biol Macromol. 120: 310-17.

Ruel M, Bianchi C, Khan TA, et al. 2003. Gene expression profile after cardiopulmonary bypass and cardioplegic arrest. J Thorac Cardiovasc Surg. 126: 1521-30.

Sawai M, Ishikawa Y, Ota A, Sakurai H. 2013. The proto-oncogene JUN is a target of the heat shock transcription factor HSF1. Febs Journal. 280: 6672-80.

Soraya AS, Tali H, Rona S, et al. 2021. ATF3 expression in cardiomyocytes and myofibroblasts following transverse aortic constriction displays distinct phenotypes. Int J Cardiol Heart Vasc. 32: 100706.

Squiccimarro E, Labriola C, Malvindi PG, et al. 2019. Prevalence and Clinical Impact of Systemic Inflammatory Reaction After Cardiac Surgery. J Cardiothorac Vasc Anesth. 33: 1682-90.

Tanaka Y, Nakamura A, Morioka MS, et al. 2011. Systems analysis of ATF3 in stress response and cancer reveals opposing effects on pro-apoptotic genes in p53 pathway. PLoS One. 6: e26848.

Trizzino M, Zucco A, Deliard S, et al. 2021. EGR1 is a gatekeeper of inflammatory enhancers in human macrophages. Science Advances. 7.

Trop-Steinberg S, Azar Y. 2017. AP-1 Expression and its Clinical Relevance in Immune Disorders and Cancer. Am J Med Sci. 353: 474-83.

Uluckan O, Guinea-Viniegra J, Jimenez M, Wagner EF. 2015. Signalling in inflammatory skin disease by AP-1 (Fos/Jun). Clin Exp Rheumatol. 33: S44-9.

Wang N, Verna L, Hardy S, et al. 1999. c-Jun triggers apoptosis in human vascular endothelial cells. Circ Res. 85: 387-93.

Wang NP, Pang XF, Zhang LH, et al. 2014. Attenuation

of inflammatory response and reduction in infarct size by postconditioning are associated with downregulation of early growth response 1 during reperfusion in rat heart. Shock. 41: 346-54.

Wei Z, Qiao S, Zhao J, et al. 2019. miRNA-181a overexpression in mesenchymal stem cell-derived exosomes influenced inflammatory response after myocardial ischemia-reperfusion injury. Life Sci. 232: 116632.

Wisdom R, Johnson RS, Moore C. 1999. C-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. EMBO J. 18: 188-97.

Wu H, Lei S, Yuan J, et al. 2013. Ischemic postconditioning downregulates Egr-1 expression and attenuates postischemic pulmonary inflammatory cytokine release and tissue injury in rats. J Surg Res. 181: 204-12.

Yan SF, Harja E, Andrassy M, Fujita T, Schmidt AM. 2006. Protein kinase C beta/early growth response-1 pathway: a key player in ischemia, atherosclerosis, and restenosis. J Am Coll Cardiol. 48: A47-55.

Yeh KY, Yeh M, Glass J, Granger DN. 2000. Rapid activation of NF-kappaB and AP-1 and target gene expression in postischemic rat intestine. Gastroenterology. 118: 525-34.

Zhang Y, Shi G, Zheng J, et al. 2008. The protective effect of Egr-1 antisense oligodeoxyribonucleotide on myocardial injury induced by ischemia-reperfusion and hypoxia-reoxygenation. Cell Physiol Biochem. 22: 645-52.

Zhou Y, Shi G, Zheng J, et al. 2010. The protective effects of Egr-1 antisense oligodeoxyribonucleotide on cardiac microvascular endothelial injury induced by hypoxia-reoxygenation. Biochem Cell Biol. 88: 687-95.

Zhu XY, Yan XH, Chen SJ. 2008. H(2)S protects myocardium against ischemia/reperfusion injury and its effect on c-Fos protein expression in rats. Sheng Li Xue Bao. 60: 221-7.