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Research Paper

Exploring the underlying molecular mechanisms of acute myocardial infarction after SARS-CoV-2 infection

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ABSTRACT

An increase in acute myocardial infarction (AMI)-related deaths has been reported during the COVID-19 pandemic. Despite evidence suggesting the association between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and AMI, the underlying mechanisms remain unclear. Here, we integrated mRNA and microRNA expression profiles related to SARS-CoV-2 infection and AMI from public databases. We then performed transcriptomic analysis using bioinformatics and systems biology approaches to explore the potential molecular mechanisms of SARS-CoV-2 infection affects AMI. First, twenty-one common differentially expressed genes (DEGs) were identified from SARS-CoV-2 infection and AMI patients in endothelial cells datasets and then we performed functional analysis to predict the roles of these DEGs. The functional analysis emphasized that the endothelial cell response to cytokine stimulus due to excessive inflammation was essential in these two diseases. Importantly, the tumor necrosis factor and interleukin-17 signaling pathways appeared to be integral factors in this mechanism. Interestingly, most of these common genes were also upregulated in transcriptomic datasets of SARS-CoV-2-infected cardiomyocytes, suggesting that these genes may be shared in cardiac- and vascular-related injuries. We subsequently built a protein-protein interaction network and extracted hub genes and essential modules from this network. At the transcriptional and post-transcriptional levels, regulatory networks with common DEGs were also constructed, and some key regulator signatures were further identified and validated. In summary, our research revealed that a highly activated inflammatory response in patients with COVID-19 might be a crucial factor for susceptibility to AMI and we identified some candidate genes and regulators that could be used as biomarkers or potential therapeutic targets.

1. Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread rapidly and widely around the world and poses a threat to human health. As of August 2022, COVID-19 has killed over 1 million individuals in the United States [1]. It is estimated that there were 180 excess deaths per 100,000 individuals in the United States in 2020 and 2021 [2]. An

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earlier study showed that 64 of 138 (46.4 %) hospitalized patients had comorbidities, including cardiovascular disease, diabetes, hypertension, and chronic obstructive pulmonary disease [3]. A meta-analysis and a study that analyzed 1590 SARS-CoV-2-infected hospitalized patients observed that cardiovascular disease was the third most common complication [4,5].

Acute myocardial infarction (AMI) represents a major cause of morbidity and mortality worldwide and has attracted considerable attention in patients with COVID-19 [6]. Prior epidemiological studies have linked SARS-CoV-2 infection to acute cardiovascular syndrome, and the excess rise in AMI-related deaths in the United States has been reported during the COVID-19 pandemic [6,7]. A high incidence rate of myocardial infarction, myocarditis, and cardiac arrest has been recorded in patients with COVID-19 [8]. Moreover, AMI may be represented as the first clinical manifestation of COVID-19, and the potential role of genetic susceptibility in COVID-19-related cardiac complications has been reported [9,10]. Based on these findings, we hypothesized that common molecular pathways shared between COVID-19 and AMI might be activated during SARS-CoV-2 infection and result in cardiovascular complications.

SARS-CoV-2 infects host cells by binding to angiotensin-converting enzyme 2 receptors, which are expressed in the lungs, vascular system (endothelial cells, vascular smooth muscle cells, and migratory angiogenic cells, *etc.*), and the heart (cardiofibroblasts, cardiomyocytes, endothelial cells, and epicardial adipose cells, *etc.*) [11]. Therefore, although the primary target of SARS-CoV-2 in patients with COVID-19 is the respiratory tract, the cardiovascular system can be involved. A prior study highlighted that SARS-CoV-2 could infect endothelial cells, leading to diffuse endothelial inflammation [12]. Moreover, substantial evidence indicates that endothelial dysfunction, especially changes in vascular integrity and coagulation ability, may be the pivotal factor in determining clinical outcomes for COVID-19 patients [12,13]. Endothelial cell also plays the frontline role in initiating and modulating the pathogenesis of AMI. From this perspective, analyzing the endothelial cells from COVID-19 and AMI patients is key to understanding the pathogenesis of these two diseases.

The development of microarray and accurate RNA-sequencing technology provides an excellent opportunity to understand pathogenic molecular mechanisms further. In this study, bioinformatics analysis was used to determine the key biological factors and pathways between SARS-CoV-2 infection and AMI, which could contribute to the early detection and treatment of COVID-19-related AMI.

2. Materials and methods

2.1. Overview of the analysis workflow of the study

The analysis workflow of this study is shown in Fig. 1. Briefly, two datasets from the Gene Expression Omnibus (GEO) database were downloaded first, including 1) GSE178331, an RNA-Seq dataset that represents the transcriptomic data of endothelial cells in patients with SARS-CoV-2 infection, and 2) GSE66360, a microarray dataset that describes the transcriptomic data of endothelial cells in patients with AMI. Then the differentially expressed genes (DEGs) were extracted for GSE178331 and GSE66360 datasets, respectively. The common DEGs were defined as the overlapped DEGs between the two datasets. To explore the biological functions of the common DEGs at the pathway level, the pathway enrichment analysis was utilized (using Gene Ontology [GO] and Kyoto Encyclopedia of Genes and Genomes [KEGG] databases). Next, to explore if the common DEGs were also induced in the SARS-CoV-2-infected cardiomyocyte cells, we further downloaded two more transcriptomic datasets (GSE150392 and GSE162736) to validate our results. Finally, to extract the critical genes in the regulation network of common DEGs, a protein-protein interaction (PPI) network was constructed to discover the hub genes and gene modules. Additionally, we further detected and validated the key transcription factors (TFs) and microRNAs (miRNAs) that regulate the expression of these hub genes by analyzing the TF-gene interaction network, the miRNA-

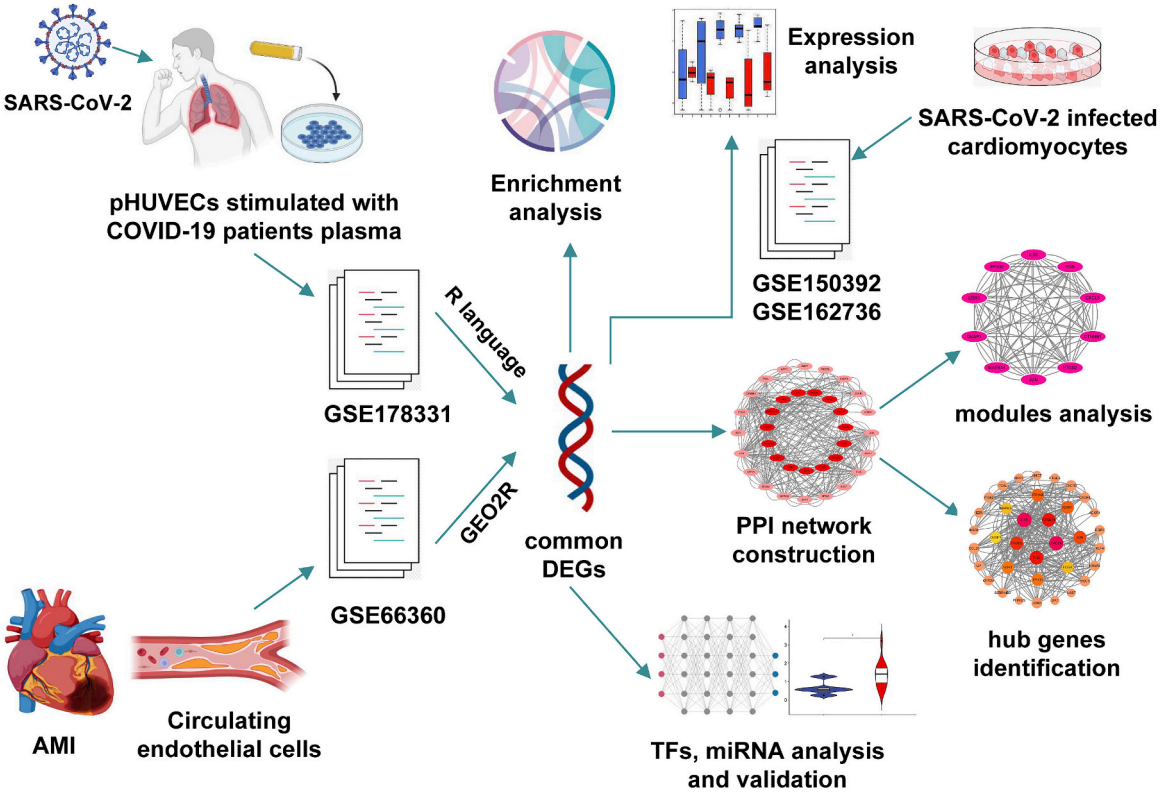

Fig. 1. Flowchart of the bioinformatics analysis.

target gene network, and corresponding datasets.

2.2. GEO datasets in the study

We used the keywords "acute myocardial infarction" or "SARS-CoV-2 or COVID-19" to search publicly available COVID-19 and AMI transcriptomic datasets from the GEO (https://www.ncbi.nlm.nih.gov/geo/). Datasets GSE178331, GSE66360, GSE150392, GSE162736 and GSE178246 were selected. GSE178331 is an expression dataset generated by high-throughput sequencing, including the sequencing of human umbilical vein endothelial cells (HUVECs) stimulated with plasma from patients with COVID-19 [14]. In the AMI dataset (GSE66360) [15], circulating endothelial cells were isolated from patients experiencing AMI and healthy subjects. The GSE150392 was derived from human induced pluripotent stem cell-derived cardiomyocytes infected with SARS-CoV-2 [16]. The GSE162736 dataset was also selected, which included transcriptomic data of SARS-CoV-2-infected cardiomyocytes differentiated from embryonic stem cell [17]. And the GSE178246 dataset was downloaded, which included the whole transcriptome miRNA profile from the plasma of COVID-19 patients. The detailed information on the datasets used in this study is provided in Supplementary Table 1. All data are available online for free. This study did not include any newly generated datasets on humans or animals.

2.3. General statistical analysis, bioinformatics and data visualization

The most statistical analysis and data visualization were performed using R language (version 4.1.3). The multiple comparisons were adjusted by the Benjamini & Hochberg method (BH) using the "p.adjust" function in R. Differences in quantitative genes expression in both datasets were analyzed using Student's *t*-test, Welch *t*-test and Mann-Whitney *U* test, with normality evaluated by Shapiro-Wilk normality test and variance homogeneity tested through Leven's test. In addition, the Kruskal-Wallis test for multiple comparisons of genes expression were also performed. p-Values < 0.05 were considered statistically significant. The R package "ggplot2" was used to perform most of the data visualization in this study.

2.4. Identification of DEGs and common DEGs between COVID-19 and AMI datasets

For the GSE178331, the raw counts were extracted, and the R package DESeq2 [18] was used to identify DEGs from pooled pHUVECs either unstimulated or stimulated with plasma from patients with COVID-19. For the AMI GSE66360 dataset, the microarray data was analyzed using the GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) web tool to identify the DEGs. The conditions for identification of DEGs were: BH-adjusted p-value < 0.05 and absolute logFC ≥ 1.0 (FC: fold change). Common DEGs between the two datasets were acquired using the UpSetR package.

2.5. GO and KEGG pathway analysis

GO analysis is a standard method for large-scale functional enrichment research. Gene functions can be divided into biological process, molecular function, and cellular component. KEGG pathway analysis is used to understand genomes, biological pathways, and other high-level functions from molecular-level information. In this study, the GO annotation and KEGG pathway enrichment analyses were performed using the R package clusterProfiler [19]. Briefly, symbol gene IDs were first converted to Entrez gene IDs and enrichment analysis was performed with clusterProfiler by mapping the gene to the background set. Based on the threshold p-value < 0.05, GO terms and signal pathways with significant enrichment were screened out.

2.6. Analysis of the expression of identified genes in cardiomyocytes infected with SARS-CoV-2

We detected whether the expression of the identified genes was also induced specifically in SARS-CoV-2-infected cardiomyocytes. The expression of the identified common genes in the GSE150392 and GSE162736 datasets, which included data from cardiomyocytes infected with SARS-CoV-2 *in vitro*, was further explored.

2.7. PPI network construction and hub genes identification

Cell viability depends on a complex web of functional associations between biomolecules. A protein interacts with many other proteins inside the cell, and this interaction is important for the function and regulation of the protein. The Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/) was designed to construct the PPI network [20]. To evaluate potential PPI relationships, first, the common DEGs were mapped to the STRING database. Next, we added an additional first shell of interactors (only direct interactors) to the number of DEGs in the network to increase the network size. We set the confidence score to 0.4, considered a medium confidence score. Subsequently, the PPI network was visualized using Cytoscape software (www.cytoscape.org/). CytoHubba, a plugin in Cytoscape, offers 11 algorithms for hub gene identification. We employed its degree algorithm to determine the top five genes based on protein node connectivity. This algorithm effectively highlights genes with the highest number of connections, pinpointing key players in gene interaction networks. To achieve more robust conclusions, we further validated their importance using the remaining ten algorithms. Based on these hub genes, we established a coexpression network and performed functional analysis using GeneMANIA (http://www.genemania.org/). GeneMANIA uses different parameters, including genetic and protein interaction, coexpression, colocalization, pathways, and protein domain similarities, to predict the interaction of the input gene with other genes [21]. Molecular Complex Detection, another plugin in Cytoscape (http://apps.cytoscape.org/apps/mcode), was used to detect the highly interconnected portion of the PPI network with default parameters.

Construction of the transcriptional regulation network and verification of TFs.

The Transcriptional Regulatory Relationships Unraveled by Sentence-based Text-mining(TRRUST, http://www.grnpedia.org/trrust/) database presents the relationships between TFs and genes based on the existing literature [22]. Using the TRRUST database of human transcriptional regulatory interactions, potential key TFs of the common genes were identified to build a TF-gene interaction network. FDR (false discovery rate) < 0.05 was considered significant. Subsequently, we verified the expression levels of these TFs in the GSE178331 and GSE66360 datasets.

2.8. Construction of the miRNA-gene interaction network and validation of miRNAs

TFs and miRNAs coregulate the expression of genes at the transcriptional and post-transcriptional levels, respectively. The roles of miRNAs are being studied in almost every area of biology. We used the NetworkAnalyst (https://www.networkanalyst.ca) platform to construct the miRNA-gene interaction network [23]. The network produced for the miRNA-gene interaction network was obtained from the miRTarBase, which is included in the NetworkAnalyst platform. The Cytoscape software was used to visualize the results. The expression of crucial identified miRNAs was verified in the GSE178246 dataset.

3. Results

3.1. Common dysregulated genes were identified between COVID-19 and AMI

We hypothesized that COVID-19 affects the risk factors of AMI, which aggravate the mortality of AMI patients. So, we first explore if COVID-19 and AMI could dysregulate the same genes in the patients. For the COVID-19 (GSE178331) dataset, 360 DEGs were identified, including 266 upregulated and 94 downregulated genes (Fig. 2A). For the AMI dataset (GSE66360), 657 DEGs were identified, including 463 upregulated and 194 downregulated genes (Fig. 2B). Interestingly, 21 common DEGs were identified between COVID-19 and AMI datasets (Fig. 2C, and Supplementary Table 2). Among the 21 common DEGs, 20 genes were upregulated (*CCL20*, *CH25H*, *CXCL8*, *DUSP1*, *EGR1*, *EGR3*, *EIF1AY*, *FOSB*, *ICAM1*, *KLF4*, *LIF*, *MGP*, *NR4A3*, *PFKFB3*, *PLXDC2*, *PTGS2*, *RPS4Y1*, *SERPINB2*, *SIK1*, and *SULF1*) and one gene was downregulated (*PATJ*, also designated as *INADL*) in both two datasets. This result demonstrated that the COVID-19 and AMI patients share the same dysregulated genes (common DEGs), which indicates that COVID-19 may aggravate the AMI disease *via* those genes.

3.2. Endothelial cell response to inflammatory stimulus in COVID-19 drive the occurrence of AMI

To explore the biological function of the 21 common DEGs between COVID-19 and AMI at a higher level, the pathway enrichment analyses (GO and KEGG database) of the common DEGs were performed. Interestingly, most of the enriched GO terms and pathways from the 21 common DEGs are related to inflammation and immune responses (Fig. 3A-D). For example, in biological process category, the cellular response to chemical stimulus, cellular response to endogenous stimulus, and cellular response to cytokine stimulus were significantly enriched. For cellular component category, the membrane raft, membrane microdomain, and membrane region were enriched, and they play important roles in signal transduction of immune responses [24]. For the molecular function category, the histone acetyltransferase binding, transcription cofactor binding, promoter-specific chromatin binding, chemokine receptor binding and cytokine receptor binding were enriched. Additionally, for the KEGG pathway enrichment analysis, the tumor necrosis factor (TNF) signaling pathway and the interleukin (IL)-17 signaling pathway were enriched, which play a central role in the control of infections [25]. In summary, all the pathway enrichment results strongly suggest that endothelial cell response to inflammatory stimulus mediated by cytokines in COVID-19 may drive the occurrence of AMI.

3.3. SARS-CoV-2 cause the cardiovascular complications in patients

As cardiovascular complications in COVID-19 has been recognized as a predictor of mortality, we also carried out *in silico* analysis of publicly available datasets derived from cardiomyocytes infected with SARS-CoV-2 to decipher the molecular basis in the pathogenesis of cardiac and vascular injuries in COVID-19. Specifically, we want to know if the common dysregulated genes can also be found in the cardiomyocyte infected by SARS-CoV-2. The GSE150392 and GSE162736 datasets were utilized and analyzed. Interestingly, we found that the vast majority of common dysregulated genes (17/21, 81.0 %, including *CH25H*, *CXCL8*, *DUSP1*, *EGR1*, *EGR3*, *FOSB*, *ICAM1*, *KLF4*, *LIF*, *MGP*, *NR4A3*, *PFKFB3*, *PLXDC2*, *PTGS2*, *SERPINB2*, *SIK1*, and *SULF1*) in AMI and COVID-19 patients were also significantly upregulated (SARS-CoV-2-infected compared to mock-infected cells, Supplementary Fig. 1). According to biological function analysis of the 21 common DEGs as previously mentioned, it is obvious that the inflammation caused by SARS-CoV-2 is also the potential cause of cardiovascular complications in patients with COVID-19, which may contribute to myocardial injury.

3.4. Identification of the hub genes in the PPI network

We then want to explore the hub genes in the PPI network constructed using the common DEGs. We first predicted the protein interactions among the DEGs using the STRING tool. Nineteen proteins in *Homo sapiens* were matched with our input genes. After removing the disconnected nodes in the network, a PPI network with 35 nodes and 352 edges was constructed (Fig. 4A). We initially assessed the importance of nodes by their connectivity degree. The top five genes identified as hub genes in the PPI network (IL1B, CXCL8, CTNNB1, FOS, and PTGS2, as shown in Table 1) were then validated using ten additional algorithms. A comprehensive analysis was conducted by extracting the top ten genes from each algorithm. It's noteworthy that these five genes were featured in 10 out of the 11 algorithms utilized, although they were not included in the DMNC algorithm (Supplementary Fig. 2A). Their consistent high ranking across these algorithms confirms their reliability as hub genes (Supplementary Fig. 2B). We then extracted the sub-PPI network containing the hub genes and their directly connected genes (Fig. 4B). Based on GeneMANIA, we analyzed the coexpression network and related functions of these genes. A total of 20 predicted genes were included in this coexpression pattern. These genes showed the complex network with physical interactions of 77.64 %, coexpression of 8.01 %, predicted components of 5.37 %, colocalization of 3.63 %, genetic interactions of 2.87 %, pathway of 1.88 % and shared protein domains of 0.60 % (Fig. 4C). Functional annotation analysis showed that those 5 hub genes were involved in inflammatory processes, including the

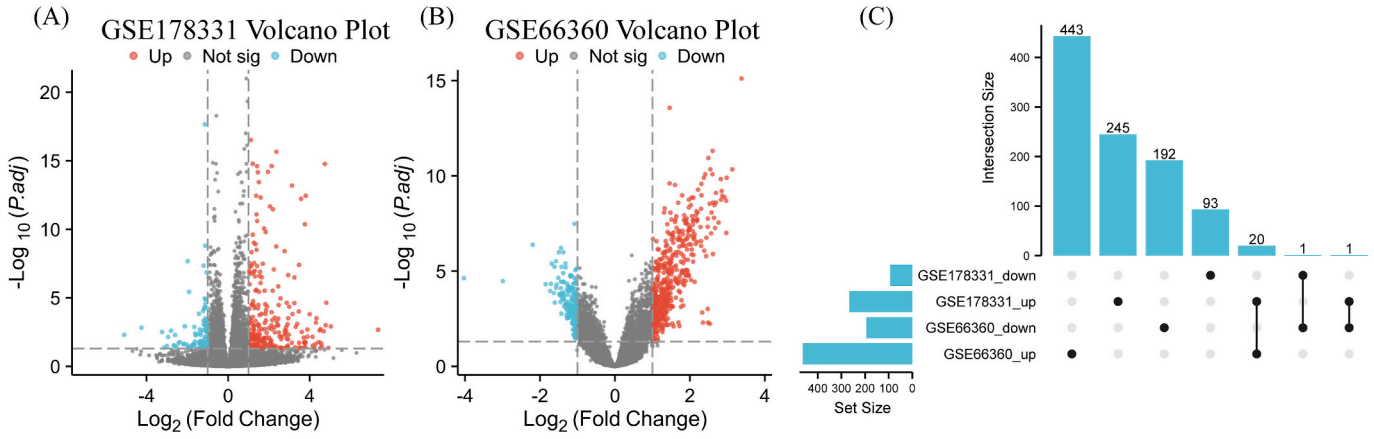

Fig. 2. Differentially expressed genes in the COVID-19 and AMI datasets. (A–B) Volcano plots. (C) An UpSet diagram representing the differentially expressed genes derived from the two datasets.

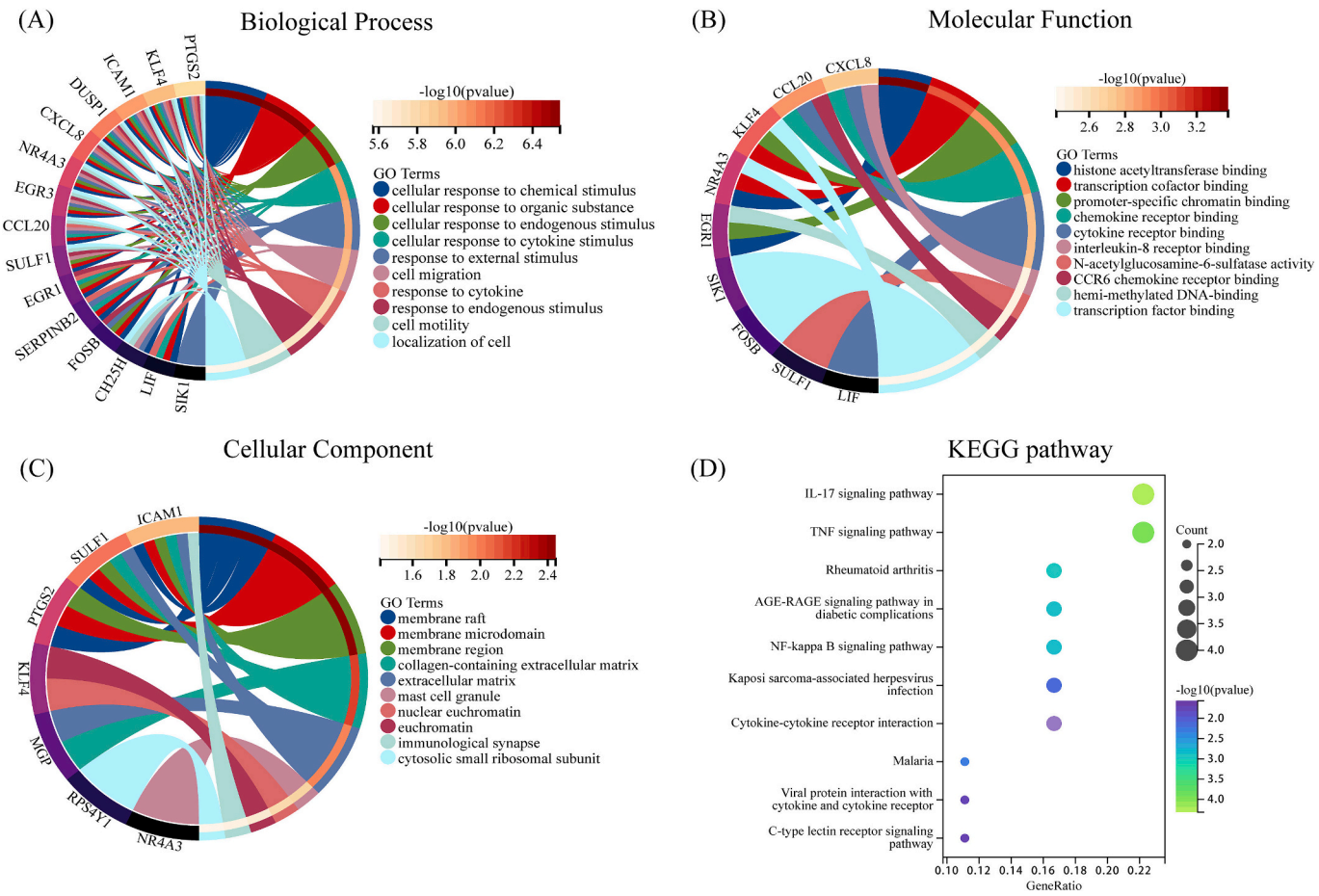

Fig. 3. Enrichment analysis of the common differentially expressed genes. (A) Biological process; (B) molecular function; (C) cellular component; (D) Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis.

prostaglandin biosynthetic process, cytokine-related response, and granulocyte migration, which was consistent with our previous results. Subsequently, a vital module was extracted from the whole PPI network using the Molecular Complex Detection algorithm, which contains 10 nodes and 45 edges (Fig. 4D). The GO enrichment analysis for the key module emphasized the role of cytokine stimulus and cellular response to stress in COVID-19 and AMI (Fig. 4E). In addition, the analysis of the KEGG pathway demonstrated that the IL-17 and TNF signaling pathways were the most prominent (Fig. 4F). It is noteworthy that six genes (*IL1B*, *FOS*, *CTNNB1*, *JUN*, *MAPK14*, and *DUSP1*) were also involved in fluid shear stress and atherosclerosis. At the same time, the module was also correlated with several types of infections, like Kaposi sarcoma-associated herpesvirus infection, pertussis, leishmaniasis, and Chagas disease. These results suggest that there may be molecular crosstalk between COVID-19 and some infection diseases. And these findings are also supported by previous studies. For example, systemic reactivation of herpesviruses has been reported in COVID-19 patients [26] and a multi-omics-based research found that bacterial, parasitic, and protozoan infection pathways are shared in SARS-CoV-2 infection [27].

3.5. Identification and verification of key transcriptional factors

We next want to know what are the related key transcriptional regulators of the common dysregulated genes between COVID-19 and AMI using the TRRUST database. Finally, 30 key transcriptional regulator factors were identified using the 21 common DEGs (Table 2). The TF-target gene transcriptional regulation network is shown in Fig. 5A. Interestingly, three key TFs (EGR2, NFKBIA, ZFP36) were all significantly upregulated in the endothelial cells of patients with AMI and COVID-19 (Figs. 5B, C). They participated in the regulation of five genes (*SERPINB2*, *LIF*, *PTGS2*, *ICAM1*, and *CXCL8*), which were involved in inflammation-related signal pathways.

3.6. MiRNA-gene interaction network construction and validation

The discovery and development of miRNAs have been viewed as potential sources of genomic medicine after viral infection, based on their gene silencing functions. Exploring the function of miRNAs after viruses invade will assist in providing novel and effective targets for the exploration and development of innovative therapeutic strategies. More importantly, miRNAs have been proved to be closely associated with diseases, which can not only provide detailed prognosis information, but also provide insight into the mechanism [28]. In our study, a set of 405 miRNAs was identified. Fig. 6A showed the miRNA-gene interaction network, which had 426 nodes and 500 edges. The miRNAs with the greatest degree were miR-26b-5p, miR-335-5p, miR-124-3p, let-7b-5p, let-7a-5p and miR-146a-5p (Supplementary Table 3). A hierarchical clustering heatmap of key miRNAs is shown in Supplementary Fig. 3. Interestingly, we found that as the severity of the disease increased, the expression of these six miRNAs in the plasma of patients with COVID-19 decreased significantly (Fig. 6B).

4. Discussion

While respiratory failure remains the primary complication of SARS-CoV-2 infection, the cardiovascular complications arising from the virus have garnered significant attention. Many COVID-19 patients exhibit cardiovascular involvement, exacerbating the risk of complications

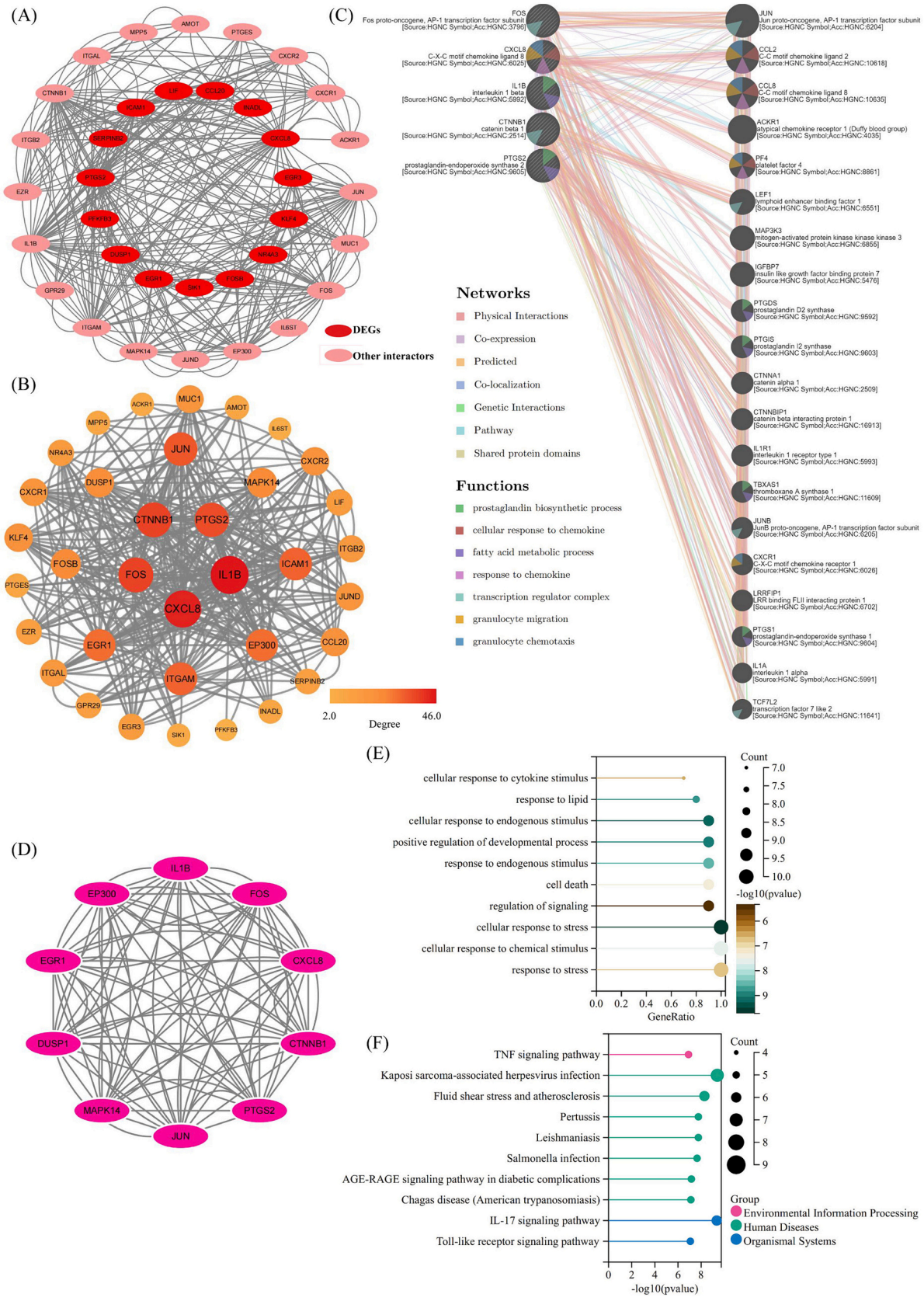

Fig. 4. (A) PPI network of common DEGs among COVID-19 and AMI. (B) Identification of hub genes from the PPI. (C) Function analysis of the hub genes using GeneMANIA. (D) Vital module obtained from the PPI. (E–F) GO and KEGG analysis of the modular genes.

Table 1
Top five hub genes with higher degrees of connectivity.

Gene symbol	Gene description	Degree
IL1B	Interleukin 1 beta	46
CXCL8	C-X-C motif chemokine ligand 8	44
CTNNB1	Catenin beta 1	38
FOS	Fos proto-oncogene	38
PTGS2	Prostaglandin-endoperoxide synthase 2	36

Table 2
Search results of key regulators using the Transcriptional Regulatory Relationships Unraveled by Sentence-based Text-mining database.

Key TF	FDR	Target genes
HDAC1	3.80E−07	KLF4, EGR1, CXCL8, PTGS2, ICAM1
STAT6	9.78E−07	FOSB, CXCL8, PTGS2, DUSP1
ETS2	5.77E−05	EGR1, CXCL8, ICAM1
RELA	0.000102	EGR1, CXCL8, ICAM1, PTGS2, CCL20
NFKB1	0.000102	EGR1, CXCL8, ICAM1, CCL20, PTGS2
NFATC1	0.000123	EGR3, ICAM1
ZFP36	0.000141	SERPINB2, CXCL8
EGR2	0.000159	LIF, PTGS2
PPARG	0.000173	KLF4, PTGS2, ICAM1
APC	0.000232	PTGS2, KLF4
ING4	0.000335	CXCL8, PTGS2
CEBPD	0.000397	CCL20, PTGS2
NFKBIA	0.00046	ICAM1, CXCL8
HDAC4	0.000687	PTGS2, KLF4
PGR	0.000697	PTGS2, DUSP1
ERG	0.000719	ICAM1, CXCL8
HDAC2	0.000719	CXCL8, KLF4
STAT3	0.000826	PTGS2, ICAM1, CXCL8
ATF2	0.000826	PTGS2, DUSP1
CDX2	0.000826	PTGS2, KLF4
JUN	0.000826	CXCL8, PTGS2, MGP
ATF4	0.000936	PTGS2, CXCL8
EP300	0.00227	PTGS2, CXCL8
FOS	0.00227	PTGS2, CXCL8
CEBPB	0.00241	CXCL8, PTGS2
STAT1	0.00449	ICAM1, PTGS2
EGR1	0.00474	CXCL8, PTGS2
CREB1	0.00477	NR4A3, PTGS2
TP53	0.0146	EGR1, DUSP1
SP1	0.0148	ICAM1, EGR1, PTGS2

TF: transcription factor; FDR: false discovery rate.

related to SARS-CoV-2 [29]. In alignment with our prior research findings [6,30], which linked the increase in AMI-related mortality with the COVID-19 pandemic, we proposed a hypothesis centered around the interaction between AMI and COVID-19. This hypothesis posits that SARS-CoV-2 infection may trigger shared molecular pathways in both AMI and COVID-19. Although earlier study has attempted to decipher the molecular mechanisms of cardiac injury post-COVID-19 [31], they were limited by the use of restricted datasets and lacked comprehensive analysis, such as restricted analytical scope, no independent data validation, and insufficient identification of regulatory elements. In this study, we adopted a bioinformatics approach from the perspective of AMI, utilizing multiple datasets to explore the molecular mechanisms of cardiac injury following SARS-CoV-2 infection. Our aim is to gain a deeper understanding of the high incidence of cardiovascular complications in COVID-19 patients.

Endothelial function is a primary determinant in the outcome of COVID-19, and endothelial cell injury is the earliest event of atherosclerosis and subsequent AMI complications. We first analyzed endothelial cells from COVID-19 and AMI datasets. A total of 21 common DEGs were identified in the COVID-19 and AMI datasets. GO, and KEGG pathway enrichment analysis revealed that these genes were significantly enriched in inflammatory and immune pathways. Endothelial cell response to cytokine stimulus due to excessive inflammation was involved in the development of these two inflammatory diseases. Recent literature on the pathogenesis of COVID-19 has demonstrated that the induction of severe acute respiratory distress phenotype is driven by a mismatched inflammatory response and extensive vascular dysfunction [32]. Thrombosis, fluid exosmosis and microvascular lesions observed in small blood vessels and capillaries of the lung also support the view that patients with severe disease have strong vascular reactions [33]. Study has shown that with the aggravation of the severity of COVID-19, endothelial cell dysfunction, cell death, and metabolic changes increase [34]. The lack of integrity or dysfunction of normal endothelium leads to a susceptibility of atherosclerotic plaque rupture [35]. Moreover, cytokines act as the major stimulators of atherosclerosis. The inflammatory state that leads to the production of circulating cytokines, such as ILs and TNF, can activate inflammatory cells in atherosclerotic plaques [36]. Thus, atherosclerotic patients infected with SARS-CoV-2 may be more susceptible to COVID-19-mediated AMI. We observed that the TNF and IL-17 signaling pathways, which are the main mechanisms involved in the production of inflammatory cytokines, were the most significantly enriched pathways, indicating their critical roles in the two diseases. Correspondingly, some studies have also observed the importance of the relationship between these signaling pathways, SARS-CoV-2 infection and AMI [37,38]. Overall, our findings could explain molecular pathogenesis of the two diseases, including acute inflammatory reactions caused by infection, which may induce endothelial dysfunction, hypercoagulability and acute thrombosis, leading to an increased risk of severe cardiac ischemic injury.

Increased troponin levels in COVID-19 are associated with severe illness and higher mortality, which emphasizes that myocardial injury is a prognostic factor [39]. In SARS-CoV-2-infected cardiomyocytes, our analysis showed that 17 of the 21 DEGs were upregulated, suggesting that these genes may be shared in cardiac and vascular-related injuries. In fact, in addition to the myocardial infarction caused by ischemia, SARS-CoV-2 can aggravate cardiac damage by inducing myocardial inflammation. Endomyocardial biopsy of a patient with COVID-19 showed diffuse T lymphocytic inflammatory infiltrates, which provided evidence of myocardial inflammation [40]. In another study, endomyocardial biopsy of a patient with COVID-19 demonstrated low-grade myocardial inflammation, while SARS-CoV-2 particles were observed in interstitial macrophages but not in cardiac myocytes [41]. In general, SARS-CoV-2 could contribute to myocardial inflammation, leading to cardiac dysfunction. Prevention of cytokine-induced cardiac dysfunction may limit severe outcomes in inflammatory diseases.

A PPI network was constructed to investigate the interrelationship of the DEGs. Five hub genes, including *IL1B*, *CXCL8*, *CTNNB1*, *FOS*, and *PTGS2*, were identified. IL1B, the primary form of circulating IL-1, is critically involved in inflammatory processes that lead to the development of atherosclerotic plaques and acute coronary syndrome [42]. Huang et al. [43] reported higher blood levels of IL1B in patients infected with SARS-CoV-2 than in healthy adults. The protein encoded by *CXCL8* (also known as IL-8) is a member of the C-X-C chemokine family and is a major mediator of the inflammatory response. This proinflammatory protein was been demonstrated to play a role in AMI and COVID-19 [44,45]. *CTNNB1*, targeted by SARS-CoV-2 miRNAs [46], encodes β-catenin, which is a key integral part of the canonical Wnt/β-catenin pathway. The activation of the Wnt/β-catenin pathway enhances the transcription of target genes involved in inflammation, endothelial dysfunction, and vascular smooth muscle cell proliferation [47]. The protein encoded by the *FOS* gene is closely related to cell apoptosis. FOS is a key biomarker of coronary artery disease progression and AMI occurrence [48]. COX-2 (encoded by *PTGS2*) can be induced in various cell types (including monocytes/macrophages, vascular endothelial cells, and colorectal cancer cells) in response to inflammatory cytokines, laminar shear stress, and growth factors. It has been shown to regulate lung inflammation and injury observed in patients with COVID-19 [49]. The functional analysis of these genes suggests that they are likely to play a central role in the occurrence and development of AMI after SARS-CoV-2 infection. Furthermore, highly dense modules were

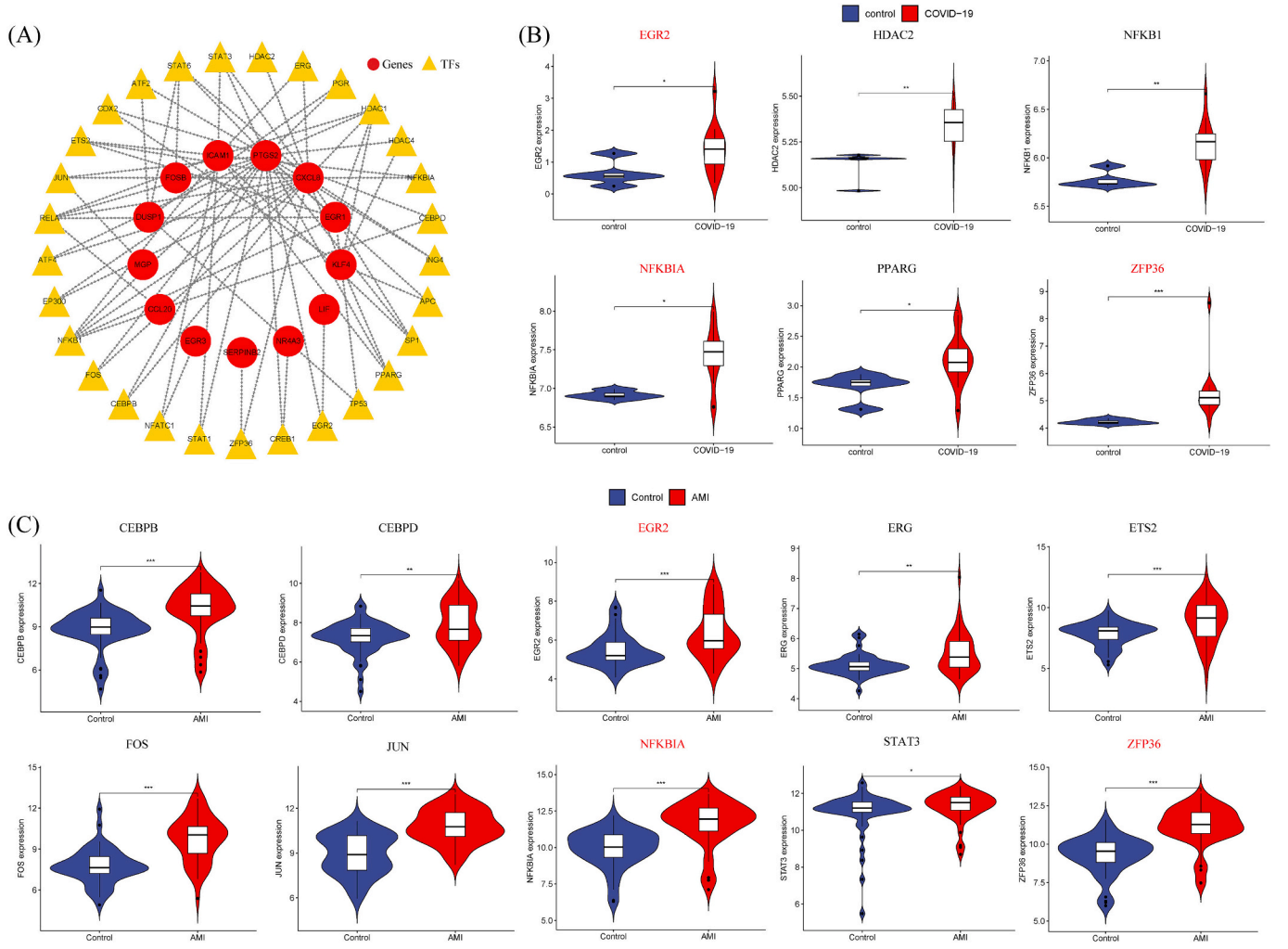

Fig. 5. (A) The gene regulatory network of the genes and key TFs. (B–C) The expression level of TFs with statistical significance in GSE178331 and GSE66360 respectively. Genes in red were upregulated in both COVID-19 and AMI. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extracted from the PPI network. Notably, the five hub DEGs were all involved in the modules. The functional analysis of the hub genes and key modules emphasized the core pathogenesis of inflammatory stimulus mediated by cytokines. Specifically, the pathway of fluid shear stress and atherosclerosis was highly enriched. The pathway is involved in the tangential stress due to the friction of flowing blood on the endothelial surface of the arterial wall [50]. Disturbed flow patterns induce inflammatory activation of the endothelium [51], and the influence of this pathway in COVID-19 should be examined further.

In addition, we found that 30 key TFs may regulate these common genes. By further verification, three TFs (EGR2, NFKBIA and ZFP36) are highly expressed in AMI and COVID-19, and they could be major drivers for the progression of COVID-19 complicated with AMI by promoting the expression of proinflammatory genes. Circulating profiles of plasma miRNAs are associated with SARS-CoV-2 infection [52]. As a potential biomarker, miRNA could provide a breakthrough therapeutic strategy for the diagnosis and management of COVID-19. Therefore, we further studied miRNAs associated with the common DEGs. By analyzing the miRNA-gene interaction network, miR-26b-5p, miR-335-5p, miR-124-3p, let-7b-5p, let-7a-5p, and miR-146a-5p had the highest connectivity, indicating that they may play important regulatory roles in the shared pathogenesis. More importantly, we found that these miRNAs in the plasma were significantly related to the severity of COVID-19. This observation could lead to the development of biomarkers to determine the severity of COVID-19 from patient blood samples collected at admission.

Our research has some limitations. First, this is a retrospective study, and more studies are needed to verify our findings. Second, some datasets are derived from *in vitro* models. Therefore, data from *in vivo* samples may better explain the microenvironment changes of COVID-19. Last, the association between the identified biomarkers and clinical outcomes was not experimentally verified. This will be the focus of our future work.

In summary, our study identified common DEGs between SARS-CoV-2 infection and AMI, followed by an extensive bioinformatics analysis. Our research revealed that a highly activated inflammatory response in patients with COVID-19 might be a crucial factor for susceptibility to AMI. Furthermore, we pinpointed potential genes, transcription factors (TFs), and miRNAs that hold promise as biomarkers or therapeutic targets. This research offers novel insights, paving the way for further exploration of the intricate molecular mechanisms underlying AMI in the context of SARS-CoV-2 infection.

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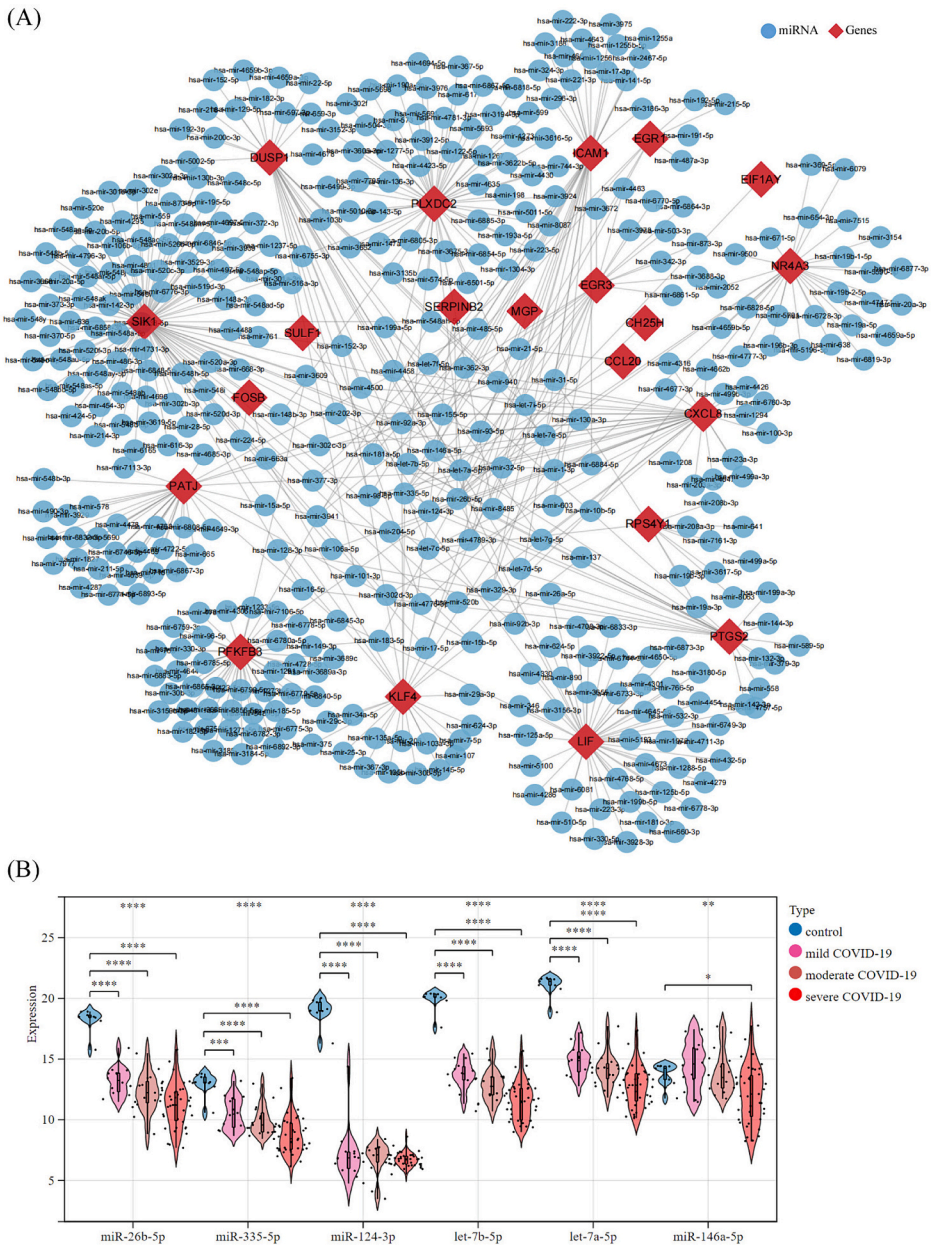

Fig. 6. (A) MiRNA-gene interaction network. (B) The expression level of six key miRNAs in varying degrees of COVID-19 severity. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Ethical statement

This study did not require ethics committee approval.

CRediT authorship contribution statement

Enrui Xie: Conceptualization, Data curation, Formal analysis. **Xiaotao Shen:** Conceptualization, Data curation, Formal analysis. **Yee Hui Yeo:** Conceptualization, Data curation, Formal analysis. **Zixuan Xing:** Conceptualization, Data curation. **Joseph E. Ebinger:** Investigation. **Yixuan Duan:** Investigation. **Yue Zhang:** Investigation. **Susan Cheng:** Investigation. **Fanpu Ji:** Conceptualization, Funding acquisition. **Jie Deng:** Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data used to support the findings of this study are available from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ahjo.2024.100417.

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