scientific reports

Check for updates

OPEN

RNA-Seq analysis of human heart tissue reveals SARS-CoV-2 infection and inappropriate activation of the TNF-NF-κB pathway in cardiomyocytes

Kirtan Dave[1,2]✉, Mukul Jain[1,3], Meenakshi Sharma[4], Anil Kumar Delta[4], Chittaranjan Kole[5] & Prashant Kaushik[6]✉

The negative impact of SARS-CoV-2 virus infection on cardiovascular disease (CVD) patients is well established. This research article explores the cellular pathways involved in underlying heart diseases after infection. The systemic inflammatory response to SARS-CoV-2 infection likely exacerbates this increased cardiovascular risk; however, whether the virus directly infects cardiomyocytes remains unknown due to limited multi-omics data. While public transcriptome data exists for COVID-19 infection in different cell types (including cardiomyocytes), infection times vary between studies. We used available RNA-seq data from human heart tissue to delineate SARS-CoV-2 infection and heart failure aetiology specific gene expression signatures. A total of fifty-four samples from four studies were analysed. Our aim was to investigate specific transcriptome changes occurring in cardiac tissue with SARS-CoV-2 infection compared to non-infected controls. Our data establish that SARS-CoV-2 infects cardiomyocytes by the TNF-NF-κB pathway, potentially triggering acute cardiovascular complications and increasing the long-term cardiovascular risk in COVID-19 patients.

Keywords Cardiomyocytes, Gene, RNA-seq, COVID-19, Heart failure, TNF-NF-κB, Bioinformatics

Abbreviations

HF	Heart failure
TNF-NF-κB	Tumor necrosis factor (TNF) and nuclear factor-kappa B (NF-κB)
iPSC	Induced pluripotent stem cells
GO	Gene ontology
DEG	Differentially expressed genes
AP-1	Activator protein-1
TNFAIP3	Tumor necrosis factor alpha-induced protein 3
CCL2	Chemokine (C–C motif) ligand 2
NFKBIA	Nuclear factor-kappa-B inhibitor alpha
CXCL8	Interleukin-8 (IL-8)

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), can lead to a wide range of clinical outcomes, from asymptomatic infection to severe illness and death[1]. Severe cases are often associated with respiratory pathology[2]. As COVID-19 is a relatively new disease, long-term follow-up data are limited, though many studies have assessed its acute phase[3]. Notably, COVID-19 has been linked to frequent cardiac complications[4]. The pathophysiology of SARS-CoV-2 infection may involve

[1]Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, Vadodara, Gujarat 391760, India. [2]Bioinformatics Laboratory, Research & Development Cell, Parul University, Vadodara, Gujarat 391760, India. [3]Cell & Developmental Biology Lab, Research & Development Cell, Parul University, Vadodara, Gujarat 391760, India. [4]Department of Chemistry, Ranchi University, Ranchi 834001, India. [5]The Neotia University, Sarisha 743368, India. [6]Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, India. ✉email: kirtandave11@gmail.com; prakau@hau.ac.in; prashantumri@gmail.com

several factors, including modifications to the immune system, persistence of viral fragments or proteins in various tissues, and vascular injury leading to blood clotting[5]. Moreover, SARS-CoV-2 infection has long-term cardiovascular effects even in individuals without prior cardiovascular conditions, indicating a strong affinity for the cardiovascular system[6]. The interplay between cardiomyocytes, cardiac fibroblasts, endothelial cells, and cytokines is a complex network that significantly influences heart function[7]. Cardiomyocytes act as the heart's contractile units, orchestrating synchronized contractions for effective blood circulation[8]. Cardiac fibroblasts contribute to the structural framework by secreting extracellular matrix proteins, maintaining myocardial integrity[9], and facilitating tissue healing and scar production when the heart is injured or stressed[10]. Cytokines are key mediators in this network, playing roles in immune responses, inflammation, and cellular communication within the cardiac microenvironment[11,12]. (Fig. 1) provides a visual representation of the specific cellular pathways that are altered as a result of the interaction between SARS-CoV-2 and cardiomyocytes.

Under physiological conditions, cytokines contribute to tissue homeostasis[13] and repair processes[14]. However, in the context of cardiac pathology, dysregulated cytokine signaling may lead to chronic inflammation[15], adverse remodeling, and ultimately, heart failure[16].

Therefore, the function of the implicated genes is not well understood in terms of their particular pathogenic significance, and multiomics analysis[17] is necessary to identify the malfunction caused by the virus[18]. So, investigation of genetic changes and molecular pathways in various diseases, including neoplastic and non-neoplastic disorders[19], has new avenues because of the recent and rapid advancement of transcriptome sequencing technology[20 21]. Since HF and COVID-19 genes linked to CVD are of increasing interest, a growing amount of RNA sequencing (RNA-seq) and microarray datasets[22] have been uploaded to the Gene Expression Omnibus (GEO) database[23], offering prospects for thorough meta-analysis using bioinformatics data mining. The cardiovascular disease etiology leads to a deep molecular level. This study sheds light on the nexus of COVID-19 inflammation and endothelial dysfunction. The study required a comparison of normal human transcriptome vs. COVID-19 infected cell transcriptome data from a publicly submitted sample. In the current investigation, DEG analysis has been carried out initially using 54 different samples of RNA-seq datasets in the GEO database

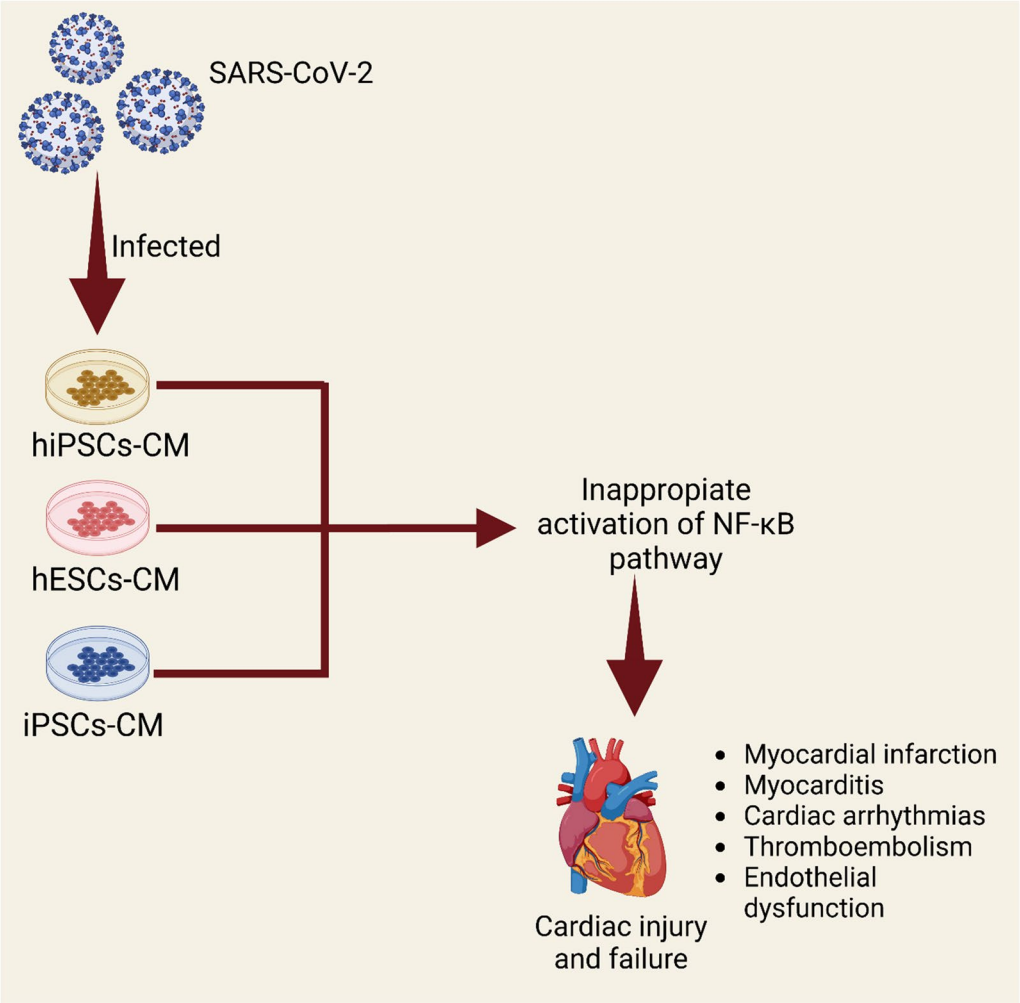

Figure 1. Putative signaling pathway of SARS-COV-2 interaction with heart tissue causing heart damage and heart failure.

to identify common upregulation and downregulation in HF normal samples and COVID-19 infected samples. Higher infection is connected to a pro-inflammatory immune phenotype known as "inflammation," in which T cells develop a pro-inflammatory[24] phenotype more innate than that of NK cells and are linked to the elevation of markers for both T cell senescence and exhaustion[25]. Additionally, elevated chemokine receptor expression, including CXCL2 on T cells, has been demonstrated in the iPSC-derived cardiomyocytes (iPSC-CMs) model[26]. The study was also followed by Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis[27], Gene Ontology (GO)[28], for the DEGs, which validate the role for TNF-A-NKB inappropriate pathway activation.

Materials and methods

Data collection

Gene expression datasets for this study were obtained from the National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO) database. We downloaded RNA-seq datasets to identify genes shared between COVID-19 and heart failure (HF).The search used keywords such as "SARS-CoV-2," "cardiomyocytes," and "cardiac." The Fastq files from GEO accession IDs GSE150392[29], GSE151879[30], and GSE156754[31] were downloaded. These datasets included RNA-seq expression data from various tissue types, including fibro fatty plaque, human pluripotent stem cell-derived cardiomyocytes (hPSC-derived CMs), and adult cardiomyocytes (CMs), as well as iPSC-derived cardiomyocytes, cardiac fibroblasts (CFs), and endothelial cells (ECs). A total of 54 samples from iPSC-CM model studies were analysed, comparing infected samples with mock samples. Table S1 provides a comprehensive overview of the RNA-seq data utilized in this study, encompassing both mock-infected and infected cell samples across various studies. The table includes detailed information on GEO IDs, sample titles, run accessions, and the specific study classifications. Please refer to Supplementary Table S1 for a detailed listing of the RNA-seq samples used.

Sequence alignment and transcriptome assembly

For the fifty-four downloaded RNA-seq runs, a standardized RNA-seq analytic process was used. In the first phase of quality control, RNA-seq runs were downloaded and analysed for sequencing errors using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc).

Second, quality control involved trimming the transcript abundance from single samples using Fastp (version 0.23.0)[32]. Specifically, we used quality-controlled RNA-seq runs with parameters of phred scores of 15 and a minimum length requirement of 20[33], considering the presence of infected and mock cells from various studies. We then utilized transcripts per million (TPM) to calculate the expression levels in each sample[34]. We compiled the quantitative data of all samples into an expression matrix and used a trimmed mean of M values (TMM) to normalize the expression levels between samples[35]. We determined the τ value for every gene to assess the level of tissue specificity in gene expression[36]. For the analysis, we used the R Studio version 3.4 (Bioconductor Package 3.18). The heatmap was created using the "GSVA" R package (https://bioconductor.org/packages/release/bioc/html/GSVA.html)[37], and the bar plot showcasing the top 20 significantly enriched pathways was produced using the "enrichplot" package (https://bioconductor.org/packages/release/bioc/html/enrichplot.html)[38]. A schematic bioinformatics pipeline is provided as a supplementary file attachment (Bioinformatics workflow). This workflow is designed to conduct transcriptome research on raw RNA-seq data for both mock-infected and infected cell samples.

Results

Identification of shared differentially expressed genes

Differential expression between COVID-19 infected cardiomyocytes and mock-infected cells was determined. The number of significant differentially expressed transcript clusters is compared in Table S2. Analysis was performed using DESeq2[39] with an adjusted p-value < 0.05 in R Studio. Upon mapping genomic reads from infected hiPSCs, macrophages, cardiac fibroblasts, and endothelial cells across multiple heart areas, heatmaps of differentially expressed genes demonstrated clustering based on conditions (mock or infected) and transcriptomic profiles. Figure 2 shows a volcano plot where each gene is represented as a point in a two-dimensional space of statistical significance (P-value or FDR) vs. log fold change, allowing visualization of expression changes and their significance across the entire gene set. Figure 3 presents a heatmap of differentially expressed genes, providing a detailed visual representation of expression patterns. Hierarchical clustering was used to cluster data by both columns (samples) and rows (genes).

Gene ontology of genes and genome pathway analyses

In all three investigations, 297 genes were found to be up-regulated in common by a differential expression analysis among infected cardiac cells. A venn diagram suggested that more genes were up-regulated in common among different groups. Together, GO enrichment analysis was conducted for these three gene categories, revealing genes were strongly linked to infected cells and have a significant role in the network regulated by NF-κB signaling. Biological processes frequently include the regulation of small GTPase-mediated signal transduction, as shown by nine GO annotations. It displays that one of the main mechanisms controlling inflammation is the nuclear factor-kappa B (NF-κB) pathway. The GO bar chart and ppi network shown in Fig. 4 represent the top functional category derived from elevated genes according to KEGG analysis.

Involvement of inflammatory genes in the regulation of cardiac inflammation and remodeling in heart failure

The SARS-CoV-2 infection induced significant changes in gene expression within cardiomyocytes. The most notable expression change, as indicated by adjusted p-values, was observed in a gene encoding an immune

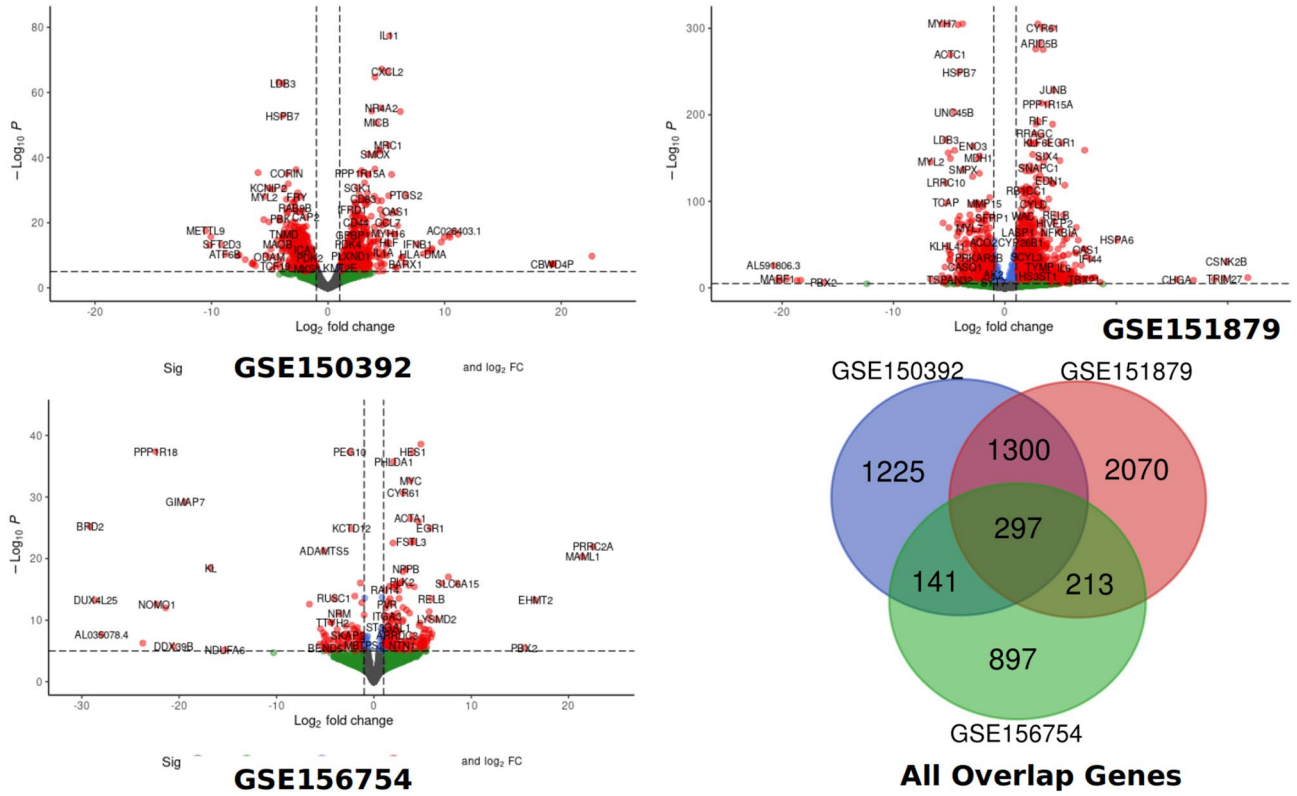

Figure 2. Volcano plots of the differential gene expression data from GSE150392, GSE151879, and GSE156754. In the volcano plots, the red points show up-regulated genes (log2FC ≥ 0.5 and adjusted P-value < 0.05). The Venn map of the differentially expressed genes, however, shows the up-regulated genes as red dots. The number within each circle denotes the quantity of genes that exhibit differential expression across the various comparisons. We took into account only the designated genes. The genes specific to each condition are indicated by the non-overlapping numbers, whereas the overlapping number represents the genes that are mutually differentially expressed between the various comparisons.

cytokine known to be transcriptionally upregulated during SARS-CoV infection—CXCL2, which recruits immune cells to sites of inflammation. Increased levels of inflammatory cytokines may contribute to cardiac dysfunction. Additionally, NF-κB plays a key role in regulating inflammation, and its activation has been linked to cardiac inflammation and remodeling in heart failure. The NF-κB pathway plays a central role in the regulation of immune responses, inflammation, cell survival, and other cellular processes.

FOS and JUN were also significantly increased in all infected samples, as these genes are components of the AP-1 transcription factor complex. They are involved in regulating gene expression in response to various stimuli, including stress and inflammation. AP-1 activation has been associated with cardiac hypertrophy and heart failure. Analysis of the top common genes in each sample revealed a highly upregulated proinflammatory and inflammatory modulation pathway. Moreover, the higher expression of TLR2 leads to the recruitment of various downstream signaling molecules, such as IRAKs (Interleukin-1 receptor-associated kinases) and TRAF6 (TNF receptor-associated factor 6), which further propagate the signaling cascade. This process also involves the recruitment and activation of protein kinases, such as MAPKs (Mitogen-Activated Protein Kinases), leading to the TNF-NF-κB pathway activation of transcription factors and the induction of pro-inflammatory gene expression, as illustrated in Fig. 5.

Discussion

By integrating bulk RNA-sequencing data from various studies, we have created an innovative immune signature atlas of COVID-19infected cardiomyocytes and mock cells, comprising 54 samples. This comprehensive analysis allowed us to identify key mechanisms that could drive the severity of the SARS-CoV-2 infection and its impact on heart health.

Our study primarily highlights the significant role of TLR2 and downstream NF-κB signaling in the context of SARS-CoV-2 infection. TLR2 (Toll-like receptor 2) is known to be essential for recognizing pathogens and initiating immune responses. Previous studies have highlighted the protective function of NF-κB in the heart during stressful conditions, such as myocardial infarction or cardiac hypoxia[40]. NF-κB, a transcription factor, regulates genes involved in inflammation, immune response, and cell survival. However, our findings suggest that hyperactive TLR2/NF-κB signaling significantly contributes to the onset and progression of heart failure

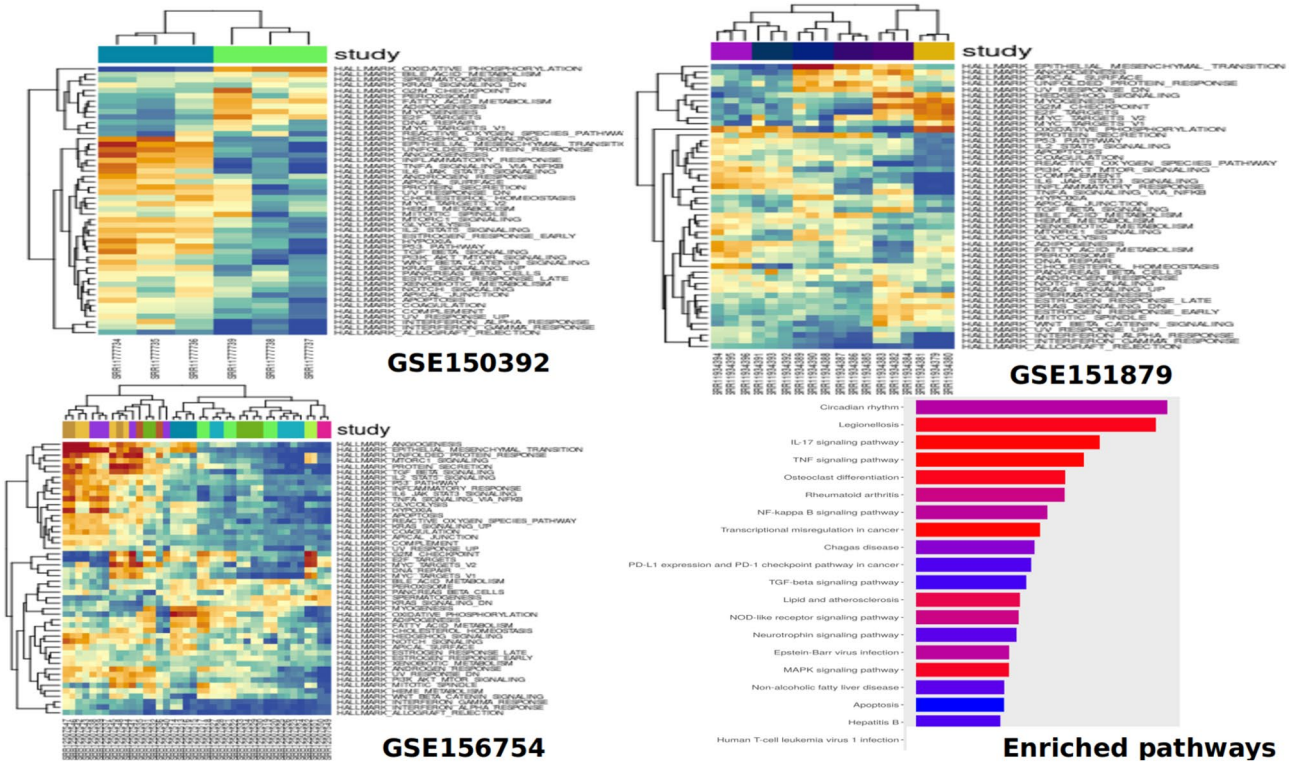

Figure 3. Heat map of the top differentially expressed genes based on GSE150392, GSE151879, and GSE156754. The colour intensity suggests the higher to lower expression pathway and pathway enrichment analysis.

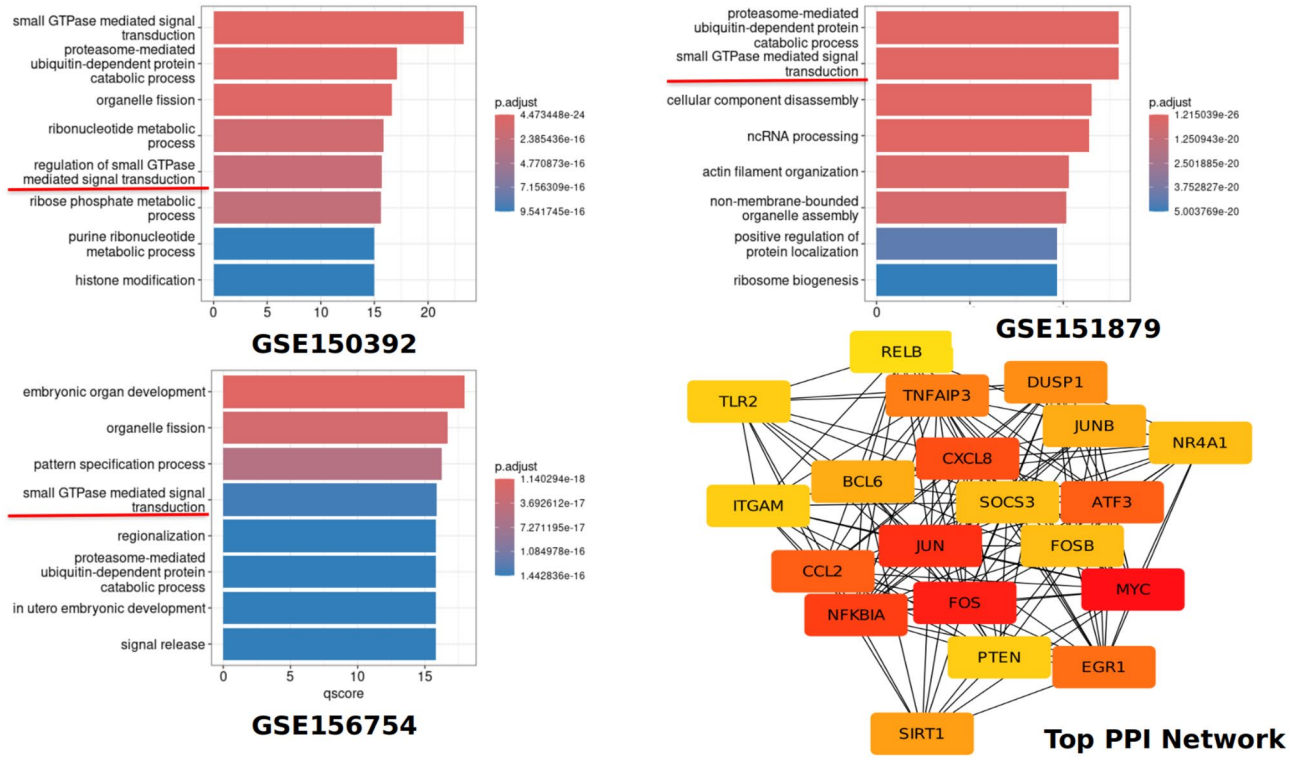

Figure 4. GO analyses of genes that are elevated and downregulated and analysis of the GO function enrichment (including BP, MF, and CC). B. Analysis of KEGG pathway enrichment. The gene ratio is displayed on the x-axis, while the GO category is displayed on the y-axis. The size of the bar represents the number of genes included in the relevant pathway, and the significance of enrichment progressively grows from blue to red.

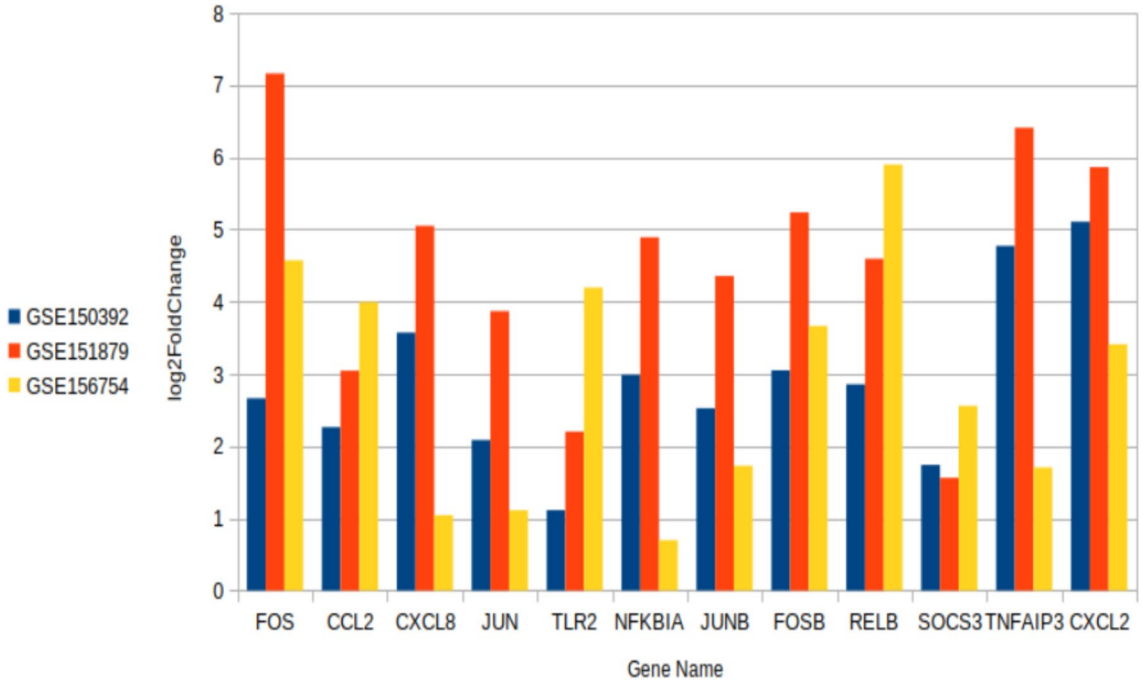

Figure 5. Differentially expression of TNF-NF-κB pathway genes from DEG analysis.

through several pathways, including oxidative stress, inflammation, myocardial fibrosis, endothelial damage, myocardial hypertrophy, and apoptosis[41].

Oxidative stress is a critical factor in the pathogenesis of cardiovascular diseases. During SARS-CoV-2 infection, excessive production of reactive oxygen species (ROS) can lead to oxidative damage in cardiomyocytes. This oxidative stress can further activate NF-κB signaling, creating a vicious cycle that exacerbates inflammation and cell death. Inflammation, another major contributor, involves the activation of various cytokines and chemokines. Our analysis identified several inflammatory mediators, such as CXCL2, which recruits immune cells to sites of inflammation, and MCP-1 (CCL2), which attracts monocytes[45,46]. These inflammatory responses can lead to chronic inflammation, adverse cardiac remodeling, and ultimately heart failure.

Myocardial fibrosis, characterized by excessive deposition of extracellular matrix proteins by cardiac fibroblasts, can lead to stiffening of the heart tissue and impaired cardiac function. NF-κB activation in fibroblasts promotes the production of pro-fibrotic factors, contributing to fibrosis. Endothelial damage, another critical aspect, involves injury to the blood vessels, leading to impaired vascular function and increased risk of thrombosis. The activation of NF-κB in endothelial cells can exacerbate this damage by promoting inflammation and coagulation.

Myocardial hypertrophy, the enlargement of cardiomyocytes, is a compensatory response to increased workload or stress. However, sustained hypertrophy can lead to heart failure. Our data indicate that NF-κB signaling, along with other transcription factors like AP-1 (formed by FOS and JUN), plays a significant role in regulating genes associated with hypertrophy[43]. Apoptosis, or programmed cell death, is another critical factor. Excessive apoptosis of cardiomyocytes can lead to a reduction in functional heart tissue, contributing to heart failure.

In addition to the heart, TLR4/NF-κB signaling is crucial for the functioning of other organs, such as the kidneys, neurological system, digestive system, and lungs. This widespread involvement underscores the importance of NF-κB as a central regulator of immune and inflammatory responses. An intricate network of genes is involved in the TNF-NF-κB pathway, highlighting its role as a critical regulator of cellular functioning. MYC, initially discovered as a proto-oncogene, becomes a key actor capable of stimulating the NF-κB pathway and promoting cell division[42]. FOS and JUN work together to generate the AP-1 transcription factor, which responds to a variety of stimuli, including TNF, and plays a vital role in the precise control of target genes for NF-κB[43].

The integral inhibitor of NF-κB, IκBα, encoded by NFKBIA, functions by confining NF-κB within the cytoplasm and obstructing its activation[44]. This regulatory mechanism is crucial for maintaining a balance between immune activation and inhibition. Interleukin-8 (IL-8) or CXCL8, facilitates the recruitment of immune cells to areas of inflammation, further amplifying the inflammatory response[45]. The transcription factor ATF3 shapes the cellular response to inflammation by regulating NF-κB activation. This modulation is critical for controlling the extent and duration of inflammatory responses.

MCP-1 (CCL2) is a chemokine that draws monocytes to areas of inflammation, playing a key role in the recruitment of immune cells during the inflammatory process[46]. Genes involved in cell proliferation and differentiation are regulated by EGR1, which is activated by TNF. EGR1 plays a pivotal role in the cellular response to injury and stress. TNFAIP3 (A20) appears as a negative regulator, providing feedback inhibition to reduce NF-κB activation and avoid over-inflammation[47]. This feedback loop is essential for preventing excessive inflammatory responses that could lead to tissue damage.

The phosphatase-encoding gene DUSP1 inhibits MAP kinases, which affects how cells react to TNF. This regulation is important for modulating the cellular responses to inflammatory stimuli[48]. SIRT1, a member of the sirtuin family, has anti-inflammatory properties through NF-κB activity modulation[49]. SIRT1's role in reducing inflammation highlights its potential as a therapeutic target for inflammatory diseases. The transcriptional repressor BCL6 modifies NF-κB signaling in a subtle way, ensuring precise control overinflammation responses. Members of the AP-1 transcription factor family, JUNB and FOSB, actively participate in the control of NF-κB target genes. These transcription factors integrate various signaling pathways, contributing to the complexity of the inflammatory response.

SOCS3, a cytokine signaling suppressor, has inhibitory effects on pathways such as NF-κB, providing another layer of regulation to prevent excessive inflammation. NR4A1 (Nur77) regulates NF-κB activity, affecting apoptosis and cellular responses. The tumour suppressor PTEN interacts with NF-κB signaling to negatively control the PI3K/AKT pathway, highlighting its role in balancing cell survival and apoptosis[50]. The toll-like receptor TLR2 is essential for identifying pathogens and has the ability to trigger NF-κB in reaction to infections. Lastly, integrin ITGAM influences NF-κB activation and is involved in cell adhesion, further contributing to the regulation of immune responses[51].

The intricate relationships between these genes demonstrate how they cooperate to subtly and crucially regulate the TNF-NF-κB pathway for inflammatory responses, immune responses, and cellular survival. Therefore, to prevent heart failure, cardiomyocytes, cardiac fibroblasts, and endothelial cells must maintain a delicate balance and coordinate their responses. Understanding the mechanisms behind both clinical disorders and physiological cardiac function requires an understanding of the TNF-NF-κB pathway and cytokines.

Furthermore, the limited number of existing datasets hinders the ability to draw definitive conclusions. To bridge these knowledge gaps, additional data is needed. Current therapeutic strategies targeting these pathways have shown limited success. However, further research employing single-cell proteomics and genomics to investigate cardiomyocyte infection by COVID-19 holds promise for developing more selective and targeted approaches, potentially leading to improved clinical outcomes. Future research focusing on novel therapeutic agents, personalized medicine, and combination therapies may offer new hope for heart failure patients. These follow-up studies would be crucial to solidifying our current understanding. Also, the existing datasets may be limited in terms of sample diversity, so there is a need to reduce gaps in dataset availability to draw more precise conclusions. While current therapies targeting these pathways have had limited success, more single-cell proteomic and single-cell genomics research on cardiomyocyte infection by COVID-19 into more selective and targeted approaches holds promise for improved clinical outcomes. Future research focusing on novel therapeutic agents, personalized medicine, and combination therapies may offer new hope for heart failure patients.

Conclusion

The complicated links between these genes show how they work together to carefully and precisely manage the TNF-NF-κ pathway. This pathway is in charge of immune system responses, inflammatory responses, and cell survival. Hence, in order to avert heart failure, it is imperative for cardiomyocytes, cardiac fibroblasts, and endothelial cells to uphold a fragile equilibrium and synchronize their reactions. To comprehend the workings of clinical diseases and physiological heart function, one must have a grasp of the cytokines in the TNF-NF-κB pathway. Moreover, the scarcity of available statistics impedes the capacity to reach conclusive findings. In order to fill these knowledge gaps, more data is required. Existing treatment approaches aimed at these pathways have demonstrated minimal efficacy. Additional research utilizing single-cell proteomics and genomes to study the COVID-19 infection of cardiomyocytes shows potential for establishing more precise and focused strategies, which could result in enhanced therapeutic results. Subsequent investigations that prioritize innovative therapeutic substances, tailored medical treatments, and the use of several therapies may provide renewed optimism for those suffering from heart failure. These subsequent investigations will be critical in consolidating our current understanding.

Data availability

All data and materials used in this research are freely available and can be obtained upon request from the corresponding author.

Received: 18 March 2024; Accepted: 7 August 2024
Published online: 27 September 2024

References

1. Infectious Disease Subdivision Leadership. The Journal of molecular diagnostics : JMD. 2020;22(8):968-74. https://doi.org/10.1016/j.jmoldx.2020.06.003.
2. Afewerky, H. K. Pathology and pathogenicity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Exp. Biol. Med. (Maywood)* **245**(15), 1299–1307. https://doi.org/10.1177/1535370220942126 (2020).
3. Schwartz, D. A. & Levitan, D. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infecting pregnant women and the fetus, intrauterine transmission, and placental pathology during the coronavirus disease 2019 (COVID-19) pandemic: It's complicated. *Arch. Pathol. Lab. Med.* **145**(8), 925–928. https://doi.org/10.5858/arpa.2021-0164-ED (2021).
4. Vosko, I., Zirlik, A. & Bugger, H. Impact of COVID-19 on cardiovascular disease. *Viruses* https://doi.org/10.3390/v15020508 (2023).
5. Wiersinga, W. J., Rhodes, A., Cheng, A. C., Peacock, S. J. & Prescott, H. C. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): A review. *JAMA* **324**(8), 782–793. https://doi.org/10.1001/jama.2020.12839 (2020).
6. Abbasi, J. The COVID heart—One year after SARS-CoV-2 infection, patients have an array of increased cardiovascular risks. *JAMA* **327**(12), 1113–1114. https://doi.org/10.1001/jama.2022.2411 (2022).

7. Takeda, N. & Manabe, I. Cellular interplay between cardiomyocytes and nonmyocytes in cardiac remodeling. *Int. J. Inflamm.* **2011**, 535241. https://doi.org/10.4061/2011/535241 (2011).
8. Pinnell, J., Turner, S. & Howell, S. Cardiac muscle physiology. *Contin. Educ. Anaesth. Crit. Care Pain* **7**(3), 85–88. https://doi.org/10.1093/bjaceaccp/mkm013 (2007).
9. Ivey, M. J. & Tallquist, M. D. Defining the cardiac fibroblast. *Circ. J.* **80**(11), 2269–2276. https://doi.org/10.1253/circj.CJ-16-1003 (2016).
10. Psarras, S., Beis, D., Nikouli, S., Tsikitis, M. & Capetanaki, Y. Three in a box: Understanding cardiomyocyte, fibroblast, and innate immune cell interactions to orchestrate cardiac repair processes. *Front. Cardiovasc. Med.* https://doi.org/10.3389/fcvm.2019.00032 (2019).
11. Frangogiannis, N. G. The extracellular matrix in ischemic and nonischemic heart failure. *Circ. Res.* **125**(1), 117–146. https://doi.org/10.1161/circresaha.119.311148 (2019).
12. Hanna, A. & Frangogiannis, N. G. Inflammatory cytokines and chemokines as therapeutic targets in heart failure. *Cardiovasc. Drugs Ther.* **34**(6), 849–863. https://doi.org/10.1007/s10557-020-07071-0 (2020).
13. Wanjare, M. & Huang, N. F. Regulation of the microenvironment for cardiac tissue engineering. *Regen. Med.* **12**(2), 187–201. https://doi.org/10.2217/rme-2016-0132 (2017).
14. Sun, K., Li, Y. Y. & Jin, J. A double-edged sword of immuno-microenvironment in cardiac homeostasis and injury repair. *Sig. Transduct. Target. Ther.* **6**(1), 79. https://doi.org/10.1038/s41392-020-00455-6 (2021).
15. Tripathy, A. S. *et al.* Pro-inflammatory CXCL-10, TNF-α, IL-1β, and IL-6: biomarkers of SARS-CoV-2 infection. *Arch. Virol.* **166**(12), 3301–3310. https://doi.org/10.1007/s00705-021-05247-z (2021).
16. Meizlish, M. L., Franklin, R. A., Zhou, X. & Medzhitov, R. Tissue homeostasis and inflammation. *Annu. Rev. Immunol.* **39**(1), 557–581. https://doi.org/10.1146/annurev-immunol-061020-053734 (2021).
17. Overmyer, K. A. *et al.* Large-scale multi-omic analysis of COVID-19 severity. *Cell Syst.* **12**(1), 23-40.e7. https://doi.org/10.1016/j.cels.2020.10.003 (2021).
18. Yancy, C. W. & Fonarow, G. C. Coronavirus disease 2019 (COVID-19) and the heart—Is heart failure the next chapter?. *JAMA Cardiol.* **5**(11), 1216–1217. https://doi.org/10.1001/jamacardio.2020.3575 (2020).
19. Srivastava S, Grizzle WE. Biomarkers and the genetics of early neoplastic lesions. (1875–8592 (Electronic)).
20. Casamassimi, A., Federico, A., Rienzo, M., Esposito, S. & Ciccodicola, A. Transcriptome profiling in human diseases: New advances and perspectives. *Int. J. Mol. Sci.* https://doi.org/10.3390/ijms18081652 (2017).
21. Tu D, Ma C, Zeng Z et al. Identification of hub genes and transcription factor regulatory network for heart failure using RNA-seq data and robust rank aggregation analysis. (2297–055X (Print)).
22. Clough, E. & Barrett, T. The gene expression omnibus database. *Methods Mol. Biol. (Clifton, NJ).* **1418**, 93–110. https://doi.org/10.1007/978-1-4939-3578-9_5 (2016).
23. Edgar, R., Domrachev, M. & Lash, A. E. Gene expression omnibus: NCBI gene expression and hybridization array data repository. *Nucl. Acids Res.* **30**(1), 207–210. https://doi.org/10.1093/nar/30.1.207 (2002).
24. Li, Y. *et al.* SARS-CoV-2 early infection signature identified potential key infection mechanisms and drug targets. *BMC Genom.* **22**(125), 1–13 (2021).
25. van Eeden, C., Khan, L., Osman, M. S. & Cohen Tervaert, J. W. Natural killer cell dysfunction and its role in COVID-19. *Int. J. Mol. Sci.* https://doi.org/10.3390/ijms21176351 (2020).
26. Sharma, A. *et al.* Human iPSC-derived cardiomyocytes are susceptible to SARS-CoV-2 Infection. *Cell Rep. Med.* **1**(4), 100052. https://doi.org/10.1016/j.xcrm.2020.100052 (2020).
27. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucl. Acids Res.* **28**(1), 27–30. https://doi.org/10.1093/nar/28.1.27 (2000).
28. Ashburner, M. *et al.* Gene ontology: Tool for the unification of biology. *Gene Ontol. Consort. Nat. Genet.* **25**(1), 25–29. https://doi.org/10.1038/75556 (2000).
29. Kumar, S. *et al.* Disease modeling and disease gene discovery in cardiomyopathies: A molecular study of induced pluripotent stem cell generated cardiomyocytes. *Int. J. Mol. Sci.* https://doi.org/10.3390/ijms22073311 (2021).
30. Chen S, Yang L, Nilsson-Payant B et al. SARS-CoV-2 Infected Cardiomyocytes Recruit Monocytes by Secreting CCL2. Research square. https://doi.org/10.21203/rs.3.rs-94634/v1. (2020)
31. Perez-Bermejo, J. A. *et al.* SARS-CoV-2 infection of human iPSC-derived cardiac cells reflects cytopathic features in hearts of patients with COVID-19. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.abf7872 (2021).
32. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560 (2018).
33. Mbandi, S., Hesse, U., Rees, D. J. & Christoffels, A. A glance at quality score: Implication for de novo transcriptome reconstruction of Illumina reads. *Front. Genet.* https://doi.org/10.3389/fgene.2014.00017 (2014).
34. Patro, R., Duggal, G., Love, M. I., Irizarry, R. A. & Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods* **14**(4), 417–419. https://doi.org/10.1038/nmeth.4197 (2017).
35. Robinson, M. D. & Oshlack, A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol.* **11**(3), R25. https://doi.org/10.1186/gb-2010-11-3-r25 (2010).
36. Kryuchkova-Mostacci, N. & Robinson-Rechavi, M. A benchmark of gene expression tissue-specificity metrics. *Brief. Bioinform.* **18**(2), 205–214. https://doi.org/10.1093/bib/bbw008 (2016).
37. Hänzelmann, S., Castelo, R. & Guinney, J. GSVA: Gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinform.* https://doi.org/10.1186/1471-2105-14-7 (2013).
38. Yu, G. Enrichplot: Visualization of functional enrichment result. *R Packag. Vers.* **1**, 24. https://doi.org/10.18129/B9.bioc.enrichplot (2024).
39. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**(12), 550. https://doi.org/10.1186/s13059-014-0550-8 (2014).
40. Nolte, F. S. *et al.* Responding to the challenges of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Perspectives from the association for molecular pathology infectious disease subdivision leadership. *J. Mol. Diagn.* **22**(8), 968–974. https://doi.org/10.1016/j.jmoldx.2020.06.003 (2020).
41. Tang, Y. *et al.* Effects of enalapril on TLR2/NF-κB signaling pathway and inflammatory factors in rabbits with chronic heart failure. *Evid. Based Complement Alternat Med.* https://doi.org/10.1155/2021/9594607 (2021).
42. Huang, H. *et al.* NF-κB1 inhibits c-Myc protein degradation through suppression of FBW7 expression. *Oncotarget* **5**(2), 493–505. https://doi.org/10.18632/oncotarget.1643 (2014).
43. McBride, K., Robitaille, L., Tremblay, S., Argentin, S. & Nemer, M. fos/jun repression of cardiac-specific transcription in quiescent and growth-stimulated myocytes is targeted at a tissue-specific cis element. *Mol. Cell. Biol.* **13**(1), 600–612. https://doi.org/10.1128/mcb.13.1.600-612.1993 (1993).
44. Yu, H. *et al.* Targeting NF-κB pathway for the therapy of diseases: Mechanism and clinical study. *Sig. Transduct. Target Ther.* **5**, 209. https://doi.org/10.1038/s41392-020-00312-6 (2020).
45. Segiet, O. A., Piecuch, A., Mielanczyk, L., Michalski, M. & Nowalany-Kozielska, E. Role of interleukins in heart failure with reduced ejection fraction. *Anatol. J. Cardiol.* https://doi.org/10.14744/AnatolJCardiol.2019.32748 (2019).

46. Sung, F. L., Zhu, T. Y., Au-Yeung, K. K., Siow, Y. L. & Karmin, O. Enhanced MCP-1 expression during ischemia/reperfusion injury is mediated by oxidative stress and NF-kappaB. *Kidney Int.* **62**(4), 1160–1170 (2002).
47. He, K. L. & Ting, A. T. A20 inhibits tumor necrosis factor (TNF) alpha-induced apoptosis by disrupting recruitment of TRADD and RIP to the TNF receptor 1 complex in Jurkat T cells. *Mol. Cell Biol.* **22**(17), 6034–6045. https://doi.org/10.1128/MCB.22.17.6034-6045.2002 (2002).
48. Chen, H. F., Chuang, H. C. & Tan, T. H. Regulation of dual-specificity phosphatase (DUSP) ubiquitination and protein stability. *Int. J. Mol. Sci.* **20**(11), 2668. https://doi.org/10.3390/ijms20112668 (2019).
49. Lin, B. *et al.* Sirt1 improves heart failure through modulating the NF-κB p65/microRNA-155/BNDF signaling cascade. *Aging (Albany NY)* **13**(10), 14482–14498. https://doi.org/10.18632/aging.103640 (2020).
50. Medzikovic, L., de Vries, C. J. M. & de Waard, V. NR4A nuclear receptors in cardiac remodeling and neurohormonal regulation. *Trends Cardiovasc. Med.* **29**(8), 429–437. https://doi.org/10.1016/j.tcm.2018.11.015 (2019).
51. Huang, H., Wang, Q., Ma, L. & Wu, Y. ITGAM: A pivotal regulator in macrophage dynamics and cardiac function during sepsis-induced cardiomyopathy. *Cureus* **16**(4), e59342. https://doi.org/10.7759/cureus.59342 (2024).

Acknowledgements

Author would like to express gratitude to Research and Development cell, Parul University for their support on this project.

Author contributions

Conceptualization and methodology K.D., PK.; writing, review and editing, K.D., P.K, C.K, A.D, M.S and M.J.; supervision, K.D All authors have read and agreed to the published version of the manuscript.

Funding

The study was conducted without external financial support.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-69635-6.

Correspondence and requests for materials should be addressed to K.D. or P.K.